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The posterior pituitary

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1 **The posterior pituitary, from Geoffrey Harris to our present understanding**

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Abstract

Geoffrey Harris pioneered our understanding of the posterior pituitary, mainly by experiments involving electrical stimulation of the supraoptico-hypophysial tract. Here we explain how his observations included key clues to the pulsatile nature of the oxytocin signal, clues which were followed up by subsequent workers including his students and their students. These studies ultimately led to our present understanding of the milk-ejection reflex and of the role of oxytocin in parturition. Key discoveries of wide significance followed: the recognition of the importance of pulsatile hormone secretion, the recognition of the importance of stimulus-secretion coupling mechanisms in interpreting patterned electrical activity of neurons, the physiological importance of peptide release in the brain, the recognition that peptide release comes substantially from dendrites and can be regulated independently of nerve terminal secretion, and the importance of dynamic morphological changes to neuronal function in the hypothalamus, all followed from the drive to understand the milk-ejection reflex. We also reflect on Harris' observations on vasopressin secretion, on the effects of stress, and on oxytocin secretion during sexual activity.

Introduction

The comfortable view of science is of a uniquely disinterested activity, gathering objective and unbiased observations which, by the selfless collaboration and co-operation of transnational armies of scientists, lead us ever closer to objective truth. A less comfortable view was expressed by Karl Popper: "Science does not rest upon solid bedrock. The bold structure of its theories rises, as it were, above a swamp", and in his view, it is the "bold ideas, unjustified anticipations and speculative thought" of individual scientists that mark the best science and which drive progress (Popper 1959). There is certainly a *flow* in our understanding: one observation leads to the next and each question answered raises another, and that flow is certainly perturbed (if not quite guided) by those whose bold ideas gain currency. In this essay, we trace the impact of the work of Geoffrey Harris on our understanding of the posterior pituitary gland, though whether our understanding would be different had Harris become an accountant instead of a scientist is something we can't say: that is one experiment we can't yet perform.

Harris won his reputation as the "father of neuroendocrinology" by incisive experiments which showed that the endocrine cells of the anterior pituitary are regulated by products of hypothalamic neurones that are secreted into the hypothalamo-pituitary portal circulation (Raisman 1997). If he was bold in this, he was more conservative when it came to the theories of

71   others: in his 1955 monograph he still at this time inclined to the view that the posterior pituitary

72   contained endocrine cells that were innervated by hypothalamic neurones (Harris 1955). While

73   conceding that the neurosecretory origin of the posterior pituitary hormones (Leveque and

74   Scharrer 1953) was an "attractive hypothesis", he stated that "sweeping statements have been

75   made at various times by the protagonists of the neurosecretory hypothesis" and warned that

76   "such claims as these, which run contrary to a great deal of established data should be taken with

77   reserve" (Harris 1955 p264). In particular, Harris rejected the notion that the Gomorri-stainable

78   material present in the hypothalamo-hypophysial tract was the histological representation of

79   antidiuretic hormone as argued by the Scharrers. He thought that the amount of oxytocic and

80   antidiuretic activity present in the hypothalamus was too low to be consistent with the

81   hypothalamus being the site of production. Finally, he disputed the evidence that neural stalk

82   section could be followed by a partial regeneration of the neural lobe - evidence which

83   suggested that regeneration of nerve terminals was sufficient to support secretion in the absence

84   of endocrine cells (Harris 1955 p262-265).

85   Nevertheless, Harris pioneered our understanding of the posterior pituitary, mainly by

86   experiments involving electrical stimulation of the supraoptico-hypophysial tract. At the outset

87   of those experiments it was known that extracts of the posterior pituitary could stimulate the let-

88   down of milk in lactating animals, and Ely and Peterson (1941) had shown that the blood of

89   cows which had been milked contained something that could evoke milk let-down in the isolated

90   udder. They proposed that this substance came from the posterior pituitary and was released by

91   suckling, but Selye (1934) had earlier proposed that lactation could be explained by the

92   stimulation of prolactin production from the *anterior* pituitary, and several reports had appeared

93   that lactation could proceed normally even after sectioning the neural stalk.

94   Accordingly, with his student Barry Cross, Harris set out to test these two hypotheses.

95   He had concluded (Harris 1948a) that direct electrical stimulation was ineffective in triggering

96   secretion from the anterior pituitary, but the posterior pituitary was innervated by a nervous tract

97   - the supraoptico-hypophysial tract. Cross and Harris (1950, 1952) showed that electrical

98   stimulation of this tract caused an increase in intramammary pressure in lactating rabbits -

99   showing that the pituitary contains a releasable factor that can induce milk let-down. Harris et al.

