Coherent Peptide-mediated Activity in a Neuronal Network Controlled by Subcellular Signaling Pathway: Experiments and Modeling

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Abstract: Classically, information processing in the brain involves fast signaling mechanisms at a vast number of discrete sites, via spike-dependent neurotransmitter release at synapses. However neurons also use a huge diversity of slower analog signaling mechanisms, these chemical signaling pathways, acting in a more global spatial scale and on a longer temporal scale, are closely related with social behaviors and emotion. How do these parallel signaling systems interact to give rise to coherent behavioral consequences? In this review, we consider the role of the neuropeptide oxytocin in the milk-ejection reflex as an example of how a complex neural network involving a peptidergic signaling pathway underlies the complex physiological behavior.

Keywords: Neuropeptide; oxytocin; vasopressin; emergent bursting; dendrites; priming.

1. **Introduction**

Neurons process and transmit information by electrochemical signaling (Zigmond et al. 1999; Feng, 2003). Classically, neurons receive synaptic input from other neurons via their dendrites, and encode it as very brief electric events (action potentials, also called spikes). These spikes are propagated along nerve axons to the nerve endings, where the signals are transmitted to other neurons via release of neurotransmitters at synapses. Most neurons in the central nervous system (CNS) use either the excitatory transmitter glutamate or the inhibitory transmitter GABA, but some use a relatively small number of alternative neurotransmitters, the best known of which are acetylcholine, histamine, noradrenaline, serotonin and dopamine. In addition, many neurons also use other messenger systems that do not involve vesicular compartmentalization; these include adenosine, endogenous cannabinoids (Piomelli, 2003; Mackie, 2005), and some gases – most notably nitric oxide (Lipton et al. 1994; Calabrese et al. 2007); these messengers are produced "on demand" in an activity-dependent manner and primarily act as short loop negative feedback signals, restraining activity either by inhibition of the cell of origin or by presynaptic actions to inhibit afferent synaptic input.

However, in all brain areas, neurons also use another slow chemical signaling system. Most if not all neurons in the CNS, in addition to a conventional neurotransmitter, make and release one or more neuropeptides. More than 100 neuropeptides are released by neuronal populations in the CNS (see Leng & Ludwig 2006); for example, the arcuate nucleus of the hypothalamus alone includes neuroendocrine neurons that release growth-hormone releasing peptide; two populations of appetite regulating neurons, one of which makes both melanocyte stimulating hormone (α-MSH) and cocaine-and amphetamine related transcript, the other of which makes agouti-related peptide and neuropeptide Y; kisspeptin neurons that regulate the activity of the gonadotrophin axis, and interneurons that make somatostatin.

Conventional neurotransmitters are packaged in small synaptic vesicles and are released selectively from axonal terminals into synaptic clefts, where they act with a half-life of ~5ms, constrained by highly efficient re-uptake mechanisms. By contrast, neuropeptides are packed in large

dense core vesicles (LDCVs) that can be released from all parts of a neuron - axons, cell bodies and dendrites. These vesicles carry much more cargo than conventional synaptic vesicles - a LDCV can contain about 85,000 molecules of a peptide, whereas a typical synaptic vesicle contains about 5,000 molecules of glutamate (Leng and Ludwig, 2008), and peptides usually act with much longer half-lives, of ~20 min in the brain in some cases (Mens et al., 1983), which means that they can diffuse to act at distant targets. Conventional neurotransmitters bind to their receptors with relatively low affinity, typically in the micromolar range, but neuropeptide receptors have very high (nanomolar) affinity, and are coupled to intracellular pathways that can amplify their effects in diverse ways. These effects can be very long lasting, and can alter the information processing abilities of neurons. For example oxytocin can mobilize intracellular Ca^{2+} to prime dendritic stores of peptides for subsequent activity-dependent release - an effect that can last for at least 90 min. This ability of neuropeptides is important because it could functionally reorganize neural networks to exert long-lasting effects (Ludwig and Leng, 2006). Indeed, neuropeptides act on a different spatial and temporal scale to conventional neurotransmitters, and provide the brain with another powerful ability in information processing. They act as autocrine agents - mediating feedback to the cell of origin, as paracrine agents - "local" hormones that act on nearby cells, and as neurohormonal agents - acting at distant sites within the brain. Some neuropeptides, and even nitrix oxide (Trabace and Kendrick, 2000), can also function as a potent modulator of classical neurotransmitter release, notably noradrenaline, dopamine, serotonin and GABA, and many of their behavioral actions are dependent upon this (von Bohlen und Halbach and Dermietzel, 2006).

Thus neuropeptides have more diverse effects and are especially involved in behavioral and physiological functions. For example, α-MSH is a potent inhibitor of feeding by its actions at MC4 receptors, while its actions are opposed by AgRP, an inverse agonist at the MC4 receptor and by NPY, a potent stimulator of appetite. Neuropeptide Y is a potent stimulator of appetite, but many other neuropeptides, including apelin, dynorphin, galanin, orexin, galanin-like peptide, prolactin-releasing peptide, oxytocin and cholecystokinin, are also involved in the control of feeding behavior and energy expenditure (Schwartz et al., 2000; Morton et al., 2006). Exactly why so many peptides are apparently

important for feeding may seem surprising, but it should be noted that feeding comprises a complex sequence of behaviours including food seeking or appetitive behaviours as well as ingestive behaviours. It also demands a co-ordinated set of physiological responses, including efferent control of glucose homeostasis, reflex natriuresis, and prandial drinking. Maintaining energy homeostasis means that energy intake must be balanced by increased energy expenditure, including increased metabolic rate and locomotor activity. Finally, feeding behaviour requires a co-ordinated "switching off" of other competing motivations. Notably, there is a competition in the mammalian brain between the drive to eat and the drive to reproduce, and this is apparent in the contrasting effects of neuropeptides on these drives – for example, α-MSH is not only a powerful inhibitor of feeding – it is also a powerful stimulator of sexual behavior (Hadley, 2005).

Neurons in the brain are phenotypically diverse; they express different signaling molecules, different membrane receptors, have different morphologies, and have different intrinsic electrical properties. Nowhere is this more apparent than in the hypothalamus. Indeed, this ancient, highly conserved part of the brain has essential roles in many processes fundamental to survival and reproduction. Increasingly, we are coming to understand the roles played by different neuronal populations in terms of peptide-mediated actions. Among these, oxytocin and vasopressin are two of the best studied neuropeptides. Classically, these are hormones secreted from the posterior pituitary gland into the blood. In mammals, oxytocin mediates milk let-down in response to suckling, and stimulates uterine contractions during parturition (Russell et al., 2003), while vasopressin acts at the kidney to promote water reabsorption and antidiuresis. However, oxytocin and vasopressin also have complex behavioral effects including on emotional and social behaviors (see Lee et al., 2009; Donaldson and Young, 2008; Caldwell et al., 2008; Raggenbass, 2008; Lim and Young, 2006; Landgraf et al., 2005).

Recent experimental studies using fMRI have revealed that the peptide oxytocin also modulates the neural circuitries associated with human behaviors such as trust and trust adaptation (Baumgartner et al., 2008), social cognition and fear (Kirsch et al., 2005). Vasopressin has different, but related behavioral effects. It promotes aggressive behavior, particularly the male-male "territorial" aggression

that is a mark of the formation of a pair bond in males of monogamous species, and also the aggression of maternal females towards intruders. These behaviors are important elements of successful parenting, and they are typically most obvious when there are young to protect and nurture. A recent study on vasopressin effects in human showed differential effects in male and females responses to neutral or emotional faces (Thompson et al. 2006). These effects on social behaviours involve actions at many different sites in the brain, including the hypothalamus, amygdala and olfactory bulb; most recently, a new population of vasopressin cells has been identified in the olfactory bulb that is involved in the processing of olfactory signals that are important for social recognition (Tobin et al. 2010).

