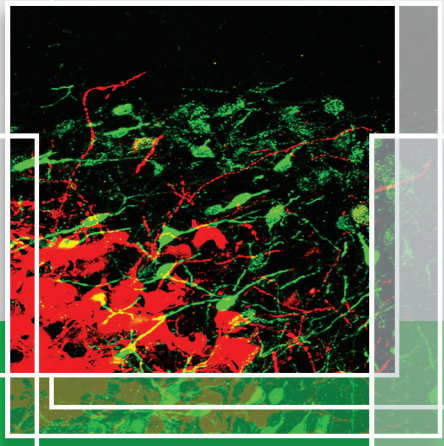
Masterclass in Neuroendocrinology Series

Neurophysiology of Neuroendocrine Neurons

Editors: William E. Armstrong & Jeffrey G. Tasker



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Series Preface

This Series is a joint venture between the International Neuroendocrine Federation and Wiley-Blackwell. The broad aim of the Series is to provide established researchers, trainees, and students with authoritative up-to-date accounts of the present state of knowledge, and prospects for the future across a range of topics in the burgeoning field of neuroendocrinology. The Series is aimed at a wide audience as neuroendocrinology integrates neuroscience and endocrinology. We define neuroendocrinology as study of the control of endocrine function by the brain and the actions of hormones on the brain. It encompasses study of normal and abnormal function, and the developmental origins of disease. It includes study of the neural networks in the brain that regulate and form neuroendocrine systems. It includes study of behaviors and mental states that are influenced or regulated by hormones. It necessarily includes understanding and study of peripheral physiological systems that are regulated by neuroendocrine mechanisms. Clearly, neuroendocrinology embraces many current issues of concern to human health and well-being, but research on these issues necessitates reductionist animal models.

Contemporary research in neuroendocrinology involves use of a wide range of techniques and technologies, from subcellular to systems and whole-organism level. A particular aim of the Series is to provide expert advice and discussion about experimental or study protocols in research in neuroendocrinology, and to further advance the field by giving information and advice about novel techniques, technologies, and inter-disciplinary approaches.

To achieve our aims each book is on a particular theme in neuroendocrinology, and for each book we have recruited an editor, or pair of editors, expert in the field, and they have engaged an international team of experts to contribute chapters in their individual areas of expertise. Their mission was to give an update of knowledge and recent discoveries, to discuss new approaches, "gold-standard" protocols, translational possibilities, and future prospects. Authors were asked to write for a wide audience to minimize references, and to consider use of video clips and explanatory text boxes; each chapter is peer-reviewed and has a glossary, and each book has a detailed index. We have been guided by an Advisory Editorial

Board. The Masterclass Series is open-ended: books in preparation include *Neuroendocrinology of Stress*; *Computational Neuroendocrinology*; *Molecular Neuroendocrinology*; and *Neuroendocrinology of Appetite*.

Feedback and suggestions are welcome.

John A. Russell, University of Edinburgh, and William E. Armstrong, University of Tennessee

International Neuroendocrine Federation – www.isneuro.org

Preface

Our understanding of the mechanisms underlying neurohormone release has evolved remarkably since the initial discovery that hypothalamic magnocellular neurons synthesizing oxytocin and vasopressin share electrical and synaptic excitability with other central nervous system neurons, and release these peptides from axon terminals in the neurohypophysis in an action potential dependent manner. In this volume, chapters range from those describing the rich history and current state of *in vivo* recordings, highlighting the precise relationship between the patterns of action potential discharge in these neurons and hormone release, to *in vitro* approaches where neuroendocrine neurons can be precisely identified and their membrane properties, morphology, and synaptic responses, directly examined. These modern approaches have led to an increased appreciation of the role the neurons play in regulating their own activity, including a new understanding of the electrical excitability and peptide-releasing capability of dendrites, and the characterization of the unique properties that axonal terminals possess to shape release. Thus, we hope that researchers and students of neuroendocrinology and neuroscience in general will glean from this volume not only an understanding of neuroendocrine cell electrophysiology, but also an appreciation of how this model system affords access to virtually all parts of the neuron for detailed study—something unique compared to most types of neurons in the brain.

