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MECHANISMS RESTRAINING OXYTOCIN NEURONES IN PREGNANCY

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Current understanding of the role and control of neurosecretory oxytocin neurones in the context of the birth of offspring is reviewed. History of key discoveries about oxytocin is outlined, and more recent animal research is described that focuses on mechanisms that prevent premature activation of the positive feedback system that drives oxytocin secretion once the birth process has been initiated. The components of this feedback system include neural pathways to the hypothalamus that convey information from the birth canal, stretched as the uterus contracts and pushes the fetus through it. Consequent excitation of the oxytocin neurones leads to oxytocin secretion from the posterior pituitary and further uterine contractions. A neural pathway from the noradrenergic neurones in the brainstem to the oxytocin neurones in the hypothalamus has a key role in exciting them. Once excited they release oxytocin from their dendrites which acts locally to further increase excitability; recent discovery that oxytocinase is produced by oxytocin neurones indicates a role in regulating this local mechanism. There is an important role for allopregnanolone, formed from progesterone in pregnancy, in restraining oxytocin neurones at several levels. Experimental evidence is presented for powerful inhibitory actions via GABA receptors on oxytocin neurones and through activation of an inhibitory opioid peptide system.

Key words: allopregnanolone; endogenous opioid; interleukin-1beta; parturition; placental leucine aminopeptidase

INTRODUCTION

It is now just more than 100 years since it was discovered that an extract of the posterior pituitary gland potently stimulates uterine contractions *in vitro* (DALE, 1906). The active substance stimulating the uterus was accordingly named oxytocin, and the antidiuretic and vasopressor activity of posterior pituitary extract was attributed to vasopressin/ antidiuretic hormone. It was subsequently demonstrated that this oxytocic activity was caused by a peptide distinct from vasopressin (PIERCE and DU VIGNEAUD, 1950; DU VIGNEAUD et al., 1954), and confirmed in 1954 with the publication by du Vigneaud and colleagues (DU VIGNEAUD et al., 1954) of the structure and synthesis of a 9-amino acid peptide with potent oxytocic activity. Subsequently, the gene for oxytocin was characterised and it became evident that the vasopressin and oxytocin genes had

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arisen in vertebrate evolution from duplication and point mutations of a common ancestral gene (Acher et al., 1995). Essentially oxytocin is only found in mammals, although in the marmoset and tree shrew oxytocin is replaced by [Pro^8] oxytocin (Wallis, 2012), and in some marsupials a similar peptide, mesotocin ([Ile^8]-oxytocin), functions like oxytocin (Siebel et al., 2005; Parry et all, 2009). Related peptides are found in other vertebrates: notably, in hens vasotocin (8-arginine-oxytocin) administration stimulates uterine contractile activity and evokes premature oviposition in hens, and is more potent than oxytocin (Rząsa and Ewy, 1970).

The biological actions of oxytocin are exerted through a 7-transmembrane G-protein coupled receptor, which can signal via several intracellular pathways, according to the phenotype of target cells (Gimpl et al., 2008; Busnelli et al., 2013). Hence oxytocin can alter contractile, electrical or secretory activity of target cells. Only a single oxytocin receptor gene has been found, contrasting with the three types of vasopressin receptor genes (V1a, V1b, V2) that have been identified (Koshimizu et al., 2012).

Oxytocin in the posterior pituitary gland is contained in the many thousands of axon terminals of neurones that have their cell bodies in the paraventricular and supraoptic nuclei in the hypothalamus. These neurones were identified as neurosecretory in morphological, histochemical, biochemical and functional studies some 40-60 years ago (Bargmann and Scharrer, 1951; Douglas, 1973; Morris, 1976; Pickering, 1976) [Figure 1A].

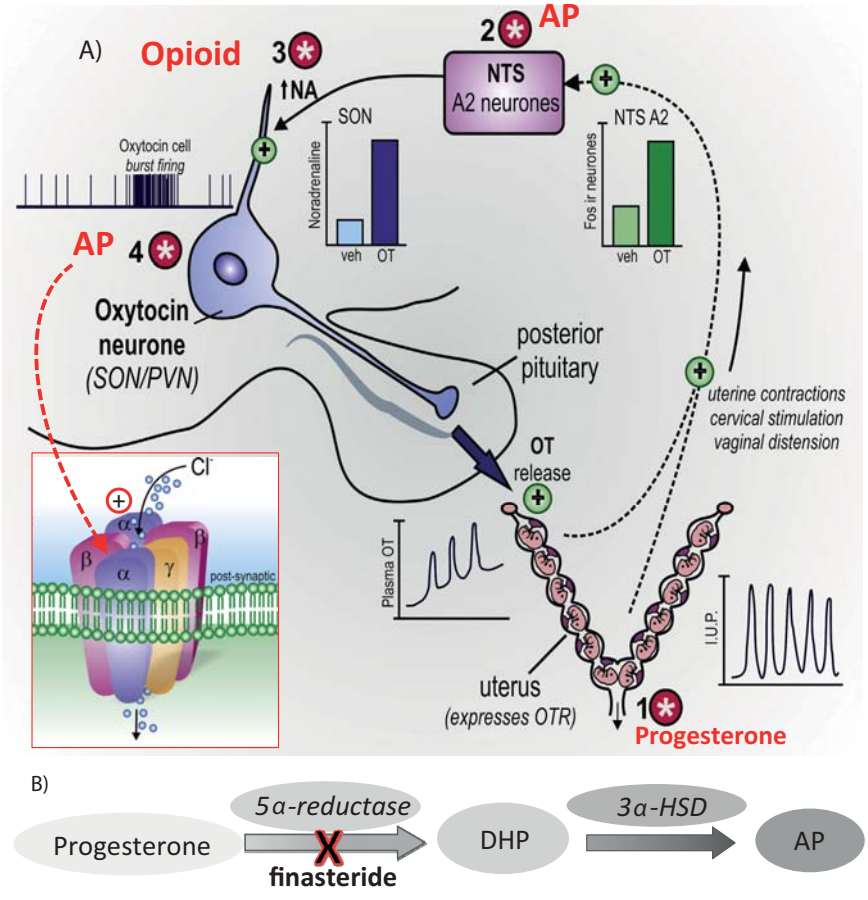

Fig. 1. Progesterone and allopregnanolone inhibit positive feedback reflex stimulation of oxytocin (OT) secretion in late pregnancy.
A) During births oxytocin neurones are stimulated by neural pathways activated by uterine cervical and vaginal distension, consequent on uterine contractions. This pathway involves A2 noradrenergic neurones in the nucleus tractus solitarii (NTS): activation during parturition (or by OT infusions at term) is shown by Fos expression in the NTS and noradrenaline (NA) release in the supraoptic and paraventricular nuclei (SON, PVN). Intermittent bursts of action potentials in oxytocin neurones cause secretion of OT pulses from the posterior pituitary gland. OT acts on oxytocin receptors (OTR) in the myometrium, increasing intrauterine pressure (IUP) and expelling fetuses and placentae. In late pregnancy, before births, progesterone acts directly on the uterus to decrease sensitivity to OT (1*), and indirectly on the neural pathway that excites oxytocin secretion through its neurosteroid metabolite, allopregnanolone (AP; see B). AP induces enkephalin gene expression in the NTS (2*), and the opioid inhibits NA release onto OT neurones (3*); AP also acts directly on OT neurones in late pregnancy (4*), acting as an enhancer of GABA action on $GABA_A$ receptors (inset). [adapted from (Brunton et al., 2014), with permission].
B) Schematic of pathway for progesterone conversion by 5α-reductase to dihydroprogesterone (DHP), and then by 3α-hydroxysteroid dehydrogenase (3α-HSD) to allopregnanolone (AP). Finasteride blocks AP synthesis by inhibiting 5α-reductase.

Thus, these neurones are highly active in synthesising oxytocin, initially within a large precursor peptide, which is cleaved in the Golgi system and the neurosecretory vesicles in which it is packaged to yield oxytocin and a higher molecular weight peptide, neurophysin (Burbach et al., 2006), which evidently is bound weakly to oxytocin but has no other known function (Kaźmierkiewicz et al., 1997). As these neurones must be capable of producing and secreting sufficient oxytocin into the systemic circulation to elicit responses in target tissues they have abundant cellular machinery for protein synthesis, which distinguishes them as magnocellular neurones (Vandesande and Dierickx, 1975).

It was established more than 40 years ago that the secretion of oxytocin by the posterior pituitary depends on the frequency and pattern of action potentials generated in the cell bodies of these neurones and propagated along their axons into the axon terminals. Here, depolarisation increases Ca^{2+} entry which triggers exocytosis (Douglas, 1973; Lemos et al., 2012). Importantly, this stimulated release of oxytocin is greater if action potentials are clustered together, reflecting frequency facilitation (Dyball et al., 1988). Hence, the capacity of the magnocellular oxytocin neurones to secrete oxytocin depends on the rate of synthesis in the cell bodies and the size of the store in the posterior pituitary gland. Given adequate production and storage, the rate and pattern of secretion of oxytocin depends on the action potentials arriving from the magnocellular neurones, and this in turn is a consequence of the balance of excitatory and inhibitory synaptic inputs to these neurones, interacting with their intrinsic electrical properties (Rossoni et al., 2008). Over the past 40 years much has been learned about these mechanisms that regulate the secretion of oxytocin from the use of *in vivo* and *in vitro* electrophysiological studies and from investigations of the neural pathways that project to these neurones (Leng et al., 1999; Hatton and Wang, 2008; Armstrong et al., 2010). Less well understood is how the production of oxytocin is regulated, in terms of the roles of transcription factors, including steroid hormone receptors, in the regulation of oxytocin gene transcription (Koohi et al., 2005); recent evidence indicates regulation by estradiol via estrogen receptor ß and a composite hormone response element (Hiroi et al., 2013), and by a specific microRNA (Choi et al., 2013).