100   (1969) later showed that the mammary response depended strongly on the stimulus frequency -

101   only at frequencies in excess of 40 Hz was there an appreciable response – a finding that was to

102   prove prescient (Fig. 1 A,B).

103   In 1966, Yagi et al. showed that electrical stimuli applied to the neural stalk would

104   trigger action potentials that were conducted antidromically to the neurosecretory cell bodies,

105 but the utility of this seemed limited as both the site of stimulation and the site of recording

106 required precise stereotaxic control. However, Barry Cross, who was now Professor of Anatomy

107 at Bristol, saw that, in lactating rats, the site of the stimulating electrode could be precisely

108 controlled by ensuring that it was positioned where stimulation would elicit a rise in

109 intramammary pressure (Sundsten et al. 1970). This opened the way to studying magnocellular

110 neurons *in vivo*, and Jon Wakerley and Dennis Lincoln, working in Cross's Department, used

111 this approach to study how the electrical activity of "antidromically identified" magnocellular

112 neurons regulate oxytocin and vasopressin secretion.

113

114 **The milk-ejection reflex**

115 There was still no real understanding of the milk-ejection reflex, and, in particular, no

116 appreciation that the reflex was intermittent. The key breakthrough came when Wakerley and

117 Lincoln (1973) showed that, during suckling, some of the antidromically identified cells in the

118 supraoptic and paraventricular nuclei showed brief, synchronised high frequency discharges (~

119 1-2 s at 5OHz) at intervals of ~ 10 min, each of which was followed, about 10s later, by an

120 abrupt increase in intramammary pressure – a marker of milk let-down in the mammary glands

121 (Fig.1D). It became clear that these bursts, which led to pulses of oxytocin secretion, were

122 approximately synchronised amongst all of the magnocellular oxytocin cells in the

123 hypothalamus. As a corollary, other magnocellular neurons that were antidromically identified

124 as projecting to the posterior pituitary but which did not participate in this bursting activity could

125 be assumed to be vasopressin cells.

126 Exactly why pulsatile secretion was a critically important phenomenon was not

127 immediately apparent, but an important clue lay in Harris' observation, alluded to earlier, that

128 electrical stimulation of the posterior pituitary would only evoke a strong intramammary

129 pressure response if relatively high frequencies of stimulation were used (Harris et al. 1969).

130 The explanation for this has two elements (Fig. 1). First, the response of the mammary gland to

131 a bolus of oxytocin is non-linear, and has quite a narrow dynamic range: there is a threshold

132 dose that must be exceeded before any effect is observed, and above this threshold the response

133 to higher doses of oxytocin rises swiftly to a maximum. Thus the mammary gland seems to

134 require pulsatile activation – especially because, if oxytocin is applied continuously rather than

135 in pulses, then the response of the gland rapidly diminishes. Second, *how much* oxytocin is

136 secreted in response to electrical stimulation strongly depends on the frequency of stimulation –

137 more is secreted per stimulus pulse when stimuli are clustered closely together (Fig. 1C). This

138 *frequency facilitation* of stimulus-secretion coupling can be attributed to several factors. A

139 solitary spike invading an axon in the pituitary will not invade all terminals of that axon, and

140 in those it does invade, it will produce only a brief rise in intracellular calcium - the essential

141 trigger for vesicle exocytosis. However, during a burst of spikes, a progressive increase in

142 extracellular $[K^+]$ depolarises the axons and endings in the neural lobe, securing a more

143 complete invasion of the terminal arborisation. Moreover, successive spikes in a burst are

144 progressively broadened, inducing a progressively larger calcium entry, giving a potentiated

145 signal for exocytosis. As a result, each spike within a burst releases much more oxytocin than

146 the isolated spikes that occur between bursts (Bourque 1991; Leng and Brown 1997).

147 The explosive nature of milk-ejection bursts suggested that some positive feedback was

148 involved, and Moss, Dyball and Cross (1972) set about to try to show that oxytocin released

149 from the posterior pituitary had that positive feedback effect. They recorded from magnocellular

150 neurons in rats and rabbits, and studied the effects of oxytocin given intravenously and

151 administered directly to the neurones by iontophoresis. The results were disconcerting –

152 oxytocin had a dramatic excitatory effect upon many magnocellular neurons, and this seemed to

153 be a specific effect, as non-neurosecretory cells were unaffected, and vasopressin applied in the

154 same way was without effect. *However,* oxytocin even at large doses had no effect at all when

155 given intravenously.

