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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

others: in his 1955 monograph he still at this time inclined to the view that the posterior pituitary contained endocrine cells that were innervated by hypothalamic neurones (Harris 1955). While conceding that the neurosecretory origin of the posterior pituitary hormones (Leveque and Scharrer 1953) was an "attractive hypothesis", he stated that "sweeping statements have been made at various times by the protagonists of the neurosecretory hypothesis" and warned that "such claims as these, which run contrary to a great deal of established data should be taken with reserve" (Harris 1955 p264). In particular, Harris rejected the notion that the Gomorri-stainable material present in the hypothalamo-hypophysial tract was the histological representation of antidiuretic hormone as argued by the Scharrers. He thought that the amount of oxytocic and antidiuretic activity present in the hypothalamus was too low to be consistent with the hypothalamus being the site of production. Finally, he disputed the evidence that neural stalk section could be followed by a partial regeneration of the neural lobe - evidence which suggested that regeneration of nerve terminals was sufficient to support secretion in the absence of endocrine cells (Harris 1955 p262-265).

Nevertheless, Harris pioneered our understanding of the posterior pituitary, mainly by experiments involving electrical stimulation of the supraoptico-hypophysial tract. At the outset of those experiments it was known that extracts of the posterior pituitary could stimulate the let-down of milk in lactating animals, and Ely and Peterson (1941) had shown that the blood of cows which had been milked contained something that could evoke milk let-down in the isolated udder. They proposed that this substance came from the posterior pituitary and was released by suckling, but Selye (1934) had earlier proposed that lactation could be explained by the stimulation of prolactin production from the *anterior* pituitary, and several reports had appeared that lactation could proceed normally even after sectioning the neural stalk.

Accordingly, with his student Barry Cross, Harris set out to test these two hypotheses. He had concluded (Harris 1948a) that direct electrical stimulation was ineffective in triggering secretion from the anterior pituitary, but the posterior pituitary was innervated by a nervous tract - the supraoptico-hypophysial tract. Cross and Harris (1950, 1952) showed that electrical stimulation of this tract caused an increase in intramammary pressure in lactating rabbits - showing that the pituitary contains a releasable factor that can induce milk let-down. Harris et al. (1969) later showed that the mammary response depended strongly on the stimulus frequency - only at frequencies in excess of 40 Hz was there an appreciable response – a finding that was to prove prescient (Fig. 1 A,B).

In 1966, Yagi et al. showed that electrical stimuli applied to the neural stalk would trigger action potentials that were conducted antidromically to the neurosecretory cell bodies,

but the utility of this seemed limited as both the site of stimulation and the site of recording required precise stereotaxic control. However, Barry Cross, who was now Professor of Anatomy at Bristol, saw that, in lactating rats, the site of the stimulating electrode could be precisely controlled by ensuring that it was positioned where stimulation would elicit a rise in intramammary pressure (Sundsten et al. 1970). This opened the way to studying magnocellular neurons *in vivo*, and Jon Wakerley and Dennis Lincoln, working in Cross's Department, used this approach to study how the electrical activity of "antidromically identified" magnocellular neurons regulate oxytocin and vasopressin secretion.

The milk-ejection reflex

There was still no real understanding of the milk-ejection reflex, and, in particular, no appreciation that the reflex was intermittent. The key breakthrough came when Wakerley and Lincoln (1973) showed that, during suckling, some of the antidromically identified cells in the supraoptic and paraventricular nuclei showed brief, synchronised high frequency discharges (~ 1-2 s at 5OHz) at intervals of ~ 10 min, each of which was followed, about 10s later, by an abrupt increase in intramammary pressure – a marker of milk let-down in the mammary glands (Fig.1D). It became clear that these bursts, which led to pulses of oxytocin secretion, were approximately synchronised amongst all of the magnocellular oxytocin cells in the hypothalamus. As a corollary, other magnocellular neurons that were antidromically identified as projecting to the posterior pituitary but which did not participate in this bursting activity could be assumed to be vasopressin cells.