For a neuropeptide-producing neuronal population to generate a hormone-like signal that is detected in distant parts of the brain requires co-ordinated release. During breast feeding (and parturition) oxytocin neurons fire together in dramatic synchronized bursts. This bursting depends on the local release of oxytocin; thus oxytocin released locally can facilitate the triggering of a large secretory event that can result in a wave of oxytocin release within the brain, a wave so large that it can reach distant sites. The key local actions of oxytocin involve "priming"; priming involves making vesicles readily available for subsequent activity-dependent release, thus enhancing the capacity for the local interactions that co-ordinate neuronal activity. By enhancing intercommunication, it 'reprograms' the behavior of a neuronal system, and as oxytocin itself can prime oxytocin release, this reprogramming can be self-sustaining, and hence long-lasting. The incorporation of experiment and computational modeling suggests that a subcellular signaling pathway that involves priming underlies the rhythmic and behavioral effects of neuropeptides (Rossoni et al., 2008).

2 Neuropeptide and peptide receptors

Neuropeptides are generated from large precursor proteins which are cleaved enzymatically to yield sometimes several biologically active peptides. For example, pro-opiomelanocortin can be cleaved to yield adrenocorticotrophic hormone, several opioid peptides including β-endorphin and

leu-enkephalin, and α-MSH (Nässle, 2002). LDCVs contain all the products of the precursor molecule, hence, when a given peptide is released from a cell, so are other fragments of the precursor molecule, and these fragments may also be biologically active.

Once released, peptides are free to bind to cell surface receptor proteins with high affinity, making them effective even at low picomolar concentrations. Neuropeptides act by binding to specific receptors, but these may have different subtypes. Oxytocin acts on oxytocin receptors (Freund-Mercier et al., 1994), and vasopressin acts on vasopressin receptors (Hurbin et al., 2002), but whereas oxytocin is currently known to act at only one receptor, there are at least three subtypes of vasopressin receptors. Usually, neuropeptide receptors are G-protein coupled receptors that are expressed on neuronal plasma membranes. These receptors are coupling elements in the transmembrane signaling pathway, where they are responsible for pre- and postsynaptic modulatory actions of short and long duration (Gilman, 1986). Interestingly, activation of these receptors can also cause Ca^{2+} release from intracellular stores, with diverse consequences. Unlike neurotransmitters, which generally act on ligand-gated or voltage-gated ion channels, neuropeptides can act as neuromodulators that regulate ion channels gated by signals such as phosphorylation, dephosphorylation, and interactions with G-proteins etc. This can affect the intrinsic electrophysiological properties of target cells, and affect how they respond to particular afferent inputs (Katz and Frost, 1996; Katz, 1998). Modulatory roles of neuropeptides have been extensively studied in the periphery, and in the CNS, neuropeptides have, for example, been shown to modulate neuronal circuits related emotional and social behaviors (Kirsch et al., 2005; Baumgartner et al., 2008). Some neuropeptides act in an autocrine and paracrine fashion to exert autoregulatory effects on the cells of origin and on neighboring neurons (Ludwig et al., 2002). Some are also hormones that are released into the circulation from neuroendocrine cells. Usually, they have long half-life time which enables these hormone-like peptides to act at peripheral targets after transport over long distances.

The cellular expression patterns of neuropeptides are highly stereotyped (Nässle, 2002), and many different neuropeptides are expressed and released by different neuronal populations. For

example, the arcuate nucleus of the hypothalamus contains separate populations of neurons that synthesize growth hormone releasing factor, neuropeptide Y, kisspeptin, somatostatin, and pro-opiomelanocortin. Different populations of peptide neurons differ from each other in their morphology, biochemical phenotype, functional connectivity, and their electrophysiological properties.

3. Release of neuropeptides

3.1. Axonal vs Dendritic release

LDCVs, like conventional small synaptic vesicles, are released by Ca^{2+}-dependent exocytosis. At nerve endings, this process is triggered by the arrival of spikes which depolarize the endings, causing the opening of voltage-dependent Ca^{2+} channels. The resulting Ca^{2+} influx causes a rise in $[Ca^{2+}]$ below the plasma membrane, which can be sufficient to trigger exocytosis of LDCVs that are ”docked” at membrane release sites. Thus LDCVs can be released at classical synapses; but while synapses contain a very large number of small synaptic vesicles, they generally contain very few LDCVs. Most LDCVs are released at non-synaptic sites such as soma, axon and especially, dendrites (Morris and Pow, 1991; Castel et al., 1996). Dendrites comprise about 80% of the volume of a typical neuron, but are usually thought as passive elements that simply receive synaptic input from other neurons and then transmit the information to the soma. However, as we will see below, dendritic peptide release is a key feature of information transfer in neuronal networks (Leng and Ludwig, 2006).

The unequivocal evidence that neuropeptides can be released in abundance from dendrites comes from studies of the hypothalamo-neurohypophysial system. This system comprises several thousand magnocellular neurons (Pow and Morris, 1989). Each has one long axon that projects to the posterior pituitary (Fig. 1), where it gives rise to more than 2000 neurosecretory terminals and swellings that are packed with LDCVs (Fig. 1). Oxytocin and vasopressin in these terminals are secreted into the blood, but do not re-enter the brain because of the brain-blood barrier (the posterior pituitary gland lies on the blood side of this barrier). However, the cell bodies of these neurons are on the brain side of the blood-brain barrier, so what is released from the soma and dendrites stays in the

brain, until it is either broken down enzymatically by aminopeptidases, or else cleared from the brain by bulk flow of cerebrospinal fluid. The dendrites in the SON project towards the ventral surface of the brain, where they form a dense plexus (Armstrong, 1995), from which release can readily be measured using intracranial microdialysis and sensitive radioimmunoassays.

Other evidence comes from luteinising hormone releasing hormone (LHRH) which is synthesized by just a few hundred neurons in the preoptic/septal area (Wray, 2002), but which is essential for reproduction, as it drives the pulsatile secretion of LH that is essential for spermatogenesis in males and for ovarian cyclicity in females. LHRH neurons have very long dendrites (Campbell et al., 2005) which contain abundant LDCVs (Goldsmith et al., 1994). In response to physiological stimuli, spikes that originate at the soma can travel along these dendrites as well as the axons to elicit secretion from both sites (Martinez-Fuentes et al., 2004). Axonally secreted LHRH is mainly released into the hypophyseal portal circulation to regulate the secretion of gonadotrophins from the anterior pituitary gland, while LHRH released from dendrites can act on their cells of origin to facilitate the development of patterned electrical activity that underlies pulsatile gonadotrophin secretion, or on their neighbors by dendro-dendritic connections to ‘bind’ the collective activity of a neuronal population into a coherent signaling entity. Moreover, centrally released LHRH can diffuse to distant targets in many brain areas to mediate behavioral effects of LHRH.