156 At that time there was no evidence that oxytocin was released centrally, and indeed it

157 seemed very unlikely that it would be – there was no strong evidence of axon collaterals, and the

158 evidence tended to suggest that if there were any recurrent collaterals then their effect was

159 probably inhibitory. Indeed several reports had appeared of "recurrent inhibition" in the

160 magnocellular system – reports later shown by Leng and Dyball (1984) to be based upon

161 misinterpreted evidence. Moss et al. (1972) recognised that the ineffectiveness of intravenous

162 oxytocin meant that oxytocin secreted from the pituitary did not find its way back into the brain.

163 Accordingly, they concluded that the excitatory action of oxytocin on oxytocin cells was a

164 pharmacological phenomenon without physiological significance.

165 However this view was soon to change. Philippe Richard and his colleagues in France

166 showed that oxytocin was released into the hypothalamus during suckling, that small amounts of

167 oxytocin injected into the brain of lactating rats dramatically facilitated the milk- ejection reflex,

168 and that central injections of oxytocin antagonist could block the reflex (Richard et al. 1991).

169 Thus it seemed that, somehow, oxytocin given centrally was able to "orchestrate" the

170 intermittent bursting activity of oxytocin cells that was first seen by Wakerley and Lincoln

171 (1973). This was the first convincing demonstration of a physiological role for a peptide in

172 the brain, and it led the way to a transformation of our understanding of information

173 processing in the nervous system. We now know that more than a hundred different

174 neuropeptides are expressed in different neuronal populations, that most if not all neurons in

175 the brain release one or more peptide messengers as well as a conventional neurotransmitter.

176 Because peptides have a relatively long half-life and act at receptors with nanomolar affinity,

177 their actions are not confined to targets in direct apposition to the site of release. Importantly,

178 peptide signals in the brain often have organisational and activational roles that seem more

179 akin to the roles of hormones in the periphery (Ludwig and Leng 2006). This understanding,

180 that peptides in the brain can have specific functional roles, we now take for granted, with our

181 knowledge of many peptides that, when injected into the brain, evoke coherent behavioural

182 responses.

183   In Germany, Rainer Landgraf and his colleagues began measuring oxytocin and

184 vasopressin release in the brain using the new technique of microdialysis (Landgraf et al.

185 1992). They at first assumed that they were measuring release from nerve terminals in the

186 brain. However, there were accumulating discrepancies between central release and

187 peripheral release of the peptides, and when Morris and Pow (1991) showed that oxytocin

188 and vasopressin could be released from all compartments of magnocellular neurons, not just

189 the nerve terminals, Landgraf's student Mike Ludwig realised that measurements of oxytocin

190 and vasopressin in the magnocellular nuclei reflected release from the soma and dendrites of

191 these neurons, not from nerve terminals (Fig. 2). Furthermore, he recognised that this

192 dendritic release must somehow be regulated independently of terminal release (Ludwig

193 1998).

194   This was a key breakthrough– but how then was dendritic release regulated?

195 Intriguing data from the laboratories of Theodosis and Hatton had indicated that in lactating

196 animals there was a morphological reorganisation of the supraoptic nucleus that might

197 facilitate dendro-dendritic interactions: normally the dendrites are separated from each other

198 by interleaved glial cell processes, but in lactation these processes are retracted, leaving the

199 dendrites of oxytocin neurons in direct apposition to each other within "bundles" of dendrites

200 (Hatton 1990; Theodosis and Poulain 1993). However, there was a stumbling block: oxytocin

201 cells only show synchronous bursting during suckling and parturition – even during lactation,

202 other stimuli would increase their activity but never elicited bursts. Dyball and Leng (1986)

203 working in Cross' group at the Babraham Institute, of which he had become the Director,

204 pursued the idea that some kind of positive feedback was involved. They thought it possible

205 that a recurrent excitatory circuit involving interneurons was responsible – but they found

206 that intense stimulation of the neural stalk, although it massively activated the cells in the

207 supraoptic nucleus, never triggered recurrent excitation in those cells. The stimulation wasn't
208 without effect on the milk-ejection reflex, but the effects were quite subtle – there was a
209 facilitation of bursting, but only when stimuli were given quite close to when a burst was
210 expected to happen anyway.

211 Leng and Ludwig began to work together to address a basic question – would intense
212 electrical stimulation of the neural stalk actually release any vasopressin or oxytocin in the
213 supraoptic nucleus? In experiment after experiment, the answer was frustratingly negative –
214 there was no sign of release measured by microdialysis following electrical activation
215 (Ludwig et al. 2002). Release could be evoked consistently by other kinds of stimulation, but
216 without a link to electrical activity of the cells, where was the positive feedback effect?

217 The next breakthrough came again from the lab of Richard, with their demonstration
218 that oxytocin could cause a mobilisation of intracellular calcium stores in oxytocin cells
219 (Lambert et al. 1994). How might that be relevant?