Understanding the regulation of the secretion of oxytocin obviously requires measurement of its concentration in the circulation under changing physiological states, and measurement of changes in the biological processes in which it has an important role. Until around 40 years ago, bioassay was the only available methodology, involving *in vitro* or *in vivo* analysis of biological responses of sensitive tissue (Holton, 1948; Niezgoda et al., 1973). In lactating animals increases in intramammary pressure during suckling can be calibrated against systemic oxytocin injections enabling estimations of endogenous oxytocin release, in real time by auto-bioassay (Lincoln et al., 1973; Tobin et al., 2014). Subsequent development of sensitive and specific radioimmunoassays, with or without extraction (Forsling et al., 1973; Robinson, 1980; Landgraf, 1981; Higuchi et al., 1985), allowed more frequent sampling. From these assays it is clear that basal circulating levels of oxytocin are in the range of a few pg/ml plasma (though some current assays evidently give spurious much higher levels, and lack specificity (Szeto et al., 2011)), and levels increase briefly during suckling, and in parturition levels increase by 10-fold with additional pulses (Higuchi et al., 1986; Leng et al., 1988). Similarly, in hens blood vasotocin levels increase about 6-fold at oviposition, and are about 50-fold greater than levels in hens not laying (Niezgoda et al., 1973).

Oxytocin and parturition

Since the characterisation and synthesis of oxytocin, synthetic oxytocin has been widely used to induce or support parturition in women and farmed and domesticated animals. This is proof of efficacy, but an important role for maternal oxytocin in normal parturition has been disputed. This doubt arose from difficulty in measuring increased oxytocin secretion before or during births, and more recently from studies of mice with targeted inactivation of the oxytocin gene or the oxytocin receptor gene, which evidently give birth normally (Nishimori et al., 1996; Takayanagi et al., 2005). Strikingly, the offspring of these mice die, unless they are cross-fostered or their mothers are given oxytocin injections, as they do not receive milk

because oxytocin is indispensable for driving the ejection of milk into the mouths of suckling young (Nishimori et al., 1996). Hence oxytocin is absolutely essential for the survival of mammals, unless succour is available by cross-fostering or artificial feeding, as is possible in women.

An alternative interpretation of the findings about parturition from the oxytocin knockout mice is that there is redundancy in the mechanisms that drive parturition, in which prostaglandins in the uterus and fetal membranes are well-established to have essential roles (Sugimoto et al., 1997), and that maternal oxytocin normally does contribute to the process. Despite the findings from the oxytocin knockout mice, there is abundant strong evidence that maternal magnocellular oxytocin neurones are activated during parturition, that oxytocin secretion is increased and that if its secretion is blocked or its action is antagonised, then parturition is slowed [Fig. 1A] (Russell et al., 2003). Thus, activation of magnocellular oxytocin neurones during parturition is revealed in rats by increased secretion of oxytocin, by their expression of the *c-fos* gene (Antonijevic et al., 1995), and in a remarkable study involving extracellular recording of the electrical activity of magnocellular neurones, by their increased rate of firing action potentials with the birth of each pup (Summerlee, 1981). This increased electrical activity can explain the increases in circulating oxytocin levels seen with the birth of each rat pup (Higuchi et al., 1985).

Pulsatile infusion is the most effective pattern of stimulation by intravenous oxytocin of the birth process, in rats and women (Randolph and Fuchs, 1989; Luckman et al., 1993), intermittent peaks are seen during parturition in pigs (Gilbert et al., 1994), and in rabbits peak circulating oxytocin levels follow a burst of action potentials within a few seconds (O'byrne et al., 1986). This finding further indicates that oxytocin is secreted in pulses during parturition, consequent on the intermittent co-ordinated burst-firing of the oxytocin neurones. Furthermore, as the bursts of firing are associated with the passage of a fetus through the birth canal, it can be surmised that each burst is triggered by distension of the birth canal which increases impulses in afferents that synapse in the spinal cord, activating excitatory projections to the magnocellular oxytocin neurones. Such a mechanism was originally proposed by Ferguson (Ferguson, 1941), who demonstrated that dilatation of the uterine cervix *in vivo* at term is followed by uterine contractions. As these contractions during parturition will cause a fetus to move into or through the birth canal, this will increase impulses in afferent nerves and stimulate further oxytocin secretion: this is a positive feedback mechanism, which continually stimulates until it is interrupted by the birth of a fetus [Fig. 1A]. A comparable mechanism operates in birds during oviposition, as uterine distension stimulates vasotocin secretion (Rząsa et al., 1979).

While pulsatile secretion of oxytocin is most efficient and effective, continuous secretion of oxytocin is also effective, as commonly mimicked by continuous intravenous infusion in human obstetrics. During parturition in animals, circulating level of oxytocin between births is also increased, and this is a result of increasing continuous firing of oxytocin neurones: indeed, parturition can be induced by infusing oxytocin in a pulsatile manner in late pregnant rats (Douglas et al., 2001).

Mechanisms of burst-firing of oxytocin neurones. Electrophysiological studies in anaesthetised lactating rats some 40 years ago first revealed the high frequency burst-firing that is characteristic of magnocellular oxytocin neurones during suckling. Each burst, a few minutes apart, leads to secretion of a pulse of oxytocin and a milk ejection – a consequence of oxytocin-induced contraction of myo-epithelial cells around the milk-secreting alveoli (Wakerley and Lincoln, 1973). The burst-firing of oxytocin neurones is driven by increased activity in afferents from the nipples during suckling; the candidate neurotransmitters mediating excitation of oxytocin neurones by suckling include noradrenaline and glutamate (Moos et al., 1997; Onaka et al., 2003).

However, the bursts, which are synchronised among the oxytocin neurones, are a result of the properties of the neurones and their interactions with each other and with their synaptic inputs, rather than patterning in the suckling input. These interactions include an essential involvement of oxytocin itself, secreted by the dendrites of these neurones during suckling (Lambert et al., 1993). Dendritic secretion of oxytocin is first primed, probably by action of another neuropeptide released by afferents (Ludwig et al., 2002; Sabatier et al., 2003), and then triggered by

action potentials invading the dendrites. This dendritically-released oxytocin acts locally on the oxytocin neurones, stimulating endocannabinoid release, which then modulates glutamatergic and GABAergic terminals on the neurones (Rossoni et al., 2008). Computer-based modelling of experimental data indicates that adjacent oxytocin neurone dendrites in bundles in the magnocellular nuclei are functionally loosely coupled during suckling (Rossoni et al., 2008). This allows mutual excitation when a critical level of local oxytocin is reached so that all the oxytocin neurones fire a co-ordinated high frequency burst of action potentials, which stimulates the release of a pulse of oxytocin from the posterior pituitary, resulting in a milk ejection.

Presumptively, the same mechanisms generate the burst-firing that underlies secretion of pulses of oxytocin during parturition. Supportive evidence is as follows [Fig. 1A]: glutamate release in the supraoptic nucleus (SON) is increased at the start of parturition (Herbison et al., 1997), noradrenergic neurones in the A2 group in the nucleus tractus solitarius [NTS] are activated during parturition (Meddle et al., 2000), and noradrenaline release in the SON is increased in natural parturition (Herbison et al., 1997) and if parturition is simulated in late pregnant rats by intravenous oxytocin infusion (Douglas et al., 2001). Furthermore, stimulation of the noradrenergic input is more effective at releasing noradrenaline in late pregnancy, indicating 'wind-up' as parturition approaches (Tobin et al., 2010); oxytocin release within the SON is increased during parturition (Neumann et al., 1993), and local infusion into the SON of an oxytocin antagonist disrupts the progress of established parturition (Neumann et al., 1996).

Restraining the oxytocin system in pregnancy. An important conclusion from consideration of the above oxytocin neurone mechanisms that mediate the Ferguson reflex to drive parturition is that the positive feedback mechanisms involved must be restrained from being activated before the young are ready to be born. Premature birth remains a major human health problem, and its prevention is difficult. The availability of synthetic peptides designed as oxytocin antagonists provides an additional tool to probe the role of oxytocin neurones in parturition and a preventive treatment for threatened pre-term labour (Manning et al., 2012). One oxytocin antagonist, atosiban, reduces electrical activity of the uterus in women in threatened pre-term labour (Hadar et al., 2013), and is partially effective in delaying pre-term birth in women, but only for a short while (Papatsonis et al, 2005; Papatsonis et al., 2013), and this at least supports other evidence for a role of oxytocin in the process. Understanding the mechanisms that normally restrain the processes involved in positive feedback activation of the magnocellular oxytocin system until full term is a potential route to developing better treatment for pre-term labour (Brunton et al., 2014).

Considered here are roles for placental leucine aminopeptidase (p-LAP); oxytocin receptor (OTR) availability; progesterone and its neuroactive metabolite, allopregnanolone [Fig. 1B] and $GABA_A$ receptors [Fig. 1A]; and a central endogenous opioid peptide mechanism.

p-LAP. This is an enzyme that effectively inactivates oxytocin, so it is known also as oxytocinase. In pregnancy it is produced by the uterus and placenta (Tobin et al., 2014), and it is present in the circulation in women in pregnancy. Hence in pregnant women circulating oxytocin is rapidly inactivated by this enzyme which can be considered to be a mechanism for preventing uterine contractions from being induced by any premature oxytocin release (Nomura et al., 2005). Accordingly, pregnant wild-type mice given repeated p-LAP injections showed delayed parturition (Ishii et al., 2009), and p-LAP gene knockout mice can have shortened pregnancy (Ishii et al., 2009), although this is not consistently found (Pham et al., 2009). A similar oxytocinase has been found in birds (Suska-Brzezińska and Ewy, 1970).