Exactly why pulsatile secretion was a critically important phenomenon was not immediately apparent, but an important clue lay in Harris' observation, alluded to earlier, that electrical stimulation of the posterior pituitary would only evoke a strong intramammary pressure response if relatively high frequencies of stimulation were used (Harris et al. 1969). The explanation for this has two elements (Fig. 1). First, the response of the mammary gland to a bolus of oxytocin is non-linear, and has quite a narrow dynamic range: there is a threshold dose that must be exceeded before any effect is observed, and above this threshold the response to higher doses of oxytocin rises swiftly to a maximum. Thus the mammary gland seems to require pulsatile activation – especially because, if oxytocin is applied continuously rather than in pulses, then the response of the gland rapidly diminishes. Second, *how much* oxytocin is secreted in response to electrical stimulation strongly depends on the frequency of stimulation – more is secreted per stimulus pulse when stimuli are clustered closely together (Fig. 1C). This *frequency facilitation* of stimulus-secretion coupling can be attributed to several factors. A

solitary spike invading an axon in the pituitary will not invade all terminals of that axon, and in those it does invade, it will produce only a brief rise in intracellular calcium - the essential trigger for vesicle exocytosis. However, during a burst of spikes, a progressive increase in extracellular [K^+] depolarises the axons and endings in the neural lobe, securing a more complete invasion of the terminal arborisation. Moreover, successive spikes in a burst are progressively broadened, inducing a progressively larger calcium entry, giving a potentiated signal for exocytosis. As a result, each spike within a burst releases much more oxytocin than the isolated spikes that occur between bursts (Bourque 1991; Leng and Brown 1997).

The explosive nature of milk-ejection bursts suggested that some positive feedback was involved, and Moss, Dyball and Cross (1972) set about to try to show that oxytocin released from the posterior pituitary had that positive feedback effect. They recorded from magnocellular neurons in rats and rabbits, and studied the effects of oxytocin given intravenously and administered directly to the neurones by iontophoresis. The results were disconcerting – oxytocin had a dramatic excitatory effect upon many magnocellular neurons, and this seemed to be a specific effect, as non-neurosecretory cells were unaffected, and vasopressin applied in the same way was without effect. *However,* oxytocin even at large doses had no effect at all when given intravenously.

At that time there was no evidence that oxytocin was released centrally, and indeed it seemed very unlikely that it would be – there was no strong evidence of axon collaterals, and the evidence tended to suggest that if there were any recurrent collaterals then their effect was probably inhibitory. Indeed several reports had appeared of "recurrent inhibition" in the magnocellular system – reports later shown by Leng and Dyball (1984) to be based upon misinterpreted evidence. Moss et al. (1972) recognised that the ineffectiveness of intravenous oxytocin meant that oxytocin secreted from the pituitary did not find its way back into the brain. Accordingly, they concluded that the excitatory action of oxytocin on oxytocin cells was a pharmacological phenomenon without physiological significance.

However this view was soon to change. Philippe Richard and his colleagues in France showed that oxytocin was released into the hypothalamus during suckling, that small amounts of oxytocin injected into the brain of lactating rats dramatically facilitated the milk- ejection reflex, and that central injections of oxytocin antagonist could block the reflex (Richard et al. 1991). Thus it seemed that, somehow, oxytocin given centrally was able to "orchestrate" the intermittent bursting activity of oxytocin cells that was first seen by Wakerley and Lincoln (1973). This was the first convincing demonstration of a physiological role for a peptide in the brain, and it led the way to a transformation of our understanding of information

processing in the nervous system. We now know that more than a hundred different neuropeptides are expressed in different neuronal populations, that most if not all neurons in the brain release one or more peptide messengers as well as a conventional neurotransmitter. Because peptides have a relatively long half-life and act at receptors with nanomolar affinity, their actions are not confined to targets in direct apposition to the site of release. Importantly, peptide signals in the brain often have organisational and activational roles that seem more akin to the roles of hormones in the periphery (Ludwig and Leng 2006). This understanding, that peptides in the brain can have specific functional roles, we now take for granted, with our knowledge of many peptides that, when injected into the brain, evoke coherent behavioural responses.