220 Working on the gonadotroph cells of the anterior pituitary gland, another of Harris'
221 students, George Fink, had shown something remarkable. In oestrogen-primed rats, the
222 secretion of luteinising hormone (LH) in response to gonadotrophin releasing hormone
223 (GnRH) increases with successive exposures to GnRH, a phenomenon that Fink called "self-
224 priming" (see Fink 1995). With Morris and others, Fink showed that, between exposures to
225 GnRH, there is a "margination" of secretory granules in gonadotrophs: how much LH is
226 secreted in response to GnRH depends on how many granules lie close to the plasma
227 membrane – and GnRH could trigger relocation of granules to these sites (Lewis et al. 1986).
228 This depends on the mobilisation, by GnRH, of intracellular calcium stores, so Leng and
229 Ludwig, knowing that the release of neurosecretory granules in response to electrical activity
230 was likely to depend upon those granules being close to the site of depolarisation-induced
231 calcium entry, wondered if something similar was happening in the dendrites of
232 magnocellular neurons. By "retrodialysis" – using microdialysis probes to deliver a substance
233 rather than to collect one - they applied thapsigargin directly to the supraoptic nucleus to
234 evoke a large increase in intracellular calcium in the magnocellular cells; then, long after the
235 direct effects of thapsigargin had worn off, they applied electrical stimulation to the neural
236 stalk. Now, finally, they could see a dramatic electrically-evoked release of both oxytocin and
237 vasopressin in the supraoptic nucleus as well as from the pituitary. They went on to show that
238 the same "priming" could be seen in response to peptides that evoked intracellular calcium
239 mobilisation – including (for oxytocin release) oxytocin itself (Ludwig et al. 2002).

240   Rossoni et al. (2008) were then able to build a computational model of the oxytocin

241   system that incorporated these phenomena, and which reproduced the bursting behaviour of

242   oxytocin neurones as observed during the milk-ejection reflex. That model explained how

243   bursts could be generated by dendro-dendritic intercommunication and could be rapidly

244   propagated through the oxytocin cells in a hypothalamic nucleus, but left unexplained how

245   oxytocin cells in the two supraoptic and two paraventricular nuclei came to be activated

246   simultaneously. One possibility lies in recognising that the appearance of separation of the

247   four nuclei is misleading –many magnocellular neurons are located between the main nuclear

248   aggregations, some as small "accessory" nuclei, and some as scattered neurons. Thus, if these

249   neurons share dendro-dendritic contacts with the major aggregations, they might complete a

250   network that links all nuclei. A second possibility arises from the work of Knobloch et al.

251   (2012) who found that the paraventricular nucleus contains some non-neuroendocrine

252   oxytocin neurons that innervate oxytocin cells in the supraoptic nucleus.

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254

255   **Parturition**

256   Oxytocin's role in milk ejection is indispensable: animals that lack oxytocin are

257   unable to feed their offspring (Nishimori et al. 1996; Young et al. 1996). By contrast,

258   although oxytocin is named after its effects on uterine contractility, mice that lack oxytocin

259   are still able to deliver young relatively normally, but whether this is generally the case in all

260   mammals remains unclear to this day. In 1941, Ferguson reported that, in the pregnant rabbit,

261   distension of the uterus and cervix could induce secretion of oxytocin (Ferguson 1941), but in

262   that same year, Dey et al. (1941) had reported on the effects of lesions to the supraoptico-

263   hypophysial tract in pregnant guinea pigs: of 16 labours studied, ten were prolonged and

264   difficult, ending in the death of the mother or delivery of dead foetuses, but six were

265   apparently normal. Harris had shown that electrical stimulation of the neural stalk could

266   evoke strong uterine contractions, but it remained unclear whether the effects of oxytocin on

267   the uterus reflected an active role of oxytocin in parturition, or a pharmacological effect

268   without real physiological significance (Harris 1948b). However, Harris' papers prompted

269   Mavis Gunther (1948) to write a letter to the *British Medical Journal*: she had observed

270   labour in a woman who was still lactating after the birth of a previous child, and noticed that

271   beads of milk appeared at the nipples during each uterine contraction. Many factors were

272   known to be capable of eliciting uterine contractions, but only oxytocin was known to induce

273 milk let-down, so Gunther speculated that the uterine contractions provoked the release of
274 oxytocin, which acted in a positive-feedback manner to support parturition.