Another aspect worth noting is that *in vivo* recording continues to provide the necessary physiological context in which we place rapidly expanding knowledge of the increasingly complex molecular characteristics of these neurons. Such work, whether it demonstrates the synchronous discharge of oxytocin neurons during lactation or the pulsatility of the gonadotropin-releasing hormone (GnRH) pulse generator, is inherently difficult, but critical to demonstrate the physiological importance of newly discovered ion channels, transporters, transmitter receptors, and transcription factors that shape the activity of these neurons.

Several chapters demonstrate the diverse power of *in vitro* techniques, whether using isolated neurohypophysial terminals, visually identified neurosecretory cells in cell cultures or *ex vivo* brain slices from transgenic rodents, or organ cultures that mimic *in vivo* activity. Whole-cell patch

recording has further allowed the identification of mRNAs in single neurons, documenting the expression of many channels and neurotransmitter receptors. These techniques have been critical for understanding the cellular physiology of neuroendocrine neurons because *in vivo* intracellular recordings are not routinely possible from these cells, due either to their deep and scattered locations in the brain, as is the case for the GnRH neurons, or to their proximity to pulsating large blood vessels that produce mechanical instability, as is the case for oxytocin and vasopressin neurons. Thus, intracellular recordings of GnRH neurons, for example, have only been accomplished *in vitro*, and there are only two short publications describing very brief *in vivo* recordings from vasopressin or oxytocin neurons.

Studies of neuroendocrine neurons have been pioneering in the discovery of the dendritic release of neurotransmitters and of the regulation of synaptic transmission by astrocytes. Several of the chapters herein consider different aspects of the release of neuropeptides and "retrograde," or backward-acting, messengers from the dendrites of neuroendocrine neurons, and describe the dynamic regulation of the actions of these retrograde messengers by astrocytes. The remarkable plasticity of the interactions between neuroendocrine neurons and their associated astrocytes under different physiological conditions makes for a fluid and ever-changing environment of synaptic modulation. In addition to modulating neurotransmission between pre- and postsynaptic neuronal elements by controlling neurotransmitter levels, glia also directly contribute to synaptic and extrasynaptic transmission via direct gliotransmitter release and actions on neurons. These are exciting times in the area of glial–neuronal interactions, and neuroendocrine neurons are at the forefront of discovery in this rapidly expanding field.

Thus, neuroendocrine neurons, "hybrids" of nerve and glandular cells that signal from the brain to the pituitary and the body, provide remarkably rich and accessible models for the study of intrinsic membrane currents, forward and backward synaptic transmission, and reciprocal neuronal–glial interactions. This volume introduces the reader to the current understanding of the physiological workings of this fascinating cell type, an introduction that hopefully will provide inspiration for further exploration into the exciting field of neuroendocrinology.

William E. Armstrong, Ph.D.
Jeffrey G. Tasker, Ph.D.

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/armstrong/neurophysiology

The website includes:

- End-of-chapter references and glossary
- Powerpoints of all figures and tables from the book
- Demonstration videos

SECTION 1A

Magnocellular Neuroendocrine Neurons: Properties and Control of Vasopressin and Oxytocin Neurons

CHAPTER 1

Electrophysiology of Magnocellular Neurons *In Vivo*

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1.1 Introduction

Neuroendocrinology is the study of things that matter: stress and appetite, metabolism, body rhythms, growth, and all aspects of reproduction from the reproductive cycle, through sexual behavior, pregnancy and parturition, to lactation and maternal behavior—things that matter for our health and happiness. However, neuroendocrine systems are also influential model systems for neuroscience generally, because of their unique value as "windows on the brain." The products of neuroendocrine systems can be measured relatively easily, and their effects are, with wit and persistence, determinable. In consequence, the neuronal activity that gives rise to those products is *interpretable* to a degree that can only be envied by colleagues in most other areas of neuroscience. If we ask of any neuron in the brain, what does it really *do*, the answers are often frustratingly incomplete: even if we know how it responds to stimuli, what it makes and where it projects, we may still not know what it does that matters to the behaving organism. By contrast, for the magnocellular vasopressin and oxytocin neurons of the hypothalamus, we can know much of what they do even before we know how they do it. All of these neurons project to the posterior pituitary gland, and what they secrete from there is measurable in the blood, and has measurable consequences for important physiological functions.