It has been recently shown that p-LAP is also expressed in magnocellular oxytocin neurones, where, as in other tissues, it is active after insertion in the plasma membrane so that its extracellular domain inactivates nearby oxytocin (and some other neuropeptides) (Rogi et al., 1996; Tobin et al., 2014); p-LAP is present in the dendrites as well as the soma of oxytocin neurones, so it is positioned to be externalised to inactivate oxytocin released from dendrites and hence limit auto-excitation by oxytocin (Tobin et al., 2014). Amastatin is an inhibitor of p-LAP and *in vitro* it enhances inhibitory presynaptic oxytocin ac-

tions on inputs to oxytocin neurones (Hirasawa et al., 2001). Given by i.c.v. injection, under urethane anaesthesia, to a suckled lactating rat it increases the frequency of milk ejections without altering the amount of oxytocin released per milk ejection [Fig. 2] (Tobin et al., 2014).

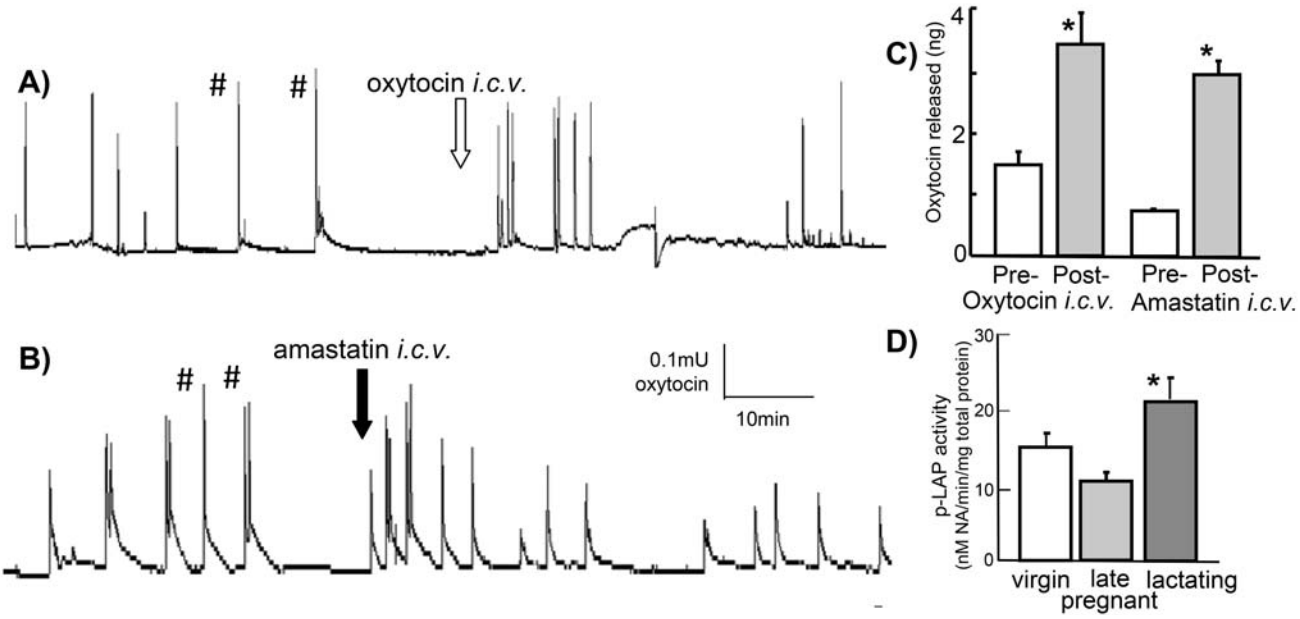

Fig. 2. Placental leucine aminopeptidase (p-LAP) regulates oxytocin neurone auto-excitability.
A), B): Recordings of intramammary pressure (i.m.p.) in a lactating suckled rat (urethane-anaesthetised). Each sharp increase in i.m.p. (#) during suckling indicates release of a pulse of oxytocin (assayed by comparing responses to intravenous oxytocin (OT) injections, inset), as a result of synchronised burst-firing of OT neurones (Lincoln et al., 1973). Intracerebroventricular (i.c.v.) injection of OT triggers the bursts (A), reflecting an essential autoexcitatory action of OT on OT neurones (Lambert et al., 1993). A similar action of i.c.v. injection of amastatin, a p-LAP inhibitor (B), indicates that p-LAP, which is produced by OT neurones, acts to limit the availability of extracellular oxytocin released by the dendrites of the OT neurones during suckling. C) Effects of i.c.v. OT and amastatin are quantitatively similar: both increase the amount of OT released by increasing the frequency of milk ejections (A, B) rather than the amount of OT per ejection. D) p-LAP activity in the hypothalamus is increased in lactation, but not in late pregnancy. *P<0.05.
[Modified from (Tobin et al., 2014) with permission].

Hence, as inhibition of central p-LAP during suckling evidently reduces the interval between the bursts of co-ordinated firing of oxytocin neurones it seems that p-LAP released by the dendrites of magnocellular oxytocin neurones acts to limit positive feedback action of oxytocin also released from the dendrites (Tobin et al., 2014).

Membrane bound and intracellular (soluble) p-LAP activities can be measured separately (Fernando et al., 2005), and in extracts of the hypothalamus from lactating rats intracellular p-LAP activity is increased [Fig. 2]. p-LAP gene (mRNA) expression in the PVN is also increased in lactation, and by confocal microscopy more p-LAP containing granules are located beneath the plasma membrane of oxytocin neurones, ready for release (Tobin et al., 2014). Together, these findings indicate up-regulation of p-LAP production and release by oxytocin neurones in lactation, when oxytocin released from dendrites is essential for co-ordinated burst-firing and pulsatile oxytocin secretion. It is not yet clear whether p-LAP in the hypothalamus has a role in preventing premature auto-excitation of oxytocin neurones in late pregnancy, although p-LAP production is evidently not increased [Fig. 2] (Tobin et al., 2014). It is also not yet known whether p-LAP production and action in the hypothalamus is increased at parturition, although it seems likely it has an important role in regulating the auto-excitation of oxytocin neurones during births.

Central OTR availability

At the level of the uterus, the myometrium is relatively unresponsive to oxytocin until late in pregnancy. This is essentially a result of inhibitory action of progesterone [Fig. 1A], which suppresses expression of contractile agonist systems in the uterus, including the expression of OTR, until near to the onset of parturition (Arthur et al., 2008). In addition, the inhibitory action of progesterone may involve inhibition of binding of oxytocin to OTRs and consequent prevention of intracellular signalling by OTRs, though this is not the case for the human OTR (Grazzini et al., 1998; Dunlap and Stormshak, 2004).

In the brain, by the end of pregnancy OTR mRNA expression is increased in the SON though not in the PVN (Meddle et al., 2007), and in the SON and PVN OTR ligand binding is increased in mid- and late pregnancy, and this may be a result of estrogen action (Bealer et al., 2006), Progesterone does not inhibit OTR binding in the brain, unlike its action in the uterus (Grazzini et al., 1998; Bealer et al., 2006). Overall, OTR expression for auto-excitatory oxytocin actions on oxytocin neurones is enhanced by the end of pregnancy, although OTR signalling mechanisms are altered at parturition [see later]. Furthermore, oxytocin neurones that express OTR are activated during parturition, as evidenced by Fos expression (Meddle et al., 2007).

Progesterone

In species such as rodents and sheep in which progesterone is mainly produced by the corpora lutea of pregnancy, the withdrawal of progesterone that follows luteolysis near the end of pregnancy is essential to sensitise the myometrium to oxytocin and hence to allow parturition (Fuchs et al., 1983; Grazzini et al., 1998; Murata et al., 2000; Arthur et al., 2008; Brunton and Russell, 2014). Similarly, in hens progesterone pretreatment reduces uterine contractile responses to vasotocin (Rząsa and Ewy, 1982). Progesterone withdrawal is also important to enable operation of the Ferguson reflex, by allowing activation by oxytocin-stimulated uterine contractions of, firstly NTS A2 neurones, and then oxytocin neurones, as shown by retrograde labelling and Fos studies (Antonijevic et al., 1995; Meddle et al., 2000) [Fig. 1A]. While uterine actions of progesterone are mediated by progesterone receptors (PR), few NTS neurones express PR and magnocellular oxytocin neurones lack PR (Antonijevic et al., 2000; Francis et al., 2002). Actions of progesterone on oxytocin neurones are instead indirect and mediated by allopregnanolone, a neuroactive metabolite of progesterone [Fig. 1B]: these allopregnanolone actions are both directly on the neurones via $GABA_A$ receptors and indirect, via induction of an endogenous opioid inhibitory mechanism.

Allopregnanolone

Allopregnanolone (3α-hydroxy-5α-pregnan-20-one, or 3α,5α-tetrahydroprogesterone) is a metabolite of progesterone that is designated as a neuroactive steroid because it acts directly and non-genomically on neurones. Allopregnanolone is an allosteric modifier of inhibitory $GABA_A$ receptors, acting to prolong the opening time of the Cl- channel in these receptors when they are activated by GABA; in several types of neurone allopregnanolone acts via the $GABA_A$ receptor ó- or α1 subunits [Fig. 1A, B] (Baulieu, 1997; Koksma et al., 2003; Hosie et al., 2006; Lambert et al., 2007; Gunn et al., 2013). In oxytocin neurones sensitivity to allopregnanolone of $GABA_A$ receptors on these neurones, and hence allopregnanolone action, involves inhibition of protein kinase C (PKC) (Koksma et al., 2003).