275 However, by the end of the 1950's it was recognised that the plasma of pregnant
276 women contained an enzyme – oxytocinase – that could potently degrade oxytocin, and that
277 the levels of oxytocinase increased markedly towards term (Melander 1961). This greatly
278 complicated measuring oxytocin in pregnancy, and also raised fresh doubt about the
279 physiological role of oxytocin – if oxytocin was important for parturition, it seemed to make
280 no sense that the placenta should produce large amounts of an enzyme that destroyed it.

281 Then, in the 1980's, Summerlee and colleagues, working in Cross' former
282 Department at Bristol, published a series of papers reporting the activity of oxytocin neurons,
283 recorded over prolonged periods in conscious rats and rabbits through parturition and
284 lactation (O'Byrne et al. 1986; Paisley and Summerlee 1984; Summerlee 1981; Summerlee
285 and Lincoln 1981). These studies achieved two things of particular importance; first, the
286 milk-ejection reflex as described in the anesthetised rat was essentially identical to the reflex
287 in conscious rats; and second, similar bursting activity was generated during parturition
288 apparently linked to the delivery of the young. The insight that oxytocin secretion was
289 pulsatile during parturition cast a new light on the high levels of oxytocinase in the plasma of
290 pregnant women, for while these diminish basal levels of oxytocin, they would also be
291 expected to "sharpen" pulses of oxytocin by shortening their half-life. By frequent blood
292 sampling combined with rigorous methods to inactivate oxytocinase in those samples, Fuchs
293 et al. (1991) confirmed that spontaneous delivery in women is indeed associated with
294 frequent short pulses of oxytocin secretion.

295 But are pulses necessary for parturition in the way that they are for milk-ejection?
296 This is less clear, as the uterus will continue to contract in the continued presence of
297 oxytocin. Nevertheless it seems that pulses are indeed a more effective way for oxytocin to
298 drive parturition. At Babraham, Luckman et al. (1993) tested this in the rat by first
299 interrupting parturition with morphine –a potent inhibitor of oxytocin neurons in the rat – and
300 then attempting to re-establish parturition by giving oxytocin either as pulses of as a
301 continuous infusion. Normal parturition could be reinstated by giving pulses of oxytocin at
302 10-min intervals, whereas much higher doses were needed to achieve a similar outcome by
303 continuous infusion of oxytocin.

304 It is now generally accepted that, in all mammalian species, oxytocin secreted from
305 the posterior pituitary has a role in the expulsive phase of labour. Apart from its direct effects
306 on the uterine myometrium, oxytocin also stimulates prostaglandin release by its actions on

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307 the decidua/uterine epithelium. Oxytocin is not strictly essential, as other mechanisms can

308 generally compensate for its absence, but it is secreted in very large amounts during labour,

309 acts on a uterus that expresses greatly increased levels of oxytocin receptor at term, and

310 acutely blocking either oxytocin release or its actions slows parturition (Blanks and Thornton

311 2003; Russell et al. 2003). The trigger for initiating parturition varies between species, but it

312 seems that oxytocin commonly is a driver for uterine contractions once parturition has begun

313 (Russell et al 2003; Arrowsmith and Wray 2014). Oxytocin may also play some part in the

314 initiation of labour, but in women, other, paracrine mechanisms are more important for this

315 (Kamel 2010), although oxytocin antagonists are used to avert threatened pre-term labour

316 (Usta et al. 2011).

317

318 **Sexual activity**

319 In 1947, Harris had shown that stimulation of the posterior pituitary evoked robust

320 uterine contractions in the oestrous or oestrogenized rabbit, and that these effects could be

321 mimicked by injections of pituitary extract (Harris 1947). He knew that this did not

322 demonstrate a physiological role for oxytocin in labour, and that Ferguson's findings were

323 more pertinent to that issue (Ferguson 1941). However, he was intrigued that oxytocin caused

324 uterine contractions in the empty, non-pregnant uterus, and speculated that coitus might

325 trigger the secretion of oxytocin to facilitate the transport of seminal fluid up the female

326 reproductive tract. He went on to find a novel way of testing whether coitus triggered

327 oxytocin secretion in women.

328 As described above, Gunther (1948) had reported the appearance of beads of milk in a

329 lactating woman during labour, and this had impressed Harris as good evidence for active

330 secretion of oxytocin. In 1953, his colleague Vernon Pickles (1953) made a similar

331 observation, this time of a lactating woman who had experienced milk let-down immediately

332 after achieving orgasm. Together, Harris and Pickles (1953) set about seeing if this was a

333 common occurrence. Their approach was wonderfully direct – they asked the wives of their

334 colleagues. Six had noticed milk let down during some stage of coitus (not necessarily at

335 orgasm), and two others reported the 'tingling experience' in their breasts that they

336 recognised as the same as they experienced during suckling. Because milk let-down is a

337 reflex for which oxytocin is essential, this "bioassay" was powerful evidence that oxytocin is

338 indeed released during coitus in women; a conclusion later confirmed by radioimmunoassay:

339 there appears to be enhanced secretion in the arousal phase before orgasm (Carmichael et al.