Neuroendocrinology began as the study of the secretion of peptides from neurons into the blood, and evolved to be also the study of secretion of peptides within the brain. In both aspects, electrophysiological recordings from single neurons *in vivo* have been fundamental in defining the physiological significance of mechanisms that have been established by cellular and molecular studies *in vitro* (Figure 1.1).

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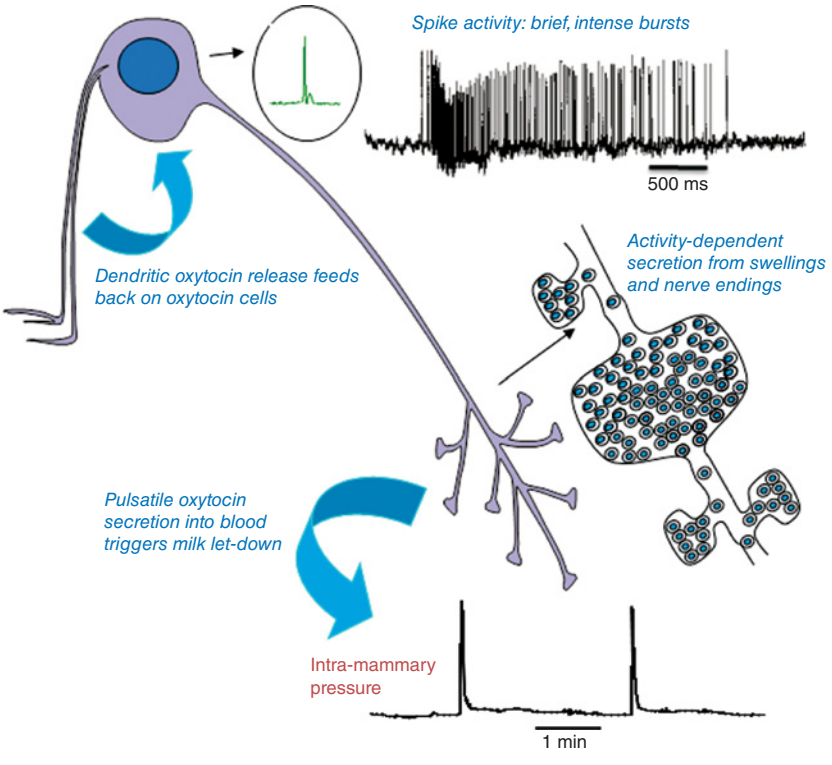

Figure 1.1 The milk-ejection reflex. The reflex was uncovered by electrophysiological studies *in vivo*. In response to suckling, oxytocin cells discharge intermittently in brief synchronized bursts that evoke secretion of pulses of oxytocin, which induce abrupt episodes of milk ejection. Dendritically released oxytocin facilitates the bursting.

1.2 Opening the window on the brain

Single-cell recording has strengths and weaknesses—and *in vivo* studies have particular problems as well as certain unique advantages. Because *in vivo* electrophysiology requires an extensive and prolonged investment in skills and expertise, it is wise to understand the nature of the investment that is involved, as well as the potential returns and the likely limitations.

The window on the brain afforded by the magnocellular system was opened by Wakerley and Lincoln (1973), when they used a technique to enable the magnocellular neurosecretory neurons to be rigorously interrogated electrophysiologically. There had been earlier attempts to study these neurons electrophysiologically, but despite careful stereotactical control, these recordings came from a mixture of neuroendocrine and non-neuroendocrine cells. This heterogeneity subverted interpretation: the breakthrough came from the ability to identify individual neurons as