In Germany, Rainer Landgraf and his colleagues began measuring oxytocin and vasopressin release in the brain using the new technique of microdialysis (Landgraf et al. 1992). They at first assumed that they were measuring release from nerve terminals in the brain. However, there were accumulating discrepancies between central release and peripheral release of the peptides, and when Morris and Pow (1991) showed that oxytocin and vasopressin could be released from all compartments of magnocellular neurons, not just the nerve terminals, Landgraf's student Mike Ludwig realised that measurements of oxytocin and vasopressin in the magnocellular nuclei reflected release from the soma and dendrites of these neurons, not from nerve terminals (Fig. 2). Furthermore, he recognised that this dendritic release must somehow be regulated independently of terminal release (Ludwig 1998).

This was a key breakthrough– but how then was dendritic release regulated? Intriguing data from the laboratories of Theodosis and Hatton had indicated that in lactating animals there was a morphological reorganisation of the supraoptic nucleus that might facilitate dendro-dendritic interactions: normally the dendrites are separated from each other by interleaved glial cell processes, but in lactation these processes are retracted, leaving the dendrites of oxytocin neurons in direct apposition to each other within "bundles" of dendrites (Hatton 1990; Theodosis and Poulain 1993). However, there was a stumbling block: oxytocin cells only show synchronous bursting during suckling and parturition – even during lactation, other stimuli would increase their activity but never elicited bursts. Dyball and Leng (1986) working in Cross' group at the Babraham Institute, of which he had become the Director, pursued the idea that some kind of positive feedback was involved. They thought it possible that a recurrent excitatory circuit involving interneurons was responsible – but they found that intense stimulation of the neural stalk, although it massively activated the cells in the

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

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

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

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

Rossoni et al. (2008) were then able to build a computational model of the oxytocin system that incorporated these phenomena, and which reproduced the bursting behaviour of oxytocin neurones as observed during the milk-ejection reflex. That model explained how bursts could be generated by dendro-dendritic intercommunication and could be rapidly propagated through the oxytocin cells in a hypothalamic nucleus, but left unexplained how oxytocin cells in the two supraoptic and two paraventricular nuclei came to be activated simultaneously. One possibility lies in recognising that the appearance of separation of the four nuclei is misleading –many magnocellular neurons are located between the main nuclear aggregations, some as small "accessory" nuclei, and some as scattered neurons. Thus, if these neurons share dendro-dendritic contacts with the major aggregations, they might complete a network that links all nuclei. A second possibility arises from the work of Knobloch et al. (2012) who found that the paraventricular nucleus contains some non-neuroendocrine oxytocin neurons that innervate oxytocin cells in the supraoptic nucleus.

Parturition

Oxytocin's role in milk ejection is indispensable: animals that lack oxytocin are unable to feed their offspring (Nishimori et al. 1996; Young et al. 1996). By contrast, although oxytocin is named after its effects on uterine contractility, mice that lack oxytocin are still able to deliver young relatively normally, but whether this is generally the case in all mammals remains unclear to this day. In 1941, Ferguson reported that, in the pregnant rabbit, distension of the uterus and cervix could induce secretion of oxytocin (Ferguson 1941), but in that same year, Dey et al. (1941) had reported on the effects of lesions to the supraoptico-hypophysial tract in pregnant guinea pigs: of 16 labours studied, ten were prolonged and difficult, ending in the death of the mother or delivery of dead foetuses, but six were apparently normal. Harris had shown that electrical stimulation of the neural stalk could evoke strong uterine contractions, but it remained unclear whether the effects of oxytocin on the uterus reflected an active role of oxytocin in parturition, or a pharmacological effect without real physiological significance (Harris 1948b). However, Harris' papers prompted Mavis Gunther (1948) to write a letter to the *British Medical Journal*: she had observed labour in a woman who was still lactating after the birth of a previous child, and noticed that beads of milk appeared at the nipples during each uterine contraction. Many factors were known to be capable of eliciting uterine contractions, but only oxytocin was known to induce