340 1987), while the rises at orgasm itself are generally very small (Blaicher et al. 1999).

341   Whether the secretion of oxytocin into blood during sexual activity has any
342 physiological role in women is still unclear: Levin (2011) has argued that it has little if any
343 role in sperm transport. Oxytocin is also secreted into the blood during coitus in female goats
344 (McNeilly and Ducker 1972), there is an inconsistent increase in rabbits (Todd and Lightman
345 1986), and in ewes, and while oxytocin secretion increases in the presence of a ram, there is
346 no further rise in secretion during mating itself (Gilbert et al. 1991). Large doses of oxytocin
347 given systemically facilitate lordosis in ovariectomised, oestrogen-primed rats; because
348 central injections of much smaller amounts of oxytocin have a similar effect it has been
349 assumed that this is an effect mainly reflecting actions within the brain, but as the effects of
350 systemically administered oxytocin appear to depend upon the presence of an intact uterus
351 and cervix, peripheral actions may also contribute (Moody and Adler 1995).
352   In men, in response to masturbation, Murphy et al. (1987) found an increase in
353 vasopressin secretion but not oxytocin secretion during sexual arousal, and a large and robust
354 increase in oxytocin secretion but not vasopressin secretion at ejaculation. Oxytocin and
355 receptors are expressed in the prostate, penis, epididymis, and testis, and there is good
356 evidence that peripheral actions of oxytocin support penile erection and ejaculation and
357 facilitate sperm transport (Corona et al. 2012).
358
359 **Vasopressin secretion**
360   While Harris (1948c) showed that electrical stimulation of the posterior pituitary in
361 rabbits resulted in the appearance of a substance in the urine that had antidiuretic activity, this
362 was not, in context, any great surprise. It was already clear that posterior pituitary extracts
363 had marked antidiuretic activity, that the hormone content of the posterior pituitary was
364 markedly depleted by dehydration, and that the urine of dehydrated animals contained a
365 substance with apparently similar antidiuretic properties to those of posterior pituitary
366 extracts. Verney (1947) had established that intracarotid infusions of hypertonic solutions
367 elicited antidiuresis in dogs, and, by experiments involving ligations of the internal carotid
368 artery and various nerve sections, he had shown that this antidiuretic response required an
369 intact posterior pituitary, and that the osmoreceptors apparently lay in a region of the
370 prosencephalon supplied by the internal carotid. The supraoptic nucleus itself was recognised
371 to be a prime candidate for the location of these osmoreceptors, particularly as it was known
372 to be exceptionally densely vascularised. Indeed this speculation was correct – the
373 magnocellular neurons of the supraoptic and paraventricular nuclei express stretch-sensitive
374 membrane channels which make them exquisitely sensitive to volume change; with raised

375 external osmolality, the cells shrink, resulting in activation of a depolarising current (Bourque

376 2008).

377   But this mechanism does not work in isolation. The direct depolarisation that results

378 from volume changes is small, and not enough in itself to increase the spiking activity of the

379 magnocellular neurons. However, if those neurons are also receiving extensive afferent input,

380 then even a small tonic depolarisation becomes effective, by increasing the probability that

381 depolarisations arising from afferent input will exceed spike threshold. Thus while the

382 magnocellular neurones are osmoreceptors, when deafferented they cannot increase their

383 firing rate in response to osmotic stimulation – this response requires at least a tonic afferent

384 input (Leng et al. 1982). They get such a tonic input from a set of anterior brain structures

385 that includes two circumventricular organs – the subfornical organ and the organum

386 vasculosum of the laminae terminalis - that are also osmoreceptive in the same way that

387 magnocellular neurons are (Bourque 2008). They project to the magnocellular nuclei, but also

388 to the nucleus medianus, a midline structure adjacent to the anterior wall of the third ventricle

389 which also projects densely to the magnocellular nuclei. Collectively these anterior regions

390 became known as the "AV3V region", and this region controls not only antidiuresis but also

391 thirst and natriuresis, and it mediates effects of angiotensin produced by the kidney, and of

392 other circulating hormones of cardiovascular origin (Johnson 1985).