Importantly, dendritic release does not necessary parallel with axonal release. Release of vasopressin and oxytocin from the dendrites and nerve endings of magnocellular neurons can be evoked on different time scales in response to the same stimulation, or can be regulated wholly independently in some cases (Ludwig, 1998). For examples, in oxytocin neurons, in response to suckling in lactating rats, oxytocin release in the SON precedes secretion into the blood (Moos et al., 1989), whereas after systemic osmotic stimulation, SON oxytocin release lags behind peripheral secretion (Voisin and Bourque, 2002; Ludwig et al., 1994). Some chemical signals, including oxytocin and vasopressin, can elicit dendritic peptide secretion without activating axonal release, while, conversely, electrical activity in the cell bodies can cause oxytocin secretion from the nerve endings without evoking release from the dendrites (Ludwig et al., 2002).

3.2. Regulation of neuropeptide release through intracellular signaling pathways

In nerve terminals, neuropeptide and neurotransmitter release is determined by the entry of Ca^{2+} through voltage-dependent channels that open as a result of spike activity (Leng and Ludwig, 2006). In dendrites, however, LDCV release can be regulated both by Ca^{2+} entry and by intracellular Ca^{2+} (Ludwig and Leng, 2006). ”Activity-dependent” release follows membrane depolarization by action potentials that trigger the entry of Ca^{2+}. This activates the release of LDCVs from a readily-releasable pool of vesicles docked at the plasma membrane, where the machinery of exocytosis has a high affinity for Ca^{2+}. The alternative route is through mobilizing Ca^{2+} from internal stores, whereby agents such as thapsigargin induce large amounts of Ca^{2+} release from the endoplasmic reticulum. This can trigger exocytosis from a reserve pool of LDCVs, and can also recruit vesicles from the reserve pool to the readily releasable pool. In oxytocin neurons, oxytocin itself can mobilize intracellular Ca^{2+}, and so, once triggered, oxytocin release is self-sustaining and hence long lasting (Ludwig et al., 2002).

One functional consequence of dendritic peptide release is to autoregulate the electrical activity of the cells of origin. In general, neurons that synthesize a neuropeptide also synthesize one or more receptors for that peptide, thus peptide release has feedback effects on the cell of origin. For example, both oxytocin and vasopressin can act on their respective cells of origin. Release of dendritic oxytocin can act back to the dendrites to stimulate further release both by mobilizing intracellular Ca^{2+} and by direct depolarizing actions, and also augments the Ca^{2+}-dependent production of endocannabinoids - another class of paracrine messengers which has specific effects on the excitability of afferent glutamatergic nerve terminals (Hirasawa et al., 2004), to suppress afferent excitation. By contrast, vasopressin can either excite or inhibit vasopressin neurons, depending on their ongoing electrical activity. As a result, fast-firing neurons slow down while slow-firing neurons are excited (Gouzenes et al., 1998); as a consequence vasopressin co-ordinates the activity level of the vasopressin neuron population as a whole.

3.3. Priming of dendritic release

Normally, dendritic release from magnocellular neurons is not much influenced by electrical activity (see Fig. 2A). However, agents such as thapsigargin or cyclopiazonic acid that mobilize intracellular Ca^{2+}, and some peptidergic inputs (and, for example, suckling input) can trigger release from dendrites independently of electrical activity (Fig. 2B), and can also prime dendritic stores of peptide (Fig. 2C), making them available for activity-dependent release (Ludwig et al., 2002; Fig. 2D). In oxytocin cells, priming potentiation lasts up to 90min after thapsigargin, much longer than the lasting time of the increase of $[Ca^{2+}]_i$, so it is not a consequence of a long-lasting elevation of $[Ca^{2+}]_i$ induced by thapsigargin. Thapsigargin increases $[Ca^{2+}]_i$ by blocking the ability of the cell to pump Ca^{2+} into the endoplasmic reticulum, causing these stores to become depleted. Release of Ca^{2+} from these stores is regulated by second messenger pathways, including the IP_3 pathway. The irreversible store depletion can also activate membrane Ca^{2+} channels, which may contribute to exocytosis, and increase the entry of Ca^{2+} into the cytosol. A recent study indicates that priming involves a relocation of LDCVs close to the dendritic plasma membrane without changing the number of LDCVs within the dendrites (Tobin et al., 2004). Priming may also involve local synthesis of proteins that support exocytosis (Ma and Morris, 2002), vesicle tethering, and/or active vesicle transport, and maturation of docked LDCVs at the plasma membrane (Ludwig and Pittman, 2003).

Peptide-mediated-priming causes a dramatic facilitation in the size of the readily releasable pool of LDCVs available for activity-dependent dendritic release, and it seems likely that this is a common mechanism throughout the CNS. As priming involves translocation of vesicles, its effects are delayed but long-lasting, which allows the neuropeptides to functionally reorganize neuronal networks through hormone-like or neuromodulatory actions on receptors which are widely expressed at diverse sites. This might result in peptide-dependent plasticity of the neuronal networks and gradually lead to coherent behavioral effects. Compared with the relatively fast neurotransmitter-mediated plasticity of synaptic connections between neurons, neuropeptide-mediated plasticity is believed to reflect the temporal modulations of neuronal circuitry rather than direct changes on membrane actions (Ludwig and Leng, 2006). Moreover, peptide priming may combine with NMDA-receptor-mediated plasticity to stimulate dendritic branching during development, and dendritically released neuropeptides such as

oxytocin in concert with sex steroids are implicated in morphological plasticity through glial cell retraction and changes in synaptic density (Ludwig and Pittman, 2003).

4. Physiological and behavioral effects of neuropeptides

The brain can be viewed as a complex endocrine organ, where neuropeptides control and modulate our mood, appetite, water and electrolyte balance, and energy level through peptides like oxytocin, vasopressin, neuropeptide Y, etc. They also regulate our social behaviors such as social recognition, aggression, social memory, and dominate reproductive behaviors such as maternal behavior and sexual behavior. Other behavioral effects of neuropeptides can be found in learning and memory forming, anxiety and depression, scent marking and grooming, etc. The reason why neuropeptides are so well suited for the above mentioned physiological and behavioral effects may be due to several specific ways that neuropeptides act:

i). the dendritic localization of neuropeptides means that the activity of peptidergic neurons can be co-ordinated through dendro-dendritic contact, in a manner that can be regulated by the availability of dendritic peptide for release.

ii). generally, neuropeptides act as potent neuromodulators or neurohormones that produce enduring effects on neural functions.

iii). some neuropeptides can prime dendritic peptide release, which can functionally reorganize neuronal networks and induce development and morphological plasticity.

Due to these characteristics, many neuropeptides exert specific, coherent effects on behavior as well as acting as regulatory hormones following release into the circulation.

4.1. Oxytocin and vasopressin; behavioral roles

In vertebrates, most of the findings relating neuropeptides to social behaviors have focused on oxytocin and vasopressin (Fig. 1). Both of these peptides are made by magnocellular neurons of the SON and PVN, and are released into the blood from nerve endings in the pituitary gland, but they have very different functions. In lactation, oxytocin is the hormonal trigger that, when releases into the

blood in response to suckling, causes milk let down. Other important peripheral actions include uterine contraction which is important for cervical dilation before birth and causes contractions during the second and third stage of labor (Russell et al., 2003). Oxytocin secretion is also elevated throughout sexual arousal (Carmichael et al., 1994). In addition, oxytocin and vasopressin released into the brain have potent effects on complex emotional and social behaviors. The diverse central effects of oxytocin are mediated in part by release from the dendrites of magnocellular neurons, and at some sites, including the brainstem and spinal cord, by release from other centrally-projecting oxytocin neurons of the paraventricular nucleus, different from those that project to the pituitary gland. Oxytocin receptors are expressed by neurons in many parts of the brain, including particularly the amygdala, ventromedial hypothalamus, septum and olfactory bulb. Notably, oxytocin has important roles in pair bonding, maternal behavior, and in humans, intranasal application of oxytocin can increase 'trust' (Kosfeld et al., 2005), reducing fear, and increasing generosity.