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

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

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

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

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

the decidua/uterine epithelium. Oxytocin is not strictly essential, as other mechanisms can generally compensate for its absence, but it is secreted in very large amounts during labour, acts on a uterus that expresses greatly increased levels of oxytocin receptor at term, and acutely blocking either oxytocin release or its actions slows parturition (Blanks and Thornton 2003; Russell et al. 2003). The trigger for initiating parturition varies between species, but it seems that oxytocin commonly is a driver for uterine contractions once parturition has begun (Russell et al 2003; Arrowsmith and Wray 2014). Oxytocin may also play some part in the initiation of labour, but in women, other, paracrine mechanisms are more important for this (Kamel 2010), although oxytocin antagonists are used to avert threatened pre-term labour (Usta et al. 2011).

Sexual activity

In 1947, Harris had shown that stimulation of the posterior pituitary evoked robust uterine contractions in the oestrous or oestrogenized rabbit, and that these effects could be mimicked by injections of pituitary extract (Harris 1947). He knew that this did not demonstrate a physiological role for oxytocin in labour, and that Ferguson's findings were more pertinent to that issue (Ferguson 1941). However, he was intrigued that oxytocin caused uterine contractions in the empty, non-pregnant uterus, and speculated that coitus might trigger the secretion of oxytocin to facilitate the transport of seminal fluid up the female reproductive tract. He went on to find a novel way of testing whether coitus triggered oxytocin secretion in women.

As described above, Gunther (1948) had reported the appearance of beads of milk in a lactating woman during labour, and this had impressed Harris as good evidence for active secretion of oxytocin. In 1953, his colleague Vernon Pickles (1953) made a similar observation, this time of a lactating woman who had experienced milk let-down immediately after achieving orgasm. Together, Harris and Pickles (1953) set about seeing if this was a common occurrence. Their approach was wonderfully direct – they asked the wives of their colleagues. Six had noticed milk let down during some stage of coitus (not necessarily at orgasm), and two others reported the 'tingling experience' in their breasts that they recognised as the same as they experienced during suckling. Because milk let-down is a reflex for which oxytocin is essential, this "bioassay" was powerful evidence that oxytocin is indeed released during coitus in women; a conclusion later confirmed by radioimmunoassay: there appears to be enhanced secretion in the arousal phase before orgasm (Carmichael et al. 1987), while the rises at orgasm itself are generally very small (Blaicher et al. 1999).

Whether the secretion of oxytocin into blood during sexual activity has any physiological role in women is still unclear: Levin (2011) has argued that it has little if any role in sperm transport. Oxytocin is also secreted into the blood during coitus in female goats (McNeilly and Ducker 1972), there is an inconsistent increase in rabbits (Todd and Lightman 1986), and in ewes, and while oxytocin secretion increases in the presence of a ram, there is no further rise in secretion during mating itself (Gilbert et al. 1991). Large doses of oxytocin given systemically facilitate lordosis in ovariectomised, oestrogen-primed rats; because central injections of much smaller amounts of oxytocin have a similar effect it has been assumed that this is an effect mainly reflecting actions within the brain, but as the effects of systemically administered oxytocin appear to depend upon the presence of an intact uterus and cervix, peripheral actions may also contribute (Moody and Adler 1995).

In men, in response to masturbation, Murphy et al. (1987) found an increase in vasopressin secretion but not oxytocin secretion during sexual arousal, and a large and robust increase in oxytocin secretion but not vasopressin secretion at ejaculation. Oxytocin and receptors are expressed in the prostate, penis, epididymis, and testis, and there is good evidence that peripheral actions of oxytocin support penile erection and ejaculation and facilitate sperm transport (Corona et al. 2012).