393

394 **Stress**

395   Harris' monograph focusses on another aspect of the regulation of vasopressin

396 secretion that is more controversial – the effect of emotional stress. He noted that there was

397 considerable evidence in man that emotional stress was accompanied by antidiuresis, that

398 Verney had shown that this also appeared to be the case in dogs, and that this seemed likely

399 to be the result of vasopressin released from the posterior pituitary. In rats, many behavioural

400 stressors have no clear effect on vasopressin secretion, although generally they do stimulate

401 oxytocin secretion (Gibbs 1986), while conditioned fear stimulates oxytocin secretion but

402 inhibits vasopressin secretion (Onaka et al. 1988) and novelty stress inhibits vasopressin

403 secretion with no effect on oxytocin secretion (Onaka et al. 2003). By contrast, in man,

404 vasopressin secretion appears to be stimulated by psychological stressors such as social stress

405 (Siegenthaler et al. 2014) and exam stress (Urwyler et al. 2015).

406   What the physiological significance of this is very uncertain. Vasopressin has an

407 important role in regulating adrenocorticotropic hormone (ACTH) secretion from the anterior

408 pituitary; it is released into the hypothalamo-hypophysial portal circulation from the

409 terminals of parvocellular and magnocellular neurones of the paraventricular nucleus, acting

410 in concert with corticotrophin releasing factor (CRF) (Antoni 1993). Circulating levels of

411 vasopressin, secreted from the posterior pituitary, are generally thought to be too low to be

412 effective. However, vasopressin and CRF interact synergistically in stimulating ACTH

413 secretion, so it is possible that in the presence of elevated CRF secretion, vasopressin

414 secretion from the pituitary might become effective. To date, this possibility has not been

415 extensively tested – and Ehrenreich et al. (1996) found no association in man between

416 increases in vasopressin secretion in response to novelty stress and ACTH secretion. Even if

417 vasopressin from the magnocellular system does influence ACTH secretion under some

418 circumstances, it is unclear what adaptive significance there might be. Similarly, the

419 increased secretion of oxytocin in response to many stressors is both without clear

420 physiological effect or adaptive significance. Oxytocin alone is an even weaker ACTH

421 secretagogue than vasopressin.

422

423

424 **The present day**

425   We now know that oxytocin and vasopressin have numerous peripheral targets that

426 were largely or completely unknown to Harris. There is evidence that, in some species at

427 least, oxytocin is involved in the regulation of natriuresis (Antunes-Rodrigues et al. 1997),

428 osteoblast activity (Di Benedetto et al. 2014) and gastric motility (Qin et al. 2009).

429 However probably the more radical change in our worldview has come from the recognition

430 that oxytocin and vasopressin are not only secreted from the posterior pituitary, but are also

431 released in the brain, where they have very diverse behavioural effects. Both oxytocin and

432 vasopressin are modulators of social behaviour (Caldwell et al. 2008; Lee et al. 2009;

433 Neumann and Landgraf 2012). Parvocellular oxytocin and vasopressin neurons in the

434 paraventricular nucleus project to many sites in the CNS and spinal cord, and vasopressin is

435 also expressed at several other sites in the brain (see De Vries 2008), including in the

436 olfactory bulb, where it has been implicated in social recognition (Tobin et al. 2010). In

437 addition, oxytocin is an important regulator of appetite (Leng et al. 2008) and sexual

438 behaviour (Baskerville and Douglas 2008). Centrally projecting parvocellular oxytocin and

439 vasopressin neurons have important roles in these, but the magnocellular neuroendocrine

440 system has also been implicated through dendritic release mechanisms. It now seems clear

441 that many neuroactive substances released in the brain, including oxytocin and vasopressin,

442 can act at a distance from their site of release (Leng and Ludwig 2008). Oxytocin and

443 vasopressin have profound effects on behaviors that are exerted at sites that, in some cases,

444 richly express peptide receptors but are innervated by few peptide-containing projections.

445 This release of these peptides is not specifically targeted at synapses, and the long half-life of

446 peptides in the CNS and their abundance in the extracellular fluid mean that, after release,

447 they can reach their sites of action by what Fuxe has called "volume transmission" (Fuxe et

448 al. 2012). At their targets, the process of priming allows peptides to functionally reorganize

449 neuronal networks, providing a substrate for prolonged behavioral effects (Ludwig and Leng

450 2006).

451   Our mechanistic understanding of the magnocellular neurons has undoubtedly

452 achieved great sophistication (Brown et al. 2013), substantially through a concerted drive by

453 many scientists over many years to meet the challenges laid down by Harris and his

454 contemporaries – to understand the milk-ejection reflex, the role of oxytocin in parturition,

455 and the nature of the osmoregulatory response of vasopressin cells. Key discoveries of wide

456 significance followed: the recognition of the importance of pulsatile hormone secretion, the

457 recognition of the importance of stimulus-secretion coupling mechanisms in interpreting

458 patterned electrical activity of neurons, the physiological importance of peptide release in the

459 brain, the recognition that peptide release comes substantially from dendrites and can be

460 regulated independently of nerve terminal secretion, and the importance of dynamic

461 morphological changes to neuronal function in the hypothalamus, all followed directly from

462 the drive to understand the milk-ejection reflex.