By contrast, vasopressin acts at the kidney to promote water reabsorption, thereby acting as the antidiuretic hormone; vasopressin also induces vasoconstriction to increase peripheral vascular resistance and thus to increase arterial blood pressure (Den Ouden and Meinders, 2005). However centrally, vasopressin also has effects on social behavior that are related to those of oxytocin. It is thought that vasopressin, released into the brain during sexual activity, initiates and sustains patterns of activity that support the pair-bond between the sexual partners; in particular, vasopressin seems to induce the male to become aggressive towards other males. More detailed accounts of the effects of oxytocin and vasopressin on emotions and behaviors can be found in recent reviews (Lee et al., 2009; Donaldson and Young, 2008; Caldwell et al., 2008; Raggenbass, 2008; Lim and Young, 2006; Landgraf et al., 2005).

Oxytocin and vasopressin secreted into the blood cannot re-enter the brain because of the blood-brain barrier (Mens et al., 1983). However, when young suckle, not only they are rewarded intermittently with a let-down of milk, but oxytocin is also secreted within the mother's brain, apparently fostering the maternal bond between mother and infant. The mystery is how breast-feeding is linked to this strong emotional bond. Previous studies on individual neurons have found no obvious

way of modifying their behavior to get the coordinated response needed to get large regular pulses of oxytocin. During breast feeding, oxytocin cells not only secrete oxytocin from their nerve ending but also recruit dendrites into secreting the hormone (Ludwig et al., 2002; Ludwig and Leng, 2006), which helps in increasing communication between neurons and thus further helps in releasing a 'flood' of the hormone at regular intervals.

5. Neuronal network dynamics mediated by neuropeptides

Normally, oxytocin cells discharge at about 1-3 spikes/s, but during suckling, every 5 min or so, they fire together in a dramatic synchronized burst of spikes This burst releases a pulse of oxytocin from the pituitary that causes the myoepithelial cells of the mammary gland to contract, intermittently letting down milk into a collecting duct. This 'milk-ejection reflex' is perhaps the best understood example of neuropeptide actions within the brain. This reflex involves a complicated neural network topology, dendritic oxytocin release, nonlinear electrical activity, and multi-scale dynamics involving both positive and negative feedbacks. Of particular interest is how exactly the few thousand oxytocin neurons are marshalled to produce a sufficiently intense burst of activity to release hormone and possibly promote maternal bonds?

5.1. Model

Constructing a reasonable model is based on the following properties of the oxytocin system:

i). The system has only a one-dimensional external input (suckling) with stochastic synaptic inputs from other neurons and a one-dimensional output (hormone).

ii). Each oxytocin cell has 2-5 dendrites filled with LDCVs that can be released by exocytosis (Pow and Morris, 1989).

iii). The oxytocin neurons intercommunicate within "bundles" of 3-8 dendrites which are directly apposed to each other (Hatton, 2004). These bundles play the role of a hub through which synchronized responses can be generated even in a sparsely connected network.

iv). Dendritic oxytocin release increases the excitability of oxytocin neurons.

v). Priming increases the readily-releasable store of oxytocin and hence increases the release rate of oxytocin, but also up-regulates the concentration of endocannabinoids which indirectly inhibit oxytocin release.

A key element of the model is the network topology (Fig.3A), which differs from all other topologies of biological networks in the literature: Each neuron in the network has two dendrites in different bundles, and it interacts with other neurons via dendro-dendritic connections through a common bundle (Fig. 3B). Mathematically, the network can be described by a bipartite graph $\mathbf{G}=\{\mathrm{N}\bigcup \mathrm{B},\mathrm{E}\}$, where $\mathrm{N}=\{i\,|1,2,\cdots,n\}$ is the set of neurons, $\mathrm{B}=\{k\,|1,2,\cdots,n_b\}$ is the set of bundles, and E is the set of connections from neurons to bundles (Fig. 3C). The topology of the network is characterized by an adjacency matrix $\mathbf{C^k}=\{c_{ij}^k\}$:

$$c_{ij}^k=\begin{cases}1, & \textit{if dendrite } j \textit{ of neuron } i \textit{ is in bundle } k,\\ 0, & \textit{otherwise.}\end{cases}$$

The structure of a single model neuron is schematically illustrated in Fig. 3D. Each neuron is treated as a leaky integrate-and-fire neuron which receives random excitatory and inhibitory synaptic inputs represented by inhomogeneous Poisson processes $N_{\{E,i\}}^j(t), N_{\{I,i\}}^j(t)$ with rates $\lambda_{\{E,i\}}^j(t), \lambda_{\{I,i\}}^j(t)$, respectively. The dynamics of the membrane potential of the i^{th} neuron in the network obeys

$$\frac{dV_i}{dt}=\frac{V_{rest}-V_i}{\tau}+\sum_{j=1}^{2}\left[a_E(V_E-V_i)\frac{dN_{E,i}^j}{dt}-a_I(V_i-V_I)\frac{dN_{I,i}^j}{dt}\right], \qquad (1)$$

where $a_E(V_E-V_i)$, $a_I(V_i-V_I)$ are the magnitude of EPSPs and IPSPs at the rest potential V_{rest} with V_E and V_I being the excitatory and inhibitory reversal potentials.

During lactation, activity-dependent changes in excitability and the effects of oxytocin are modeled as effects on spike threshold:

$$T_i=T_0+T_{AHP,i}+T_{HAP,i}-T_{OT,i} \qquad (2)$$

where T_0 is a constant, T_{HAP} accounts for a transient rise in the spike threshold due to a hyperpolarizing afterpotential (HAP) (Bourque et al., 1985; Leng et al., 2001), and T_{AHP} mimics the effect of activity-dependent hyperpolarization (AHP). The AHP can sustain a prolonged reduction in excitability after intense activation and is enhanced during lactation (Teruyama and Armstrong, 2005). For detailed mathematical equations for T_{HAP} and T_{AHP}, see Rossoni et al. (2008).

Oxytocin release from dendrites requires a large Ca^{2+} entry, achieved when spikes occur close enough together for the resulting Ca^{2+} entry from each spike to summate. So the model assumes that oxytocin is released only when two spikes are close enough, at an interval of less than τ_{rel}, and then, the release rate is proportional to the size of the readily-releasable store of oxytocin. Thus the instantaneous release rate ρ_i^j of oxytocin from dendrite j of neuron i is given by

$$\rho_i^j(t) = k_r r_i^j(t) \sum \delta(t - t_i^s - \Delta) \tag{3}$$

where k_r is a constant and Δ is the fixed time delay before release. The summation extends over the set $\{t_i^s < s, t_i^s - t_i^{s-1} < \tau_{rel}\}$, and r_i^j is the priming resulted readily-releasable store of oxytocin in dendrite j of neuron i represented by

$$\frac{dr_i^j}{dt} = -\frac{r_i^j}{\tau_r} + k_p(t) - \rho_i^j(t) \tag{4}$$

with τ_r being the time constant, and $k_p(t)$ the rate of priming due to suckling which is simply set as a positive constant during suckling and zero otherwise.