Allopregnanolone is produced by the sequential actions of 5α-reductase (which converts progesterone to dihydroprogesterone [DHP; 20α-hydroxy-4-pregnen-3-one]) and 3α-hydroxysteroid dehydrogenase (3α-HSD) which converts DHP to allopregnanolone [Fig. 1B]. These enzymes are expressed in the brain, in glia and neurones (Melcangi et al., 1993), so that the brain has the capacity to produce allopregnanolone from progesterone. In pregnancy circulating allopreganolone level increases as progesterone levels increase, and there is markedly increased allopregnanolone level in the brain as well (Concas et al., 1998). In particular the capacity of the hypothalamus to produce allopregnanolone is increased in late pregnancy, and expression of 3α-HSD mRNA in the PVN is

increased (Brunton et al., 2009). In addition, NTS neurones express both 5α-reductase and 3α-HSD, and the expression of mRNAs for both enzymes is increased near the end of pregnancy (Brunton et al., 2009). Hence, local actions of allopregnanolone generated in the PVN, SON and NTS may be involved in regulating oxytocin neurones: by enhancing inhibitory actions of GABA on these neurones in the PVN and SON, or via action on their input neurones in the NTS [Fig. 1A].

Power of allopregnanolone. The importance of the actions of allopregnanolone in enhancing inhibition by GABA of the activity of oxytocin neurones is indicated by treating late pregnant rats with finasteride, a 5α-reductase inhibitor, which by blocking conversion of progesterone to DHP blocks allopregnanolone formation [Fig. 1B] (Finn et al., 2006). Repeated administration of finasteride to rats in the last week of pregnancy (from 5 days before to 1 day before expected delivery) results in pre-term births, high post-natal offspring mortality and impaired post-natal brain development in surviving offspring [Fig. 3] Paris et al., 2011b).

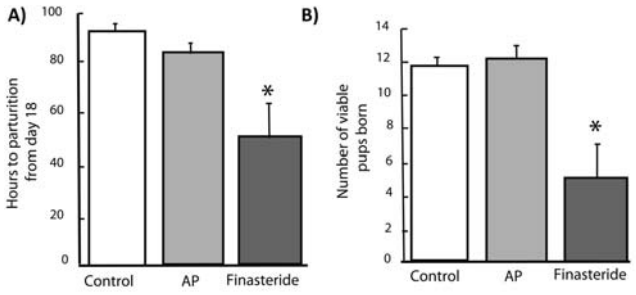

Fig. 3. Finasteride treatment in late pregnancy shortens pregnancy and reduces pup survival.
To inhibit allopregnanolone synthesis, daily injections of finasteride (50 mg/day), a 5α-reductase inhibitor, were given to pregnant rats from day 18. Finasteride reduced A) the time to parturition, and B) the number of viable pups. Daily allopregnanolone injections (10 mg/day) had no effect. *P<0.05. [Modified from (Paris et al., 2011b), with permission].

Impact on the oxytocin system in these circumstances has not been directly examined, nor has a role for premature oxytocin secretion yet been assessed.

However, the role of allopregnanolone in restraining inappropriate premature oxytocin secretion in late pregnancy has been studied in the context of challenge with systemic interleukin-1ß (IL-1ß) administration (Brunton et al., 2012). The rationale for this approach is that uterocervical infection is considered to predispose to preterm labour (Hagberg et al., 2005; Klein and Gibbs, 2005), and in non-pregnant rats acute systemic IL-1ß administration stimulates oxytocin secretion (Brunton et al., 2012). The striking finding is that in rats near the end of pregnancy there is no oxytocin secretory response to acute intravenous IL-1ß injection [Fig. 4] (Brunton et al., 2012).

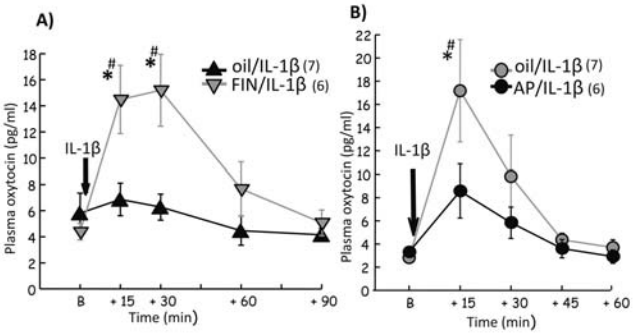

Fig. 4. Allopregnanolone (AP) inhibits stimulation of oxytocin secretion by interleukin-1ß (IL-1ß).
A) Day 21 pregnant rats were given an injection of a pro-inflammatory cytokine, IL-1ß (500 ng/kg, intravenous), after pretreatment with a 5α-reductase inhibitor, finasteride (FIN, 25 mg/kg, subcutaneous injection) 22 and 2 h previously. Venous blood samples were collected before and after IL-1ß injection for plasma oxytocin radioimmunoassay. IL-1ß had no effect on oxytocin secretion in vehicle (oil) treated pregnant rats, but after FIN pre-treatment IL-1ß strongly stimulated oxytocin secretion (as in virgin rats not given FIN: B). B) Virgin rats given AP pre-treatment (3 and 1 mg/kg) instead of FIN, had no significant oxytocin response to IL-1ß. Hence, AP is responsible for suppressed oxytocin responses to IL-1ß in late pregnancy.[Modified from (Brunton et al., 2012) with permission].

This suppressed response to IL-1ß is due to central mechanisms as, in contrast to the stimulation of electrical activity in SON oxytocin neurones and of Fos expression in PVN and SON oxytocin neurones in virgin rats, there is no electrophysiological or Fos response of oxytocin neurones to IL-1ß in late pregnant rats [Fig. 5A,B] (Brunton et al., 2006; Brunton et al., 2012).

In virgin rats, the oxytocin neurone responses to IL-1ß can be attenuated by allopregnanolone treatment and conversely the suppressed re-