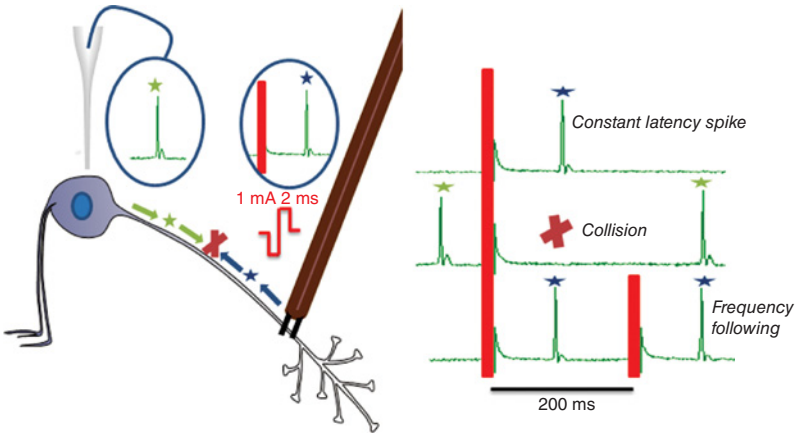

Figure 1.2 Antidromic identification. A stimulating electrode that is placed on the axon of any neuron may be used to trigger a spike that is propagated both orthodromically (green stars), toward the axonal endings, and *antidromically* (blue stars), toward the cell body. A recording electrode at the cell body will record the antidromic spike at a fixed latency following the stimulus—a latency that reflects the conduction velocity and the axonal length. In general, a stimulus pulse might evoke a spike that is generated by monosynaptic excitation, which would also arise at a nearly constant latency. Antidromic spikes can be distinguished from such orthodromic spikes by two additional tests: *frequency following* and the *collision* test. Antidromic spikes will be generated (i.e., will follow) each of a short train of stimulus pulses presented at a high frequency (50–100 Hz); these spikes will maintain a near-constant latency (there is a slight prolongation of latency with each successive pulse). A longer train of stimuli will result in fractionation of the antidromic spike—as the soma becomes refractory to antidromic stimulation, antidromic invasion is progressively delayed and may intermittently fail, while the smaller initial segment spike, which is normally hidden within the soma spike, will be preserved and become visible as a notch on the rising phase of the antidromic spike. However, antidromic spikes will not invade either the initial segment or the soma when they are extinguished by collision with a spontaneous, orthodromically propagated spike. This collision (red X) occurs when an antidromic stimulus pulse immediately follows a spontaneously generated spike—the descending spontaneous orthodromic spike meets the ascending antidromic spike along the axon, and both are extinguished by this collision.

neuroendocrine neurons immediately and unequivocally. That breakthrough came with the introduction of *antidromic identification* (Figure 1.2).

Because magnocellular neurons project to the pituitary, a stimulating electrode placed on the neural stalk can be used to trigger action potentials (spikes) in their axons, and these spikes can be detected at the soma, after antidromic (i.e., backward) propagation, as spikes that follow each stimulus at a constant latency. The rat supraoptic nucleus contains only two types of neuron—magnocellular oxytocin neurons and magnocellular vasopressin neurons, so every neuron recorded from this region that can be antidromically stimulated can be identified as one of these two types. Once Wakerley and Lincoln began to apply this approach, they saw that these

two cell types could be distinguished by other tests. These tests were refined by many others, leading to an extensive battery of tests that now allows us to talk of *identified oxytocin neurons* and *identified vasopressin neurons*.

From the ability to identify oxytocin and vasopressin neurons *in vivo* came the cornerstones of our understanding of the physiology of these systems. Wakerley and Lincoln (1973) defined the milk-ejection reflex, as it is known in all good text books (Figure 1.1). From their studies came the realization that, in response to suckling, oxytocin is not released continuously, but intermittently—in pulses that result from the brief intense synchronized bursts of action potentials generated by the oxytocin neurons (Figure 1.3). From Francois Moos and her colleagues (see Richard