The effects of oxytocin release on the excitability of the oxytocin neurons are accounted by the following equation

$$\frac{dT_{OT,i}}{dt} = -\frac{T_{OT,i}}{\tau_{OT}} + k_{OT} \sum_{k,j} \sum_{l,m=1}^{2} c_{il}^k c_{jm}^k \rho_j^m(t) \tag{5}$$

with constants τ_{OT} and k_{OT}. The increase of the instantaneous release rate will meanwhile increase the concentration of endocannabinoids in each bundle with equation given by

$$\frac{d\varepsilon_k}{dt} = -\frac{\varepsilon_k}{\tau_{EC}} + k_{EC}\sum_{i=1}^{n}\sum_{j=1}^{2} c_{ij}^{k}\rho_i^{j} \ , \qquad (6)$$

with constants τ_{EC} and k_{EC}.

Such a simplified model describing the instantaneous release of oxytocin coupled with the electric activity of neurons is well established by Rossoni et al. (2008), which makes it feasible to use the model to explore how connectivity and dynamics of intercommunication affect the behavior of the system.

5.2 What does the model tell us?

The model accounts well for the background stochastic activity as well as the bursting activity (Fig. 4A) of the oxytocin system, and explains several interesting phenomena (Rossoni et al., 2008), for example:

i). Emergent bursting accompanied by pre and post-burst silences

A burst can begin anywhere in the network, but is more likely to start in regions where dendritic bundling is more pronounced. Increasing the number of neurons or the number of dendrites in a bundle causes bursts to propagate faster and become better synchronized. As observed *in vivo*, the bursting activity shows no marked ”leader” or ”follower”, either in a homogenously connected network or in a spatially inhomogeneous network, but the system performs more optimally when the network is relatively homogeneous. With increasing spatial inhomogeneity, there are more faster firing cells as well more slowly firing cells. The coupled dynamics of the oxytocin release and the readily-releasable store (Eqs. (3,4)) means that, although fast firing neurons can generate more of the sporadic fast clusters of spikes that tend to trigger bursts, their dendritic stores of releasable oxytocin are more depleted than those of neighboring slow firing neurons. The net result is that bursts are triggered less often.

Model neurons show a brief silence immediately before many bursts (Fig. 4B) and a long silence after bursting (Fig. 4C); in the model, both are due to the inhibitory effects of endocannabinoids. The endocannabinoids released from the first bursting cells suppress synaptic input

enough to cause a brief inhibition in other oxytocin cells before they are activated by oxytocin release. The post-burst silence is mainly a consequence of the prolonged suppression of afferent input, following the large increase in endocannabinoid concentration after a burst (Rossoni et al., 2008). Thus, some oxytocin neurons, if they are relatively unresponsive to oxytocin, fall silent even though they show no bursts at milk ejection (Fig. 4C).

We may further ask how each burst is triggered, sustained and terminated. In phasically firing vasopressin neurons, phasic bursts are initiated by activity-dependent Ca^{2+} influx and terminated by a slow, progressive desensitization to Ca^{2+} of the K^+ leak current (Roper et al., 2004), and similar mechanisms may also underlie bursting mechanisms of oxytocin cells; in the present model, the AHP, which reflects the activation of Ca^{2+}-activated K^+ channels, plays an important role in sculpting the profile of temporal bursts.

In oxytocin neurons recorded in vivo, the cross-correlation of the spiking activity gradually increases between bursts while the background spike activity becomes progressively more irregular (Rossoni et al., 2008). In the model, the former is a consequence of the strengthening of the interactions between cells, while the latter is due to the increased endocannabinoid production. The inter-burst interval is highly correlated with the dendritic stores of readily-releasable LDCVs which are continuously incremented by the suckling-related 'priming'. Between bursts, the dendritic stores increase relatively steadily despite activity-dependent depletion, but corresponding to the irregular spiking activities approaching bursts, there are large fluctuations of store levels approaching the phase of oxytocin release (Fig. 4D).

ii). Paradoxical behavior

In the model, increasing the excitatory input rate can result in a paradoxical blockade of bursting (Fig. 5A left). Such a phenomenon is well established in experimental observations, for example, systemic injection of hypertonic saline which strongly excites oxytocin cells will block their ongoing burst activity (Brown et al., 2000a, Fig. 5A right). Conversely, enhancing inhibitory activity can paradoxically reduce the background firing rate and promote bursting (Fig. 5B), as observed both in the model and in *vivo* (Brown et al., 2000a). By simulating the model with varying excitatory inputs,

we see that bursts can only occur within a particular range of synaptic input levels (Fig. 5C). In the presence of endocannabinoids, the range of synaptic input rates compatible with bursting is widened and, within this range, bursts occur relatively independently of synaptic input, compared with a model without endocannabinoids (Fig. 5D).

5.3. How well do we understand the model?

The model with random dendro-dendritic connections explains the milk-ejection reflex as an emergent property of the network behavior that arises due to activity-dependent dendritic peptide release through a positive feedback mechanism followed by synaptic depression.

i). The topology of network connections

In the model, bursting is an emergent activity due to the interplay of single neuron dynamics and network dynamics. In response to suckling, fast firing neurons occasionally fire spikes with an interspike interval short enough to trigger oxytocin release from dendrites; these oxytocin neurons depolarize neighboring cells by two dendrites projecting to bundles. Thus the connected slow firing neurons tend to speed up; however, fast firing neurons tend to slow down due to activity-dependent production of endocannabinoids, which feed back to reduce the synaptic input rates. At first, only fast firing cells contribute to bursts, but progressively more and more oxytocin cells are involved until all show intense, synchronous bursts.

Random connection is a key element for propagating this bursting. To show this, let us compare two ways of assigning dendrites to bundles. One is a regular arrangement of the connection (corresponding to $p = 0$ of the network in Fig. 6A), where each bundle contains the same number of dendrites. The other is a randomly connected network but with the same degree of the bundle. This can be realized by selecting a dendrite of a neuron in a regular network uniformly at random, and projecting it to another bundle with probability $p > 0$ (but avoiding sharing the same bundle with another dendrite of the same neuron). The bursts are much more synchronized in a random network than in a regular network. As the connection probability increases, synchronization becomes more pronounced (Fig. 6B).

ii). Subcellular signaling pathway

Dendritic peptide release is a key feature in the control of information transfer in neuronal networks, through cross-talk and autocontrol by paracrine/autocrine mechanisms. Without suckling, oxytocin neurons fire slowly, asynchronously and randomly, but only when the suckling stimulus $k_p(t)$ is present, the network displays synchronized high frequency every 3-10 min. During lactation, suckling evokes the release of oxytocin from dendrites of fast firing neurons, which speeds up the firing of slow firing neurons through cross-talk. Once oxytocin is released, it acts via oxytocin receptors to depolarize the oxytocin cells and mobilize intracellular Ca^{2+}, which further promotes the release of oxytocin from the dendrites, and also primes the dendritic stores of oxytocin, making them available for subsequent activity-dependent release.

iii). Balance of positive and negative feedbacks

The core mechanism that triggers bursting in such a sparsely connected population of neurons is activity-dependent positive feedback through dendritic oxytocin release, combined with negative feedback through endocannabinoids, whose concentration increases simultaneously with the oxytocin release. This allows bursting to occur only within a particular range of values of excitatory input (Fig. 5C). At a low level of excitation, an increase in the excitatory rate favors bursting by increasing the frequency of release episodes which can trigger a burst. However, beyond a critical level of excitation, release events may be so frequent that stores are not replenished fast enough to trigger a burst. Under such conditions, bursts become rarer and less predictable, until eventually over-excitation disrupts the reflex.

iv). Multiscale dynamics

In the model, network interactions are solely mediated by spikes separated by a very short interspike interval; these spike “doublets” thus play a critical role in synchronizing the network activity. Here, the transient mechanism of the HAP is a critical factor, by limiting the occurrence of short interspike intervals. On the other hand, oxytocin neurons as neuroendocrine cells also generate a hormone-like, pulsatile signal to act at dispersed and distant targets to produce prolonged organizational changes. For endocrine cells to produce a signal strong enough to be read at distance,

the pulsatile activity of peptide cells must be coordinated in a physiologically plastic manner. Thus, dendritic priming, which persists for a prolonged period, changes the nature of interaction between oxytocin neurons and, if oxytocin has similar priming actions at its targets elsewhere in the brain, there may be an equivalent functional reprogramming of neural circuitry at those targets also.