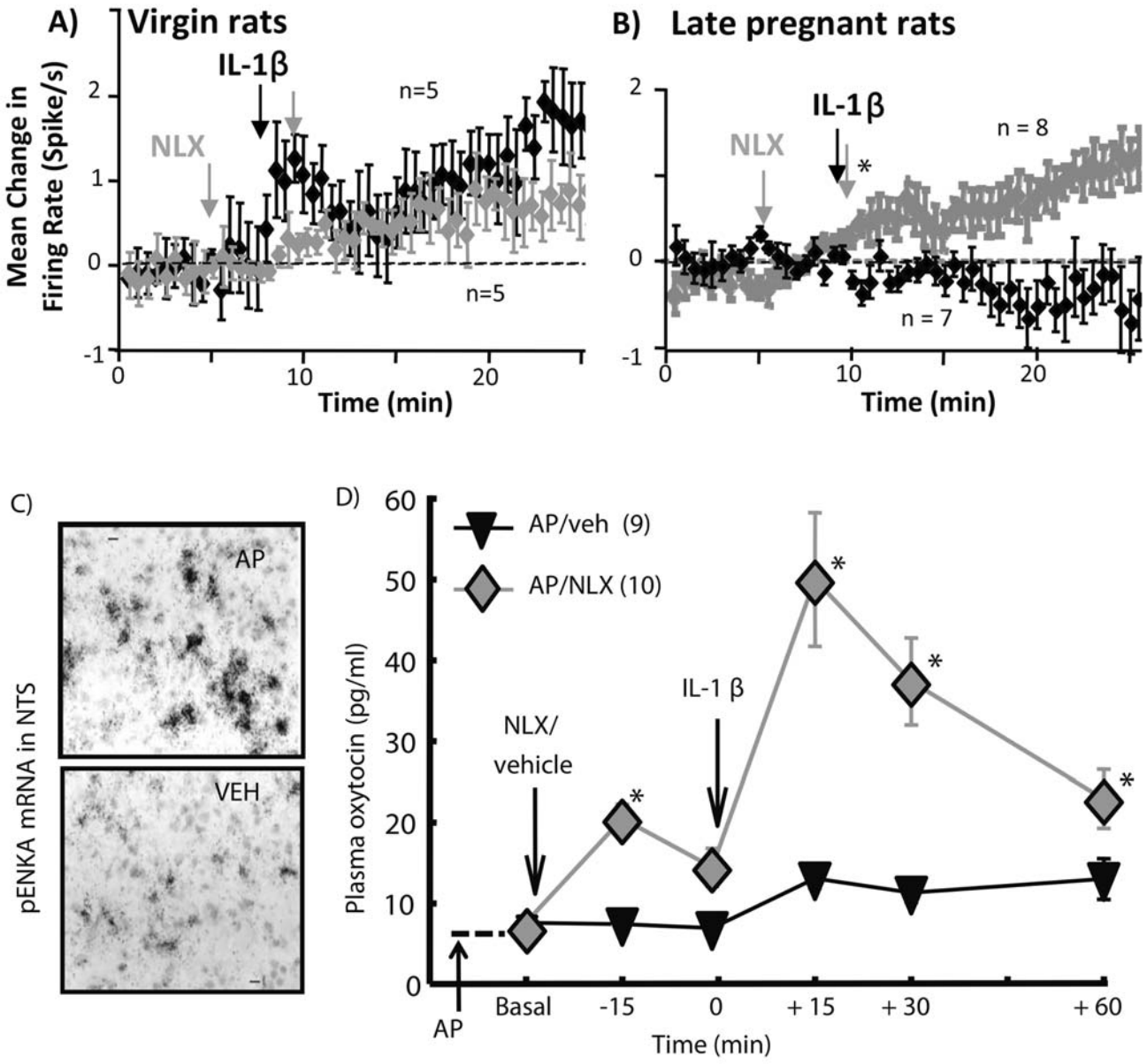

Fig. 5. Allopregnanolone inhibits oxytocin neurone responses to interleukin-1ß (IL-1ß) by inducing central opioid inhibition. A), B) Firing-rates of supraoptic nucleus oxytocin neurones in virgin (A) and late pregnant (B) rats (urethane-anaesthetised). After collecting baseline data for at least 10 min, all rats were given IL-1ß (500 ng/kg, intravenous, i.v.), and the effects were recorded for at least a further 25 min. In the rats given only IL-1ß (black data points and s.e.m. bars) the virgin rats (A) showed increased firing rate, but in the pregnant rats (B) there was no response. Naloxone (an opioid receptor antagonist, 5 mg/kg, i.v.) 5 min before IL-1ß did not alter the response (grey data points and s.e.m. bars) in the virgin rats (A), but enabled a response to IL-1ß in the pregnant rats (B). Hence, an endogenous opioid mechanism prevents excitation of oxytocin neurones by IL-1ß in late pregnancy. *P<0.05. C) *In situ* hybridisation autoradiographs for pro-enkephalin-A (pENKA) mRNA in the nucleus tractus solitarii from virgin rats. Top: allopregnanolone (AP) pre-treatment (3 and 1 mg/kg, 22 and 2 h before tissue collection); Bottom: vehicle (VEH) pre-treatment. AP clearly increased pENKA mRNA level.
D) Virgin rats were given AP treatment as in C; after a basal blood sample the rats were given naloxone (NLX, as in A, B) or vehicle, and then IL-1ß (as in A,B). Only the rats given AP and naloxone responded to IL-1ß. Hence, the opioid mechanism induced by AP can account for restraint by AP of oxytocin neurone responses to IL-1ß in pregnancy.
[A,B: adapted from (Brunton et al., 2006) with permission; C, D: adapted from (Brunton et al., 2009; Brunton et al., 2012) with permission].

sponses in pregnancy are restored by treatment with finasteride (Brunton et al., 2012) [FIG 4]. Hence, the high levels of allopregnanolone in late pregnancy can protect against premature stimulation of oxytocin secretion by acute IL-1ß exposure. Nonetheless, as noted above, repeated IL-1ß injections in late pregnancy induce pre-term delivery in rats (Paris et al., 2011a), although it is not yet known if this is a result of actions on oxytocin secretion.

Endogenous opioid mediation of allopregnanolone actions. While allopregnanolone has direct actions on oxytocin neurones (see later), it also inhibits these neurones indirectly by inducing activation of an endogenous opioid peptide inhibitory mechanism. Oxytocin neurones are highly sensitive to inhibition by μ- and κ-opioids, which act directly on these neurones and presynaptically (Brown et al., 2013). Hence, administration of agonists at μ- and κ-opioid receptors to rats in established parturition markedly slows births, as a consequence of inhibiting oxytocin secretion (Russell et al., 1989; Douglas et al., 1993a). Moreover, without pregnancy the activity of oxytocin neurones is not under regulation by central opioid mechanisms as naloxone, an opioid receptor antagonist, does not alter electrical activity or stimulate Fos expression in these neurones (Douglas et al., 1995); however, an auto-inhibitory mechanism involving κ-opioid receptors operates on oxytocin axon terminals in the posterior pituitary (Douglas et al., 1993b). By contrast, in late pregnant rats the κ-opioid mechanism in the posterior pituitary is down-regulated, which enhances fidelity of coupling between action potentials and secretory mechanisms (Douglas et al., 1993b). Instead, at this time central μ-opioid inhibition of oxytocin neurones is evident (Douglas et al., 1995). Hence, oxytocin neurone electrophysiological, Fos and secretory responses to acute IL-1ß in late pregnant rats are restored by prior naloxone injection [Fig. 5] (Brunton et al., 2006; Brunton et al., 2012). The central opioid mechanism is functional during parturition, regulating the spacing of births (Leng et al., 1988), and in rats and pigs is evidently responsible for stress-induced suspension of births by an environmental stressor (Leng et al., 1988; Lawrence et al., 1992).

The central actions of systemic IL-1ß are mediated via the NTS noradrenergic A2 neurones (Ericsson et al., 1994; Buller et al., 2001), and without pregnancy systemic IL-1ß activates Fos expression in these neurones and the release of noradrenaline in the PVN (Brunton et al., 2005). In late pregnant rats given IL-1ß NTS neurones express Fos, so they are clearly activated, but there is no release of noradrenaline in the PVN (Brunton et al., 2005). Microdialysis of naloxone into the PVN restores noradrenaline release, so this finding indicates presynaptic opioid inhibition of noradrenergic terminals in late pregnancy (Brunton et al., 2005). The source of this opioid may be the NTS A2 neurones as they express more pro-enkephalin A (PENKA) mRNA in late pregnancy, and more μ-opioid receptor mRNA (Brunton et al., 2005), which would provide μ-opioid receptor for axonal transport to terminals in the PVN and SON. Importantly, in virgin rats allopregnanolone treatment induces increased PENKA mRNA expression in the NTS and opioid inhibition of the oxytocin response to IL-1ß [Fig. 5] (Brunton et al., 2009; Brunton et al., 2012). In late pregnant rats the effect of naloxone in reversing suppression of the oxytocin response to IL-1ß is not enhanced by finasteride treatment, indicating that allopregnanolone action depends on the opioid mechanism (Brunton et al., 2012). Furthermore, finasteride treatment in late pregnancy reduces PENKA mRNA expression in the NTS (Brunton et al., 2009), indicating that allopregnanolone sustains as well as induces the endogenous opioid mechanism. Together, these findings indicate that in late pregnancy a major action of allopregnanolone is to induce an opioid shield that protects oxytocin neurones from premature activation by the brainstem pathway that will mediate the Ferguson reflex [Fig. 1A]. This opioid shield is withdrawn at the end of parturition (Douglas et al., 1993b), perhaps because of the decline in allopregnanolone levels at the end of pregnancy.

Oxytocin neurone plasticity and preparation for parturition. There are two types of change in the oxytocin neurones near the end of pregnancy that first, alleviate direct actions of allopregnanolone on the neurones, and second, enhance their excitability when they are activated by the positive feedback mechanism of the Ferguson reflex.

$GABA_A$ receptor changes. In late pregnancy, allopregnanolone effectively potentiates in-

hibitory actions of GABA on oxytocin neurones [Fig. 1A], but at the end of pregnancy, in parturition, this action is lost (Brussaard et al., 1997; Koksma et al., 2003). Clearly this will increase the excitability of the neurones. The explanation for this dramatic change is the loss of the effect of inhibition of PKC by allopregnanolone at this time, as a result of rapid local feedback action of oxytocin on stimulation of PKC and relative reduction in serine/threonine phosphatase activity, leading to phosphorylation of the $GABA_A$ receptors and desensitisation to allopregnanolone (Koksma et al., 2003). In addition, at this time, GABA receptors with different subunit compositions cluster on the oxytocin neurones in a new pattern, reducing the predominance of α1 subunits, thus effectively reducing actions of allopregnanolone (Koksma et al., 2005). Locally released oxytocin, acting with the high level of estradiol at this time, also induces this plasticity of GABA synapses (Theodosis et al., 2006). Hence, despite remaining still increased level of allopregnanolone in the brain at the end of pregnancy (Concas et al., 1998), the local dendritic release of oxytocin, which is evident in parturition and is important in regulating the process (Neumann et al., 1993; Neumann et al., 1996), ensures inaction of allopregnanolone on oxytocin neurones during births.

Electrophysiological properties. The oxytocin neurones show some subtle changes with the end of pregnancy that make them more excitable, and more prone to burst-fire effectively. Studied *in vitro* with intracellular electrophysiological recording, increased excitability is evident with the emergence of depolarising after-potentials following a train of action potentials (Teruyama et al., 2002). Conversely, oxytocin neurones also show enhanced Ca^{2+}-dependent after-hyperpolarisations (AHPs) following a burst of action potentials, and these are considered to restrict the duration of each burst (Teruyama et al., 2008). In accordance with other changes in the magnocellular system discussed above, the enhancement of AHPs is attributable to actions of locally released oxytocin in the presence of estradiol (Teruyama et al., 2008).

CONCLUSION

The magnocellular oxytocin neurone system has an undisputed essential role in the survival of mammals as it provides the means to effect the transfer of milk from the secretory alveoli in the mammary glands into the mouths of the infants. It is also important in promoting the expulsion of the fetuses and placentae in parturition, during which it releases sufficient oxytocin to drive the delivery of a ca 3.5 kg human infant, or 12 or more rat pups within a short time. For this process, and lactation, oxytocin neurones are adapted to discharge action potentials in high frequency bursts that are synchronised among the whole population, resulting in intermittent secretion of pulses of oxytocin from the posterior pituitary gland. As the stimulation of these bursts involve positive neural feedback signals, including oxytocin itself acting on the neurones after release from their dendrites, emphasis here has been on inhibitory mechanisms in the system that restrain excitation in pregnancy until the start of parturition. Such understanding may be helpful in the management of threatened pre-term labour.

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REFERENCES

Acher, R., J. Chauvet, and M.T. Chauvet. 1995. Man and the chimaera. Selective versus neutral oxytocin evolution. *Adv. Exp. Med. Biol.* 395: 615-627.

Antonijevic, I.A., G. Leng, S.M. Luckman, A.J. Douglas, R.J. Bicknell, and J.A. Russell. 1995. Induction of uterine activity with oxytocin in late pregnant rats replicates the expression of c-fos in neuroendocrine and brain stem neurons as seen during parturition. *Endocrinology* 136(1): 154-163.