463   Yet despite the intensity with which magnocellular neurons have been interrogated,

464 these neurons still have the capacity to surprise us. For example, it has only recently become

465 clear that magnocellular vasopressin neurons are exquisitely thermosensitive (Sudbury et al.

466 2010) and are regulated by circadian inputs (Trudel and Bourque 2012).

467   In this essay, and we do not pretend it to be a comprehensive review, we sought to

468 follow the impact of Harris' work. Any such venture risks reinterpreting history to suit a

469 narrative. Yet science is an inescapably social activity, and to neglect this would be a

470 mistake. For good and bad, there are "bandwagons" in our science, some of which crash in

471 blind alleys, as we suspect will be the case for the current bandwagon of attention to the

472 effects of intranasal application of oxytocin and vasopressin, the behavioural consequences of

473 which are generally ascribed, on little evidence, to central actions but which in our view are

474 more likely incidental consequences of peripheral actions. The bandwagons that Harris set

475 rolling have, however, rolled and rolled, leading us inexorably to our present sophisticated

476 and nuanced understanding of the magnocellular neurons.

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Figure Legends

Figure 1: **(A)** Harris and co-workers showed, in lactating rabbits, that electrical stimulation of the neural stalk resulted in a sharp rise in intramammary pressure, and they inferred that this was the consequence of oxytocin secreted from the posterior pituitary. They noted that the response to stimulation depended strongly on the frequency of stimulation (**A**; modified from Harris et al. 1969). The explanation for this has two components. First, the response of the mamary gland to oxytocin is non-linear. As shown in **B** (modified from Cross and Harris 1952), the rabbit mammary gland shows a threshold response to i.v. injection of 10 mU of oxytocin and a near-maximal response to a dose of 50 mU. Second, the secretion of oxytocin is greatly facilitated by increasing frequency of stimulation. As shown in **C** (modified from Bicknell 1988), the amount of oxytocin (and vasopressin) that is released from the rat posterior pituitary gland *in vitro* in response to a fixed number of electrical stimulus pulses varies markedly with the frequency at which the pulses are applied (the graph plots hormone release in response to 156 pulses at each frequency). As shown in **D** (modified from Lincoln and Wakerley 1974), during the milk-ejection reflex (MER), oxytocin neurons discharge short bursts (1-3s) at a spike frequency averaging 40-50 spikes/s, i.e. at a frequency that optimises the effeciency of secretion, and which evokes a sharp rise in intramammary pressure. As shown in **E** (modified from Higuchi et al. 1985) this response is indeed attributable to a pulse of oxytocin, as measured in blood by radioimmunoassay. As shown in **F** (modified from Summerlee et al. 1986), similar bursts are observed during parturition.

Figure 2:

(A) Vasopressin and oxytocin that circulate in the plasma are synthesized by magnocellular neurons whose cell bodies are located mainly in the paraventricular (PVN) and the supraoptic nuclei (SON) of the hypothalamus (vasopressin cells are immunostained with fluorescent green and oxytocin cells with fluorescent red). **(B)** The peptide immunostaining is punctate and represents individual or aggregates of large dense-cored vesicles and in dendrites the vesicles are particularly abundant. **(C)** Push-pull perfusion studies have shown that dendritic

oxytocin release increases before the high frequency burst activity of oxytocin neurons, which is associated with the milk-ejection reflex. **(D)** Intracerebroventricular injection of oxytocin increases the burst amplitude and the burst frequency of oxytocin cells showing that central release regulates the milk-ejection reflex. **(E)** Dendritic oxytocin release can be conditionally primed. **(1)** Under normal conditions dendritic peptide release is not activated by electrical (spike) activity. This is indicated by the lack of dendritic oxytocin release in response to electrical stimulation of the neural stalk (light grey columns **(1a)**). **(2)** A conditional signal (arrow), such as oxytocin itself triggers release from dendrites independently of the electrical activity **(2a)**. **(3)** The conditional signal also primes dendritic stores. Priming occurs partially by relocation of dendritic large dense-core vesicles closer to the dendritic plasma membrane **(3a)**. **(4)** After oxytocin-induced priming, the vesicles are available for activity-dependent release for a prolonged period **(4a)**. Adapted and modified from (Brown et al. 2000; Freund-Mercier and Richard 1984; Ludwig and Leng 2006; Ludwig et al. 2002; Moos et al. 1989; Tobin et al. 2004).

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