6. Discussion

The human brain contains about 10 billion neurons. Each of these has about 10,000 nerve endings from which neurotransmitters are released in response to spikes that in some cases can be discharged at rates exceeding 500 Hz. However, individually, neurons are very noisy, synaptic release is unreliable, and the entire brain including specification of its microcircuitry is determined by a developmental process that is controlled by just a few thousand genes. The brain is a self-organized complex system that is globally robust in its functions despite the unreliability and noisiness of its individual elements and despite random variability in its microstructure. Any individual neuron makes an insignificant contribution to information processing, but groups of neurons collectively perform complex information processing tasks robustly and reliably. To do so, groups of neurons that fulfill a common role must be "bound together" by intercommunication that allows them to perform coherently as a robust processing unit.

Over the past few decades, many efforts have been devoted to understanding how dendritic release of neuropeptides influences normal function in the nervous system. Among these, peptide neurons in the hypothalamus might be the best studied subjects of dendritic release, and computational models incorporating physiological facts play an important role in advancing our understanding. Besides the oxytocin model reviewed above, there are mathematical models of the pulsatile secretion of LHRH (Gordan et al., 1998; Scullion et al., 2004; Khadra and Li, 2006), the hypothalamic control of growth hormone secretion (MacGregor and Leng, 2005), and the bursting properties of vasopressin (Roper et al., 2004), etc. The recent model for LHRH revealed that LHRH plays the roles of feedback regulator and a diffusive synchronization effects in pulsatile secretion of LHRH from hypothalamic neurons. This also involves both positive and negative effects of autocrine regulation by neuropeptides

in generating rhythmic oscillations. Depending on the question we ask, a mathematical model could be a true biophysical model to match detailed biophysical properties, or a minimalist representation but congruent with physiological evidence to elucidate the key process, as we and others have demonstrated elsewhere (Nikitin et. al. 2008, Vavoulis et al., 2007, Davison et al., 2003). For example, the complicated biophysical model of Roper et al. (2004) seeks to understand how voltage-dependent conductances combine to form the distinctive discharge pattern of vasopressin cells, while the simplified model presented by Leng et al. (2001) seek to understand the implications for coding osmotic information through a modified leaky integrate-and-fire model. The principle of whether the constructed model is a good computational neural model depends on whether its *in silico* experimental results is direct open to *in vivo* or *in vitro* experimental interrogation.

Although the oxytocin model reviewed above does not incorporate all physiological properties of the oxytocin neurons, it provides a minimalist representation congruent with physiological evidence to elucidate the key process of how dendritic release of oxytocin coupled with non-linear electrical activity gives rise to rhythmic bursts. Several key elements contribute to the emergent bursting: the topology of the network, the stimulus-dependent process of priming of the dendritic stores, multiscale dynamics as well as the positive and negative feedbacks. This simplified model produces a close match to electrophysiological data, and explains how the connectivity of the network affects the system behavior. Nevertheless, several issues remain to be explored:

i) Information coding and decoding by spike doublets It can be easily seen from the dynamical equation for instantaneous oxytocin release that network interactions are solely mediated by the so-called spike doublets, i.e., spikes with interspike intervals less than τ_{rel}. Similar spike doublets have been observed in projection neurons in the lateral amygdala (Driesang and Pape, 2000) and many other systems which display both fast and slow rhythms (De Schutter and Maex, 1998; Kopell and Karbowski, 2000; Whittington et al., 2000), and it has been proposed that spike doublets play a critical role in overall rhythmicity. Recent experiments also show that a particular type of spike doublets, called 'theta-nested gamma', is related to object recognition as well as learning (Kendrick et al., 2009). On the other hand, spike doublets are not necessary deterministic, but can be stochastic

events. An important issue is how we can understand the encoding and decoding the input information of spike doublets, even for a population of leaky integrate-and-fire neurons (Feng and Ding, 2004; Zhang et al., 2009). Furthermore, the oxytocin model we developed exhibits a positively correlated activity (Feng and Brown 2000, Brown and Feng, 2000b). To develop a mathematical tool to deal with higher order moments in a neuronal network is also an interesting question (Deco et. al., 2008; Lu et. al., 2009).

ii). Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and priming In the oxytocin model, the dynamics of priming rate is set as a constant during suckling and is otherwise zero. Even this simplified assumption shows that activity-dependent process of priming is crucial for the occurrence of the emergent bursting. However, as discussed above, priming is a complicated dynamical process requiring the mobilization of intracellular Ca^{2+}. Modern biotechnology makes it possible to measure $[Ca^{2+}]_i$, through which different types of oscillatory Ca^{2+} signals generated in neurosecretory cells have been discovered (Li et al., 1995; Van Goor et al., 2000; LeBeau et al., 2000). These rhythmic Ca^{2+} signals are believed to be essential for peptide neurons to secrete hormonal signals to act at distant targets to produce organizational change. Certainly, they are crucial in affecting the bursting behavior of the oxytocin system.

iii). Dendritic priming and plasticity The physiological plasticity of complex behaviors presents an important challenge for neuroscientists; how can a discrete event – such as parturition - produce a long lasting change in behavior, apparently requiring a persistent reprogramming of complex neural circuitry? Parturition changes the behavior of the mother in ways that are at least partly attributable to the large release of oxytocin within the brain that accompanies parturition; some of the adaptations can be blocked by giving oxytocin antagonists into the brain, and can be mimicked by giving oxytocin into the brain. During lactation, besides producing intense bursts of hormone, the oxytocin system also mediates a remarkable activity-dependent structural plasticity, by acting either directly on oxytocin-expressing neurons and their synaptic inputs or on astrocytes to promote release of glia-derived neurotrophic factors (Theodosis, 2002). Can we develop a mathematical model to explain how the peptide-mediated priming combing both $[Ca^{2+}]_i$, electric activity of neurons as well as

the glial retraction to explain the observed experimental data and predict its behavioral consequences? This is a typical systems biological approach.

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Figure 1. Magnocellular oxytocin neurons have a single long axon that projects to the posterior pituitary gland where it gives rise to many swellings and nerve endings. These are packed with large dense core secretory vesicles that contain oxytocin. These vesicles are released by activity-dependent exocytosis in response to spikes, generated at the cell body, and propagated down the axons. During suckling, the oxytocin cells discharge brief, intense synchronised bursts of spikes, as in the inset; these bursts lead to pulses of oxytocin release into the blood that cause milk let-down at the mammary gland. However oxytocin is also released from the dendrites of oxytocin neurons, and this acts back on the oxytocin cells in a positive feedback manner, so is a major contributor to the bursting activity.