Vasopressin secretion

While Harris (1948c) showed that electrical stimulation of the posterior pituitary in rabbits resulted in the appearance of a substance in the urine that had antidiuretic activity, this was not, in context, any great surprise. It was already clear that posterior pituitary extracts had marked antidiuretic activity, that the hormone content of the posterior pituitary was markedly depleted by dehydration, and that the urine of dehydrated animals contained a substance with apparently similar antidiuretic properties to those of posterior pituitary extracts. Verney (1947) had established that intracarotid infusions of hypertonic solutions elicited antidiuresis in dogs, and, by experiments involving ligations of the internal carotid artery and various nerve sections, he had shown that this antidiuretic response required an intact posterior pituitary, and that the osmoreceptors apparently lay in a region of the prosencephalon supplied by the internal carotid. The supraoptic nucleus itself was recognised to be a prime candidate for the location of these osmoreceptors, particularly as it was known to be exceptionally densely vascularised. Indeed this speculation was correct – the magnocellular neurons of the supraoptic and paraventricular nuclei express stretch-sensitive membrane channels which make them exquisitely sensitive to volume change; with raised

external osmolality, the cells shrink, resulting in activation of a depolarising current (Bourque 2008).

But this mechanism does not work in isolation. The direct depolarisation that results from volume changes is small, and not enough in itself to increase the spiking activity of the magnocellular neurons. However, if those neurons are also receiving extensive afferent input, then even a small tonic depolarisation becomes effective, by increasing the probability that depolarisations arising from afferent input will exceed spike threshold. Thus while the magnocellular neurones are osmoreceptors, when deafferented they cannot increase their firing rate in response to osmotic stimulation – this response requires at least a tonic afferent input (Leng et al. 1982). They get such a tonic input from a set of anterior brain structures that includes two circumventricular organs – the subfornical organ and the organum vasculosum of the laminae terminalis - that are also osmoreceptive in the same way that magnocellular neurons are (Bourque 2008). They project to the magnocellular nuclei, but also to the nucleus medianus, a midline structure adjacent to the anterior wall of the third ventricle which also projects densely to the magnocellular nuclei. Collectively these anterior regions became known as the "AV3V region", and this region controls not only antidiuresis but also thirst and natriuresis, and it mediates effects of angiotensin produced by the kidney, and of other circulating hormones of cardiovascular origin (Johnson 1985).

Stress

Harris' monograph focusses on another aspect of the regulation of vasopressin secretion that is more controversial – the effect of emotional stress. He noted that there was considerable evidence in man that emotional stress was accompanied by antidiuresis, that Verney had shown that this also appeared to be the case in dogs, and that this seemed likely to be the result of vasopressin released from the posterior pituitary. In rats, many behavioural stressors have no clear effect on vasopressin secretion, although generally they do stimulate oxytocin secretion (Gibbs 1986), while conditioned fear stimulates oxytocin secretion but inhibits vasopressin secretion (Onaka et al. 1988) and novelty stress inhibits vasopressin secretion with no effect on oxytocin secretion (Onaka et al. 2003). By contrast, in man, vasopressin secretion appears to be stimulated by psychological stressors such as social stress (Siegenthaler et al. 2014) and exam stress (Urwyler et al. 2015).