Antonijevic, I., J. Russell, R. Bicknell, G. Leng, and A. Douglas. 2000. Effect of progesterone on the activation

of neurones of the supraoptic nucleus during parturition. *J. Reprod. Fertil.* 120: 367-376.

Armstrong, W.E., L. Wang, C. Li, and R. Teruyama. 2010. Performance, properties and plasticity of identified oxytocin and vasopressin neurones in vitro. *J. Neuroendocrinol.* 22(5): 330-342.

Arthur, P., M.J. Taggart, B. Zielnik, S. Wong, and B.F. Mitchell. 2008. Relationship between gene expression and function of uterotonic systems in the rat during gestation, uterine activation and both term and preterm labour. *J. Physiol.* 586(24): 6063-6076.

Bargmann, W., and E. Scharrer. 1951. The site of origin of the hormones of the posterior pituitary. *Am. Sci.* 39(2): 255-259.

Baulieu, E.E. 1991. Neurosteroids: a new function in the brain. *Biol. Cell.* 71: 3-10.

Bealer, S.L., D.L. Lipschitz, G. Ramoz, and W.R. Crowley. 2006. Oxytocin receptor binding in the hypothalamus during gestation in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 291(1): 53-58.

Brown, C.H., J.S. Bains, M. Ludwig, and J.E. Stern. 2013. Physiological regulation of magnocellular neurosecretory cell activity: integration of intrinsic, local and afferent mechanisms. *J. Neuroendocrinol.* 25(8): 678-710.

Brunton, P.J., S.L. Meddle, S. MA, T. Ochedalski, A.J. Douglas, and J.A. Russell. 2005. Endogenous opioids and attenuated hypothalamic-pituitary-adrenal axis responses to immune challenge in pregnant rats. *J. Neurosci.* 25(21): 5117-5126.

Brunton, P.J., N. Sabatier, G. Leng, and J.A. Russell. 2006. Suppressed oxytocin neuron responses to immune challenge in late pregnant rats: a role for endogenous opioids. *Eur. J. Neurosci.* 23(5): 1241–1247.

Brunton, P.J., A.J. Mckay, T. Ochedalski, A. Piastowska, E. Rebas, A. Lachowicz, and J.A. Russell. 2009. Central opioid inhibition of neuroendocrine stress responses in pregnancy in the rat is induced by the neurosteroid allopregnanolone. *J. Neurosci.* 29(20): 6449-6460.

Brunton, P.J., J. Bales, and J.A. Russell. 2012. Allopregnanolone and induction of endogenous opioid inhibition of oxytocin responses to immune stress in pregnant rats. *J. Neuroendocrinol.* 24(4): 690-700.

Brunton, P.J., J.A. Russell, and J.J. Hirst. 2014. Allopregnanolone in the brain: Protecting pregnancy and birth outcomes. *Prog. Neurobiol.* 113: 106-136.

Brunton, P.J., and J.A. Russell. 2014. Maternal Brain Adaptations in Pregnancy. In: 4th Edition of Knobil and Neill's Physiology of Reproduction: Implantation and Pregnancy Section . *Elsevier* Vol 2, Chapter 44, pp 1957-2026

Brussaard, A.B., K.S. Kits, R.E. Baker, W.P. Willems, J.W. Leyting-Vermeulen, P. Voorn, A.B. Smit, R.J. Bicknell, and A.E. Herbison. 1997. Plasticity in fast synaptic inhibition of adult oxytocin neurons caused by switch in GABA(A) receptor subunit expression. *Neuron* 19(5): 1103-1114.

Buller, K.M., Y. XU, C.V. Dayas, and T.A. Day. 2001. Dorsal and ventral medullary catecholamine cell groups contribute differentially to systemic interleukin-1beta induced HPA axis responses. *Neuroendocrinology* 73(2): 129–138.

Burbach, J.P.H., L.J. Young, and J.A. Russell. 2006. Oxytocin: Synthesis, Secretion, and Reproductive Functions in Third Edition of Knobil and Neill: the Physiology of Reproduction ed. Jimmy D. Neill, chapter 58. pp.3055-3128. Elsevier.

Busnelli, M., E. Peverelli, G. Mantovani, A. Spada, and B. Chini. 2013. Deciphering the specific role of G(αi/o) isoforms: functional selective oxytocin ligands and somatostatin SST5 receptor mutants. *Biochem. Soc. Trans.* 41(1): 166-171.

Choi, J.W., S.M. Kang, Y. Lee, S.H. Hong, N.A. Sanek, W.S. Young, and H.J. Lee. 2013. MicroRNA profiling in the mouse hypothalamus reveals oxytocin-regulating microRNA. *J. Neurochem.* 126(3): 331-337.21.

Concas, A., M.C. Mostallino, P. Porcu, P. Follesa, M.L. Barbaccia, M. Trabucchi, R.H. Purdy, P. Grisenti, and G. Biggio. 1998. Role of brain allopregnanolone in the plasticity of gamma-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. *Proc. Natl. Acad. Sci. USA* 95(22): 13284-13289.

Dale, H.H. 1906. On some physiological actions of ergot. *J. Physiol.* 34: 163-206.

Douglas, W.W. 1973. How do neurones secrete peptides? Exocytosis and its consequences, including "synaptic vesicle" formation, in the hypothalamo-neurohypophyseal system. *Prog. Brain Res.* 39: 21-39.

Douglas, A.J., G. Clarke, S.J. Macmillan, P.M. Bull, I. Neumann, S.A. Way, D.M. Wright, B.G. Mcgrory, and J.A. Russell. 1993a. Effects of the kappa-opioid agonist U50,488 on parturition in rats. *Br. J. Pharmacol.* 109(1): 251-258.

Douglas, A.J., S. Dye, G. Leng, J.A. Russell, and R.J Bicknell. 1993b. Endogenous opioid regulation of oxytocin secretion through pregnancy in the rat. *J. Neuroendocrinol.* 5(3): 307-314.

Douglas, A.J., I. Neumann, H.K. Meeren, G. Leng, L.E. Johnstone, G. Munro, and J.A. Russell. 1995. Central endogenous opioid inhibition of supraoptic oxytocin neurons in pregnant rats. *J. Neurosci.* 15(7 Pt1): 5049-5057.

Douglas, A.J., S. Scullion, I.A. Antonijevic, D. Brown, J.A. Russell, and G. Leng, 2001. Uterine contractile activity stimulates supraoptic neurons in term pregnant rats via a noradrenergic pathway. *Endocrinology* 142(2): 633-644.

Dunlap, K.A. and F. Stormshak. 2004. Nongenomic inhibition of oxytocin binding by progesterone in the ovine uterus. *Biol. Reprod.* 70(1): 65-69.

Du Vigneaud, V., Charlotte Ressler C , JM , C.W. Roberts, and P.G. Katsoyannis. 1954. The synthesis of oxytocin. *J. Am. Chem. Soc.* 76 (12): 3115-3121.

Dyball, R.E., R. Grossmann, G. Leng, and K. Shibuki. 1988. Spike propagation and conduction failure in the rat neural lobe. *J. Physiol.* 401: 241-256.

Ericsson, A., K.J. Kovacs, and P.E. Sawchenko. 1994. A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress related neuroendocrine neurons. *J. Neurosci.* 14: 897-913.

Ferguson, J.K.W. 1941. A study of the motility of the intact uterus at term. *Surg. Gynecol. Obstet.* 73: 359-366.

Fernando, R.N., J. Larm, A.L. Albiston, and S.Y. Chai. 2005. Distribution and cellular localization of insulin-regulated aminopeptidase in the rat central nervous system. *J. Comp. Neurol.* 487: 372–390.

Finn, D.A., A.S. Beadles-Bohling, E.H. Beckley, M.M. Ford, K.R. Gililland, R.E. Gorin-Meyer, and K.M. Wiren. 2006. A new look at the 5α-reductase inhibitor finasteride. *CNS Drug. Rev*. 12: 53-76.

Forsling, M.L., M.J. Martin, J.C. Sturdy, and A.M. Burton. 1973. Observations on the release and clearance of neurophysin and the neurohypophysial hormones in the rat. *J. Endocrinol.* 57(2): 307-315.

Francis, K., S.L. Meddle, V.R. Bishop, and J.A. Russell. 2002. Progesterone receptor expression in the pregnant and parturient rat hypothalamus and brainstem. *Brain Res.* 927: 18-26.

Fuchs, A.R., S. Periyasamy, M. Alexandrova, and M.S. Soloff. 1983. Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effects of ovarian steroids. *Endocrinology* 113: 742-749.

Gilbert, C.L., J.A. Goode, and T.J. Mcgrath. 1994. Pulsatile secretion of oxytocin during parturition in the pig: temporal relationship with fetal expulsion. *J. Physiol.* 475(1): 129-137.

Gimpl, G., J. Reitz, S. Brauer, and C. Trossen. 2008. Oxytocin receptors: ligand binding, signalling and cholesterol dependence. *Prog. Brain Res*. 170: 193-204.

Grazzini, E., G. Guillon, B. Mouillac, and H.H. Zingg. 1998. Inhibition of oxytocin receptor function by direct binding of progesterone. *Nature* 392(6675): 509-512.

Gunn, B.G., L. Cunningham, M.A. Cooper, N.L. Corteen, M. Seifi, J.D. Swinny, J.J. Lambert, and D. Belelli. 2013. Dysfunctional astrocytic and synaptic regulation of hypothalamic glutamatergic transmission in a mouse model of early-life adversity: relevance to neurosteroids and programming of the stress response. *J. Neurosci.* 33(50): 19534-19554.