Figure 2. Priming of dendritic peptide release. *Adapted from Leng and Ludwig, 2006.*

Figure 3. **A.** Visualization of the network with yellow circles for neurons and blue squares for bundles. **B.** Schematic diagram of the organization of the oxytocin network; the yellow boxes represent dendritic bundles. **C.** The (bipartite) adjacency matrix for a randomly generated network with 48 neurons and 12 bundles; the squares mark non-zero matrix elements. **D.** Schematic illustrating the organization of a single model neuron: it receives random excitatory and inhibitory synaptic inputs, and its excitability is modeled as a dynamically changing spike threshold that is influenced by the HAP, the AHP, and the dendritic release of oxytocin. Activity-dependent production of endocannabinoids (EC) feeds back to reduce synaptic input rates. Each neuron interacts with neighboring oxytocin neurons by two dendrites that project to bundles. *Adapted from Rossoni et al.,*

2008.

Figure 4. A. Raster plots showing the spikes generated by all the cells of the model in two bursts. **B. Pre-burst silence:** Mean instantaneous firing rates vs. time of occurrence on a semi-log plot from a real oxytocin cell (circles, red dashed line) and from a model cell (squares, blue line). **C. Post-burst silence:** Simultaneous activity of two cells in the model, in one of which the sensitivity to oxytocin has been disabled but still displays post-burst silences. **D. Role of Dendritic Release in Generating Bursts.** Upper trace: The evolution of the mean firing rate in the model (in spikes/s; average over all neurons). Bottom trace: the evolution of dendritic stores level, given as the average over all the dendrites in the network; grey bars are SD. *Adapted from Rossoni et al., 2008.*

Figure 5: A. Both model (left) and experiment (right, Brown et al., 2000) show that a large increase in excitatory input rate will stop ongoing bursting activity. **B.** Both model (left) and experiment (right, Brown et al., 2000) show that increasing the inhibitory synaptic input can paradoxically start bursting activity in the model when the suckling input is sub-threshold (kp = 1.4/s). **C.** The model shows that bursts is related to the average level of synaptic input in a biphasic manner. **D.** The effect of endocannabinoids in the model is to increase the range of synaptic input rates compatible with bursting. *Adapted from Rossoni et al., 2008.*

Figure 6: A. Sketch of two types of topology of the network. $p = 0$ corresponds to a regularly connected network, while $p > 0$ is for a random connected network. **B**. Raster plots of spiking activities show that with the increase of the probability p (from left to right, p is increased), bursts

become better synchronized.

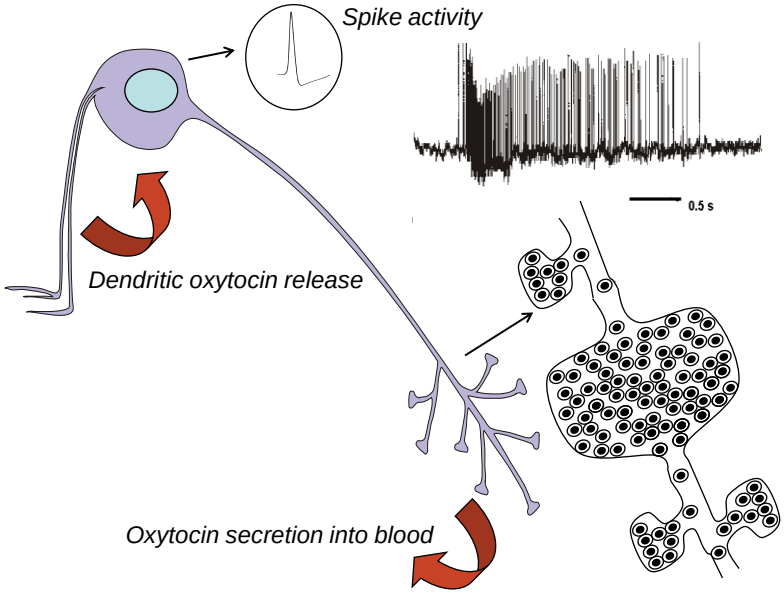
Spike activity
0.5 s
Dendritic oxytocin release
Oxytocin secretion into blood

Figure 1.

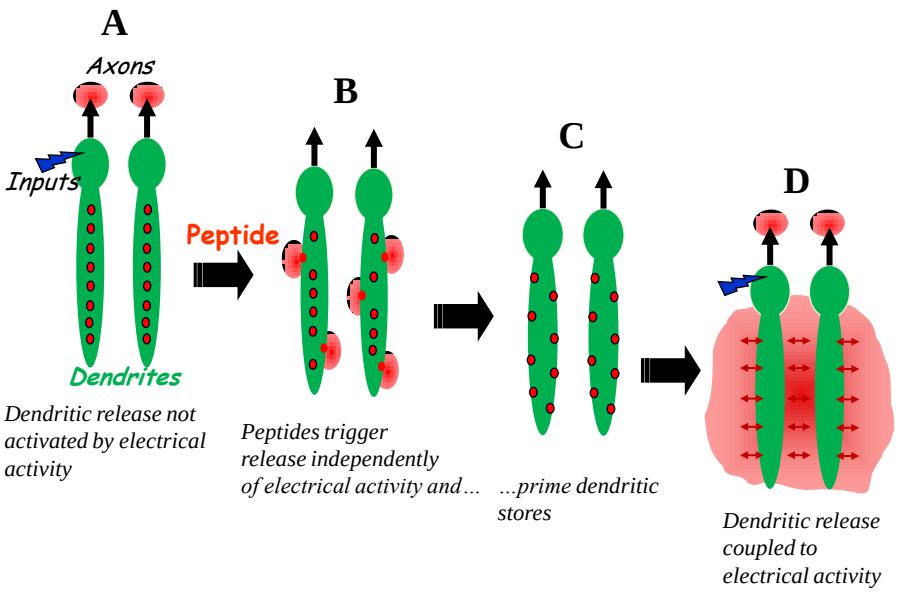
A
Axons
Inputs
Dendrites
Dendritic release not activated by electrical activity
B
Peptide
Peptides trigger release independently of electrical activity and…
C
…prime dendritic stores
D
Dendritic release coupled to electrical activity

Figure 2.

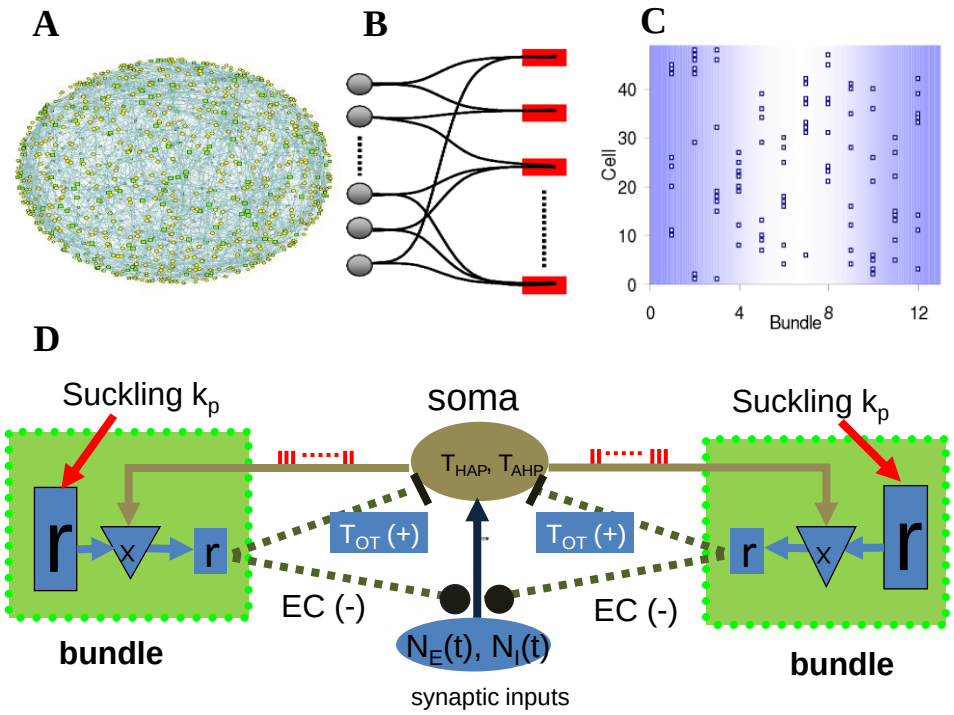
A
B
C
Cell
Bundle
D
Suckling k_p
soma
Suckling k_p
T_HAP, T_AHP
T_OT (+)
T_OT (+)
EC (-)
EC (-)
bundle
bundle
N_E(t), N_I(t)
synaptic inputs

Figure 3.