What the physiological significance of this is very uncertain. Vasopressin has an important role in regulating adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary; it is released into the hypothalamo-hypophysial portal circulation from the

terminals of parvocellular and magnocellular neurones of the paraventricular nucleus, acting in concert with corticotrophin releasing factor (CRF) (Antoni 1993). Circulating levels of vasopressin, secreted from the posterior pituitary, are generally thought to be too low to be effective. However, vasopressin and CRF interact synergistically in stimulating ACTH secretion, so it is possible that in the presence of elevated CRF secretion, vasopressin secretion from the pituitary might become effective. To date, this possibility has not been extensively tested – and Ehrenreich et al. (1996) found no association in man between increases in vasopressin secretion in response to novelty stress and ACTH secretion. Even if vasopressin from the magnocellular system does influence ACTH secretion under some circumstances, it is unclear what adaptive significance there might be. Similarly, the increased secretion of oxytocin in response to many stressors is both without clear physiological effect or adaptive significance. Oxytocin alone is an even weaker ACTH secretagogue than vasopressin.

The present day

We now know that oxytocin and vasopressin have numerous peripheral targets that were largely or completely unknown to Harris. There is evidence that, in some species at least, oxytocin is involved in the regulation of natriuresis (Antunes-Rodrigues et al. 1997), osteoblast activity (Di Benedetto et al. 2014) and gastric motility (Qin et al. 2009). However probably the more radical change in our worldview has come from the recognition that oxytocin and vasopressin are not only secreted from the posterior pituitary, but are also released in the brain, where they have very diverse behavioural effects. Both oxytocin and vasopressin are modulators of social behaviour (Caldwell et al. 2008; Lee et al. 2009; Neumann and Landgraf 2012). Parvocellular oxytocin and vasopressin neurons in the paraventricular nucleus project to many sites in the CNS and spinal cord, and vasopressin is also expressed at several other sites in the brain (see De Vries 2008), including in the olfactory bulb, where it has been implicated in social recognition (Tobin et al. 2010). In addition, oxytocin is an important regulator of appetite (Leng et al. 2008) and sexual behaviour (Baskerville and Douglas 2008). Centrally projecting parvocellular oxytocin and vasopressin neurons have important roles in these, but the magnocellular neuroendocrine system has also been implicated through dendritic release mechanisms. It now seems clear that many neuroactive substances released in the brain, including oxytocin and vasopressin, can act at a distance from their site of release (Leng and Ludwig 2008). Oxytocin and

vasopressin have profound effects on behaviors that are exerted at sites that, in some cases, richly express peptide receptors but are innervated by few peptide-containing projections. This release of these peptides is not specifically targeted at synapses, and the long half-life of peptides in the CNS and their abundance in the extracellular fluid mean that, after release, they can reach their sites of action by what Fuxe has called "volume transmission" (Fuxe et al. 2012). At their targets, the process of priming allows peptides to functionally reorganize neuronal networks, providing a substrate for prolonged behavioral effects (Ludwig and Leng 2006).

Our mechanistic understanding of the magnocellular neurons has undoubtedly achieved great sophistication (Brown et al. 2013), substantially through a concerted drive by many scientists over many years to meet the challenges laid down by Harris and his contemporaries – to understand the milk-ejection reflex, the role of oxytocin in parturition, and the nature of the osmoregulatory response of vasopressin cells. 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 directly from the drive to understand the milk-ejection reflex.

Yet despite the intensity with which magnocellular neurons have been interrogated, these neurons still have the capacity to surprise us. For example, it has only recently become clear that magnocellular vasopressin neurons are exquisitely thermosensitive (Sudbury et al. 2010) and are regulated by circadian inputs (Trudel and Bourque 2012).

In this essay, and we do not pretend it to be a comprehensive review, we sought to follow the impact of Harris' work. Any such venture risks reinterpreting history to suit a narrative. Yet science is an inescapably social activity, and to neglect this would be a mistake. For good and bad, there are "bandwagons" in our science, some of which crash in blind alleys, as we suspect will be the case for the current bandwagon of attention to the effects of intranasal application of oxytocin and vasopressin, the behavioural consequences of which are generally ascribed, on little evidence, to central actions but which in our view are more likely incidental consequences of peripheral actions. The bandwagons that Harris set rolling have, however, rolled and rolled, leading us inexorably to our present sophisticated and nuanced understanding of the magnocellular neurons.

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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