Hadar, E., N. Melamed, A. Aviram, O. Raban, L. Saltzer, L. Hiersch, and Y. Yogev. 2013. Effect of an oxytocin receptor antagonist (atosiban) on uterine electrical activity. *Am. J. Obstet. Gynecol.* 013;209(4): 384.e1-7. doi: 10.1016/j.ajog.2013.05.053. Epub 2013 Jun 15.

Hagberg, H., C. Mallard, and B. Jacobsson. 2005. Role of cytokines in preterm labour and brain injury. *BJOG*. 112(1): 16–18.

Hatton, G.I., and Y.F. Wang. 2008. Neural mechanisms underlying the milk ejection burst and reflex. *Prog. Brain Res.* 170: 155-166.

Herbison, A.E., D.L. Voisin, A.J. Douglas, and C. Chapman. 1997. Profile of monoamine and excitatory amino acid release in rat supraoptic nucleus over parturition. *Endocrinology* 138(1): 33-40.

Higuchi, T., K. Honda, T. Fukuoka, H. Negoro, and K. Wakabayashi. 1985. Release of oxytocin during suckling and parturition in the rat. *J. Endocrinol.* 105(3): 339-346.

Higuchi, T., Y. Tadokoro, K. Honda, and H. Negoro. 1986. Detailed analysis of blood oxytocin levels during suckling and parturition in the rat. *J. Endocrinol.* 110(2): 251-256.

Hirasawa, M., S.B. Kombian, and Q.J. Pittman. 2001. Oxytocin retrogradely inhibits evoked, but not miniature, EPSCs in the rat supraoptic nucleus: role of N- and P/Q-type calcium channels. *J. Physiol.* 532(P3): 595-607.

Hiroi, R., A.F. Lacagnina, L.R. Hinds, D.G. Carbone, R.M. Uht, and R.J. Handa. 2013. The androgen metabolite, 5α-androstane-3ß,17ß-diol (3ß-diol), activates the oxytocin promoter through an estrogen receptor-ß pathway. *Endocrinology* 154(5): 1802-1812.

Holton, P. 1948. A modification of the method of Dale and Laidlaw for standardization of posterior pituitary extract. B*rit. J. Pharmacol. 3*: 328-334.

Hosie, A.M., M.E. Wilkins, H.M. Da Silva, and T.G. Smart. 2006. Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. *Nature* 444(7118): 486-489.

Ishii, M., K. Naruse, A. Hattori, M. Tsujimoto, S. Ishiura, Y. Numaguchi, T. Murohara, H. Kobayashi, and S. Mizutani. 2009. Oxytocin hypersensitivity in pregnant P-LAP deficient mice. *Life Sci*. 84(19-20): 668-672.

Kaźmierkiewicz, R., C. Czaplewski, and J. Ciarkowski. 1997. Elucidation of neurophysin/bioligand interactions from molecular modeling. *Acta Biochim. Pol.* 44(3): 453-466.

Klein, L.L., and R.S. Gibbs. 2005. Infection and preterm birth. Obstet. *Gynecol. Clin. North. Am*. 32(3): 397-410.

Koksma, J.J., R.E. Van Kesteren, T.W. Rosahl, R. Zwart, A.B. Smit, H. Lüddens, and A.B. Brussaard. 2003. Oxytocin regulates neurosteroid modulation of GABA(A) receptors in supraoptic nucleus around parturition. *J. Neurosci.* 23(3):788-797.

Koksma, J.J., J.M. Fritschy, V. Mack, R.E. Van Kesteren, and A.B. Brussaard. 2005. Differential GABAA receptor clustering determines GABA synapse plasticity in rat oxytocin neurons around parturition and the onset of lactation. *Mol. Cell Neurosci.* 28(1): 128-140.

Koohi, M.K., R. Ivell, and N. Walther. 2005. Transcriptional activation of the oxytocin promoter by oestrogens uses a novel non-classical mechanism of oestrogen receptor action. *J. Neuroendocrinol*. 17(4): 197-207.

Koshimizu, T.A., K. Nakamura, N. Egashira, M. Hiroyama, H. Nonoguchi, and A. Tanoue. 2012. Vasopressin V1a and V1b receptors: from molecules to physiological systems. *Physiol. Rev*. 92(4): 1813-1864.

Lambert, R.C., F.C. Moos, and P. Richard. 1993. Action of endogenous oxytocin within the paraventricular or supraoptic nuclei: a powerful link in the regulation of the bursting pattern of oxytocin neurons during the milk-ejection reflex in rats. *Neuroscience* 57(4): 1027-1038.

Lambert, J.J., M.A. Cooper, R.D. Simmons, C.J. Weir, and D. Belelli. 2009. Neurosteroids: endogenous allosteric modulators of GABA(A) receptors. *Psychoneuroendocrinology* 34(1): 48-58.

Landgraf, R. 1981. Simultaneous measurement of arginine vasopressin and oxytocin in plasma and neurohypophyses by radioimmunoassay. *Endokrinologie* 78(2-3): 191-204.

Lawrence, A.B., J.C. Petherick, K. Mclean, C.L. Gilbert, C. Chapman, and J.A. Russell. 1992. Naloxone prevents interruption of parturition and increases plasma oxytocin following environmental disturbance in parturient sows. *Physiol. Behav*. 52(5): 917-923.

Lemos, J.R., S.I. Ortiz-Miranda, A.E. Cuadra, C. Velázquez-Marrero, E.E. Custer, T. Dad, and G. Dayanithi. 2012. Modulation/physiology of calcium channel sub-types in neurosecretory terminals. *Cell Calcium*. 51(3-4): 284-292.

Leng, G., S. Mansfield, R.J. Bicknell, R.E. Blackburn, D. Brown, C. Chapman, R.G. Dyer, S. Hollingsworth, K. Shibuki, J.O. Yates, and S. Way. 1988. Endogenous opioid actions and effects of environmental disturbance on parturition and oxytocin secretion in rats. *J. Reprod. Fertil.* 84(1): 345-356.

Leng, G., C.H. Brown, and J.A. Russell. 1999. Physiological pathways regulating the activity of magnocellular neurosecretory cells. *Prog. Neurobiol.* 57(6): 625-655.

Lincoln, D.W., A. Hill, and J.B. Wakerley. 1973. The milk-ejection reflex of the rat: an intermittent function not abolished by surgical levels of anaesthesia. *J. Endocrinol.* 57(3): 459-476.

Luckman, S.M., I. Antonijevic, G. Leng, S. Dye, A.J. Douglas, J.A. Russell, and R.J. Bicknell. 1993. The maintenance of normal parturition in the rat requires neurohypophysial oxytocin. *J. Neuroendocrinol.* 5(1): 7-12.

Ludwig, M., N. Sabatier, P.M. Bull, R. Landgraf, G. Dayanithi, and G. Leng. 2002. Intracellular calcium stores regulate activity-dependent neuropeptide release from dendrites. *Nature* 418(6893): 85-89.

Manning, M., A. Misicka, A. Olma, K. Bankowski, S. Stoev, B. Chini, T. Durroux, B. Mouillac, M. Corbani, and G. Guillon. 2012. Oxytocin and vasopressin agonists and antagonists as research tools and potential therapeutics. *J. Neuroendocrinol.* 24(4): 609-628.

Meddle, S.L., G. Leng, J.R. Selvarajah, R.J. Bicknell, and J.A. Russell. 2000. Direct pathways to the supraoptic nucleus from the brainstem and the main olfactory bulb are activated at parturition in the rat. *Neuroscience* 101(4): 1013-1021.

Meddle, S.L., V.R. Bishop, E. Gkoumassi, F.W. Van Leeuwen, and A.J. Douglas. 2007. Dynamic changes in oxytocin receptor expression and activation at parturition in the rat brain. *Endocrinology* 148(10): 5095-50104.

Melcangi, R.C., F. Celotti, P. Castano, and L. Martini. 1993. Differential localization of the 5 alpha-reductase and the 3 alpha-hydroxysteroid dehydrogenase in neuronal and glial cultures. *Endocrinology* 132(3): 1252-1259.

Moos, F.C., K. Rossi, and P. Richard. 1997. Activation of N-methyl-D-aspartate receptors regulates basal electrical activity of oxytocin and vasopressin neurons in lactating rats. *Neuroscience* 77(4): 993-1002.

Morris, J.F. 1976. Distribution of neurosecretory granules among the anatomical compartments of the neurosecretory processes of the pituitary gland: a quantitative ultrastructural approach to hormone storage in the neural lobe. *J. Endocrinol.* 68(2): 225-234.

Murata, T., E. Murata, C.X. Liu, K. Narita, K. Honda, and T. Higuchi. 2000. Oxytocin receptor gene expression in rat uterus: regulation by ovarian steroids. *J. Endocrinol.*166: 45-52.

Neumann, I., J.A. Russell, and R. Landgraf. 1993. Oxytocin and vasopressin release within the supraoptic and paraventricular nuclei of pregnant, parturient and lactating rats: a microdialysis study. *Neuroscience* 53(1): 65-75.

Neumann, I., A.J. Douglas, Q.J. Pittman, J.A. Russell, and R. Landgraf. 1996. Oxytocin released within the supraoptic nucleus of the rat brain by positive feedback action is involved in parturition-related events. *J. Neuroendocrinol.* 8(3): 227-233.

Niezgoda, J., J. Rząsa, and Z. Ewy. 1973. Changes in blood vasotocin activity during oviposition in the hen. *J. Reprod. Fertil.*35(3): 505-509.

Nishimori, K., L.J. Young, Q. Guo, Z. Wang, T.R. Insel, and M.M. Matzuk. 1996. Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. *Proc. Natl. Acad. Sci. USA* 93: 11699-11704.