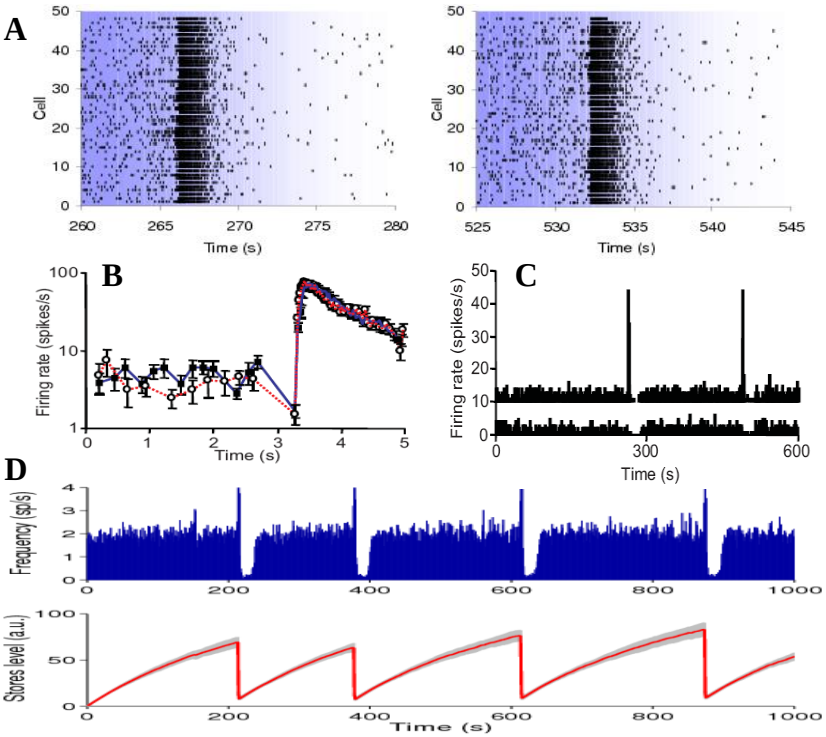
A
Cell
Time (s)
B
Firing rate (spikes/s)
Time (s)
C
Firing rate (spikes/s)
Time (s)
D
Frequency (sp/s)
Stores level (a.u.)
Time (s)

Figure 4.

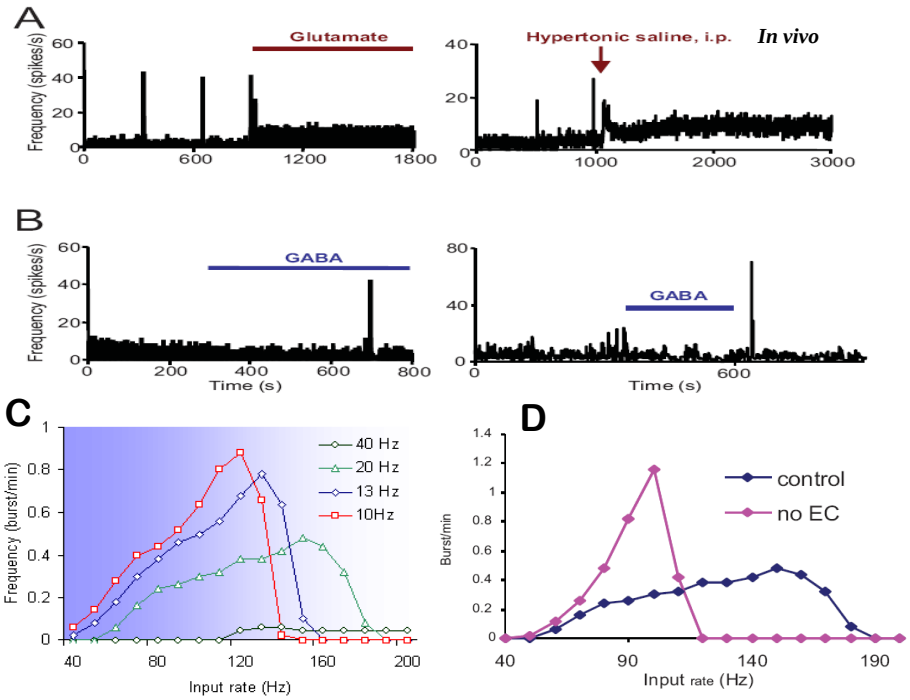
A
Glutamate
Hypertonic saline, i.p.
In vivo
Frequency (spikes/s)
B
GABA
GABA
Time (s)
C
40 Hz
20 Hz
13 Hz
10Hz
Frequency (burst/min)
Input rate (Hz)
D
control
no EC
Burst/min
Input rate (Hz)

Figure 5 .

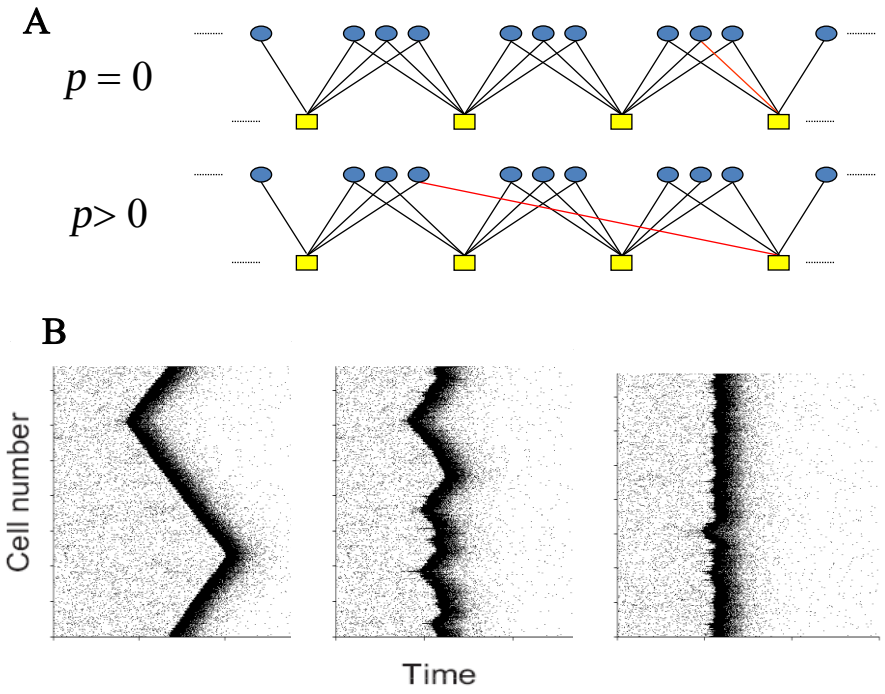

Figure 6 .