Nomura, S., T. Ito, E. Yamamoto, S. Sumigama, A. Iwase, M. Okada, K. Shibata, H. Ando, K. Ino, F. Kikkawa, and S. Mizutani. 2005. Gene regulation and physiological function of placental leucine aminopeptidase/ oxytocinase during pregnancy. *Biochim. Biophys. Acta* 1751: 19-25.

O'byrne, K.T., J.P. Ring, and A.J. Summerlee. 1986. Plasma oxytocin and oxytocin neurone activity during delivery in rabbits. *J. Physiol.* 370: 501-513.

Onaka, T., K. Ikeda, T. Yamashita, and K. Honda. 2003. Facilitative role of endogenous oxytocin in noradrenaline release in the rat supraoptic nucleus. *Eur. J. Neurosci.* 18(11): 3018-3026.

Papatsonis, D., V. Flenady, S. Cole, and H. Liley. 2005. Oxytocin receptor antagonists for inhibiting preterm labour. *Cochrane Database Syst. Rev.* (3): CD004452.

Papatsonis, D.N., V. Flenady, and H.G. Liley. 2013. Maintenance therapy with oxytocin antagonists for inhibiting preterm birth after threatened preterm labour. *Cochrane Database Syst. Rev*. 2013 Oct 13;10:CD005938. doi: 10.1002/14651858.CD005938.pub3.

Paris, J.J., P.J. Brunton, J.A. Russell, and C.A. Frye. 2011a. Immune stress in late pregnant rats decreases length of gestation, fecundity, and alters later cognitive and affective behaviour of surviving pre-adolescent offspring. *Stress* 14(6): 652–664.

Paris, J.J., P.J. Brunton, J.A. Russell, A.A. Walf, and C.A. Frye. 2011b. Inhibition of 5α-reductase activity in late pregnancy decreases gestational length and fecundity and impairs object memory and central progestogen milieu of juvenile rat offspring. *J. Neuroendocrino*l. 23(11): 1079-1090.

Parry, L.J., F.J. Guymer, T.P. Fletcher, and M.B. Renfree. 1996. Release of an oxytocic peptide at parturition in the marsupial, Macropus eugenii. *J. Reprod. Fertil.* 107(2): 191-198.

Pham, V., P. Burns, A.L. Albiston, H.R. Yeatman, L. NG, S. Diwakarla, and S.Y. Chai. 2009. Reproduction and maternal behavior in insulin-regulated aminopeptidase (IRAP) knockout mice. *Peptides* 30(10): 1861-1865.

Pickering, B.T. 1976. The molecules of neurosecretion: their formation, transport and release. *Prog. Brain Res.* 45: 161-179.

Pierce, J.G., and V. Du Vigneaud. 1950. Preliminary studies on the amino acid content of a high potency preparation

of the oxytocic hormone of the posterior lobe of the pituitary gland. *J. Biol. Chem*. 182: 359-366.

Randolph, G.W., and A.R. Fuchs. 1989. Pulsatile administration enhances the effect and reduces the dose of oxytocin required for induction of labor. *Am. J. Perinatol.* 6(2): 159-166.

Robinson, I.C. 1980. The development and evaluation of a sensitive and specific radioimmunoassay for oxytocin in unextracted plasma. *J. Immunoassay* 1(3): 323-347.

Rogi, T., M. Tsujimoto, H. Nakazato, S. Mizutani, and Y. Tomoda. Human placental leucine aminopeptidase/ oxytocinase. A new member of type II membrane-spanning zinc metallopeptidase family. *J. Biol. Chem*. 1996; 271:56–61.

Rossoni, E., J. Feng, B. Tirozzi, D. Brown, G. Leng, and F. Moos. 2008. Emergent synchronous bursting of oxytocin neuronal network. *PLoS. Comput. Biol*. 4(7): e4 e1000123.doi: 10.1371/journal.pcbi.1000123.

Russell, J.A., R.G. Gosden, E.M. Humphreys, R. Cutting, N. Fitzsimons, V. Johnston, S. Liddle, S. Scott, and J.A. Stirland. 1989. Interruption of parturition in rats by morphine: a result of inhibition of oxytocin secretion. *J. Endocrinol.* 121(3): 521-536.

Russell, J.A., G. Leng, and A.J. Douglas. 2003. The magnocellular oxytocin system, the fount of maternity: adaptations in pregnancy. *Front. Neuroendocrinol.* 4 (1): 27-61.

Rząsa, J., and Z. Ewy. 1970. Effect of vasotocin and oxytocin on oviposition in the hen. *J. Reprod. Fertil.* 21(3): 549-550.

Rząsa, J., and Z. Ewy. 1971. Effect of vasotocin and oxytocin on intrauterine pressure in the hen. *J. Reprod. Fertil.* 25(1): 115-116.

Rząsa, J., J. Niezgoda, and Z. Ewy. 1979. Changes in blood vasotocin level in response to uterine stimulation in the hen. *Acta Physiol. Pol*. 30(2): 267-272.

Rząsa, J., and Z. Ewy. 1982. The effect of ovarian steroids on the response of the hen uterus to neurohypophysial hormones. *Acta Physiol. Pol.* 33(4): 249-255.

Sabatier, N., C. Caquineau, G. Dayanithi, P. Bull, A.J. Douglas, X.M. Guan, M. Jiang, L. Van Der Ploeg, and G. Leng. 2003. Alpha-melanocyte-stimulating hormone stimulates oxytocin release from the dendrites of hypothalamic neurons while inhibiting oxytocin release from their terminals in the neurohypophysis. *J. Neurosci.* 23(32): 10351-10358.

Siebel, A.L., R.A. Bathgate, and L.J. Parry. 2005. Differential expression of mesotocin receptors in the uterus and ovary of the pregnant tammar wallaby. *Reproduction* 129(5): 639-649.

Sugimoto, Y., A. Yamasaki, E. Segi, K. Tsuboi, Y. Aze, T. Nishimura, H. Oida, N. Yoshida, T. Tanaka, M. Katsuyama, K. Hasumoto, T. Murata, M. Hirata, F. Ushikubi, M. Negishi, A. Ichikawa, and S. Narumiya. 1997. Failure of parturition in mice lacking the prostaglandin F receptor. *Science* 277: 681-683.

Summerlee, A.J. 1981. Extracellular recordings from oxytocin neurones during the expulsive phase of birth in unanaesthetized rats. *J. Physiol*. 321: 1-9.

Suska-Brzezińska, E., and Z. Ewy. 1970. Differences in the plasma L-cystine aminopeptidase activity in various species of birds. *Bull. Acad. Pol. Sci. Biol*. 18(2): 121-123.

Szeto, A., P.M. Mccabe, D.A. Nation, B.A. Tabak, M.A. Rossetti, M.E. Mccullough, N. Schneiderman, and A.J. Mendez. 2011. Evaluation of enzyme immunoassay and radioimmunoassay methods for the measurement of plasma oxytocin. *Psychosom. Med.* 73(5): 393-400.

Takayanagi, Y., M. Yoshida, I.F. Bielsky, H.E. Ross, M. Kawamata, T. Onaka, T. Yanagisawa, T. Kimura, M.M. Matzuk, L.J. Young, and K. Nishimori. 2005. Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proc. Natl. Acad. Sci. USA* 102: 16096-16101.

Teruyama, R., and W.E. Armstrong. 2002. Changes in the active membrane properties of rat supraoptic neurones during pregnancy and lactation. *J. Neuroendocrinol.* 14(12): 933-944.

Teruyama, R., D.L. Lipschitz, L. Wang , G.R. Ramoz, W.R. Crowley, S.L. Bealer, and W.E. Armstrong. 2008. Central blockade of oxytocin receptors during mid-late gestation reduces amplitude of slow afterhyperpolarization in supraoptic oxytocin neurons. *Am. J. Physiol. Endocrinol. Metab*. 295(5): E1167-1171.

Theodosis, D.T., J.J. Koksma, A. Trailin, S.L. Langle, R. Piet, J.C. Lodder, J. Timmerman, H. Mansvelder, D.A. Poulain, S.H. Oliet, and A.B. Brussaard. 2006. Oxytocin and estrogen promote rapid formation of functional GABA synapses in the adult supraoptic nucleus. *Mol. Cell Neurosci.* 31(4): 785-794.

Tobin, V.A., G. Leng, M. Ludwig, and A.J. Douglas. 2010. Increased sensitivity of monoamine release in the supraoptic nucleus in late pregnancy: region- and stimulus-dependent responses. *J. Neuroendocrinol.* 22(5): 430-437.

Tobin, V.A., G. Arechaga, P.J. Brunton, J.A. Russell, G. Leng, M. Ludwig, and A.J. Douglas. 2014. Oxytocinase in the female rat hypothalamus: a novel mechanism controlling oxytocin neurones during lactation. *J. Neuroendocrinol.* 26(4): 205-216.

Vandesande, F., and K. Dierickx. 1975. Identification of the vasopressin producing and of the oxytocin producing neurons in the hypothalamic magnocellular neurosecretroy system of the rat. *Cell Tissue Res.* 164(2): 153-162.

Wakerley, J.B., and D.W. Lincoln. 1973. The milk-ejection reflex of the rat: a 20- to 40-fold acceleration in the firing of paraventricular neurones during oxytocin release. *J. Endocrinol*. 57(3): 477-493.

Wallis, M. 2012. Molecular evolution of the neurohypophysial hormone precursors in mammals: Comparative genomics reveals novel mammalian oxytocin and vasopressin analogues. *Gen. Comp. Endocrinol.* 179(2): 313-318.