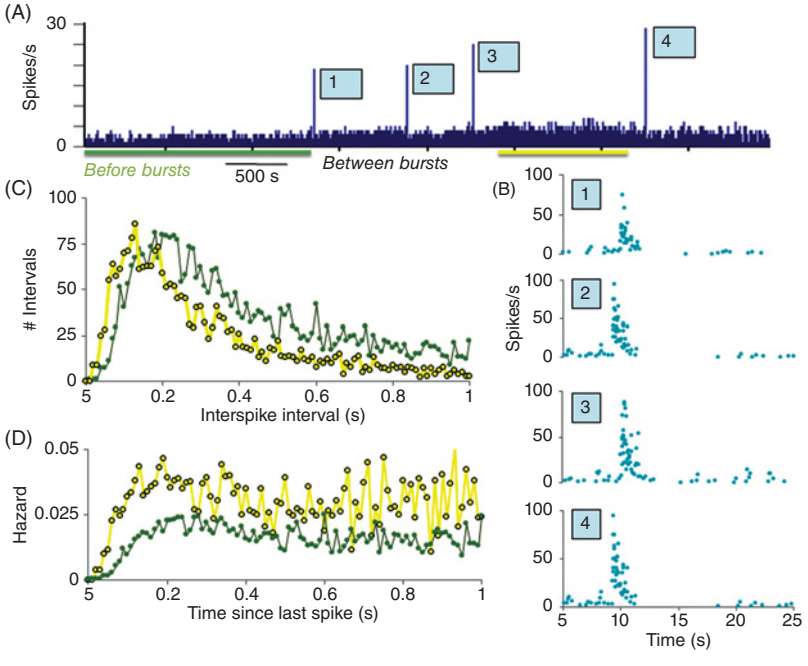

Figure 1.3 Milk-ejection bursts. Recording from an oxytocin cell in a urethane-anesthetized, lactating rat. Typically, oxytocin cells fire slowly and continuously, but, once the pups are applied, then brief intense bursts start to appear. (A) The first four bursts from one cell, progressively increasing in intensity. (B) Instantaneous frequency plots of these four bursts (the reciprocal of the interspike interval is plotted against spike time); note the consistency in the profiles of the bursts, which reveals their stereotyped structure, and the long quiet period following each burst. (C) Interspike intervals of the spontaneous activity before bursts (in green) and between bursts (in yellow). In this cell, the spontaneous activity is slightly elevated; typically, slow-firing cells become more active during suckling, while active cells become less active, but there is little change in the shape of the interspike interval distribution. (D) This is clearer in the hazard functions, which show a very similar shape except for the increase in hazard, which reflects an increase in mean firing rate.

et al., 1997), we learned that this reflex depends upon central release of oxytocin, and could be facilitated by injecting tiny amounts of oxytocin introcerebroventricularly (i.c.v.)—the first (and still the best) example of an indispensable physiological role of central peptide release. From Summerlee and Lincoln (1981), we learned that the milk-ejection reflex in conscious rats is the same as in anaesthetized rats—and that during parturition, oxytocin is again released in intermittent pulses as a result of similar synchronized bursting (Summerlee, 1981). From Jon Wakerley, Richard Dyball, and Dominique Poulain came the recognition that in response to osmotic challenge, vasopressin neurons fire not continuously, but phasically (Figure 1.4). This phasic firing is asynchronous among the vasopressin neurons, so that it leads to continuously maintained secretion—its significance is not in producing a phasic pattern of secretion, but in optimizing the efficiency of stimulus–secretion coupling at the nerve terminals. From Dyball and others, we learnt that oxytocin neurons are just as responsive as vasopressin neurons to osmotic pressure increases, leading to recognition of oxytocin's role (in many species) in regulating natriuresis. These pioneers laid the cornerstones of our current understanding, inspiring a wealth of hypotheses that have been pursued ever since by subsequent workers. Some of the questions that they posed were particular to these systems: What makes magnocellular neurons osmosensitive? Why is oxytocin released in response to osmotic stimulation? Many others were questions of general significance: Why do cells fire in bursts, and what are the mechanisms that underlie bursting? What mechanisms allow bursts to be synchronized? Why is it important that hormones are released in pulses? How can a peptide change the pattern in which cells fire? How is peptide release in the brain regulated? The mechanistic understanding that flowed from pursuit of the answers to these and related questions has changed our understanding of the brain in profound ways. Most neurons in the brain release one or more peptide messengers in addition to classical neurotransmitters, and these have a myriad of autocrine, paracrine, neuromodulatory and neurohormonal actions.

The ability to reliably identify neurons is critically important for electrophysiological studies *in vivo*: the brain is a large and diverse community, and adjacent neurons often have disparate functions, and this is especially true in the hypothalamus. In the paraventricular nucleus, for example, many different neuronal populations jostle together in distressing disharmony: there are magnocellular oxytocin neurons and vasopressin neurons like those in the supraoptic nucleus, but also populations of centrally projecting oxytocin and vasopressin neurons, neuroendocrine neurons that release thyrotropin-releasing hormone and corticotropin-releasing hormone, pre-autonomic neurons and interneurons. The supraoptic nucleus by contrast is wonderfully homogeneous, but at the margins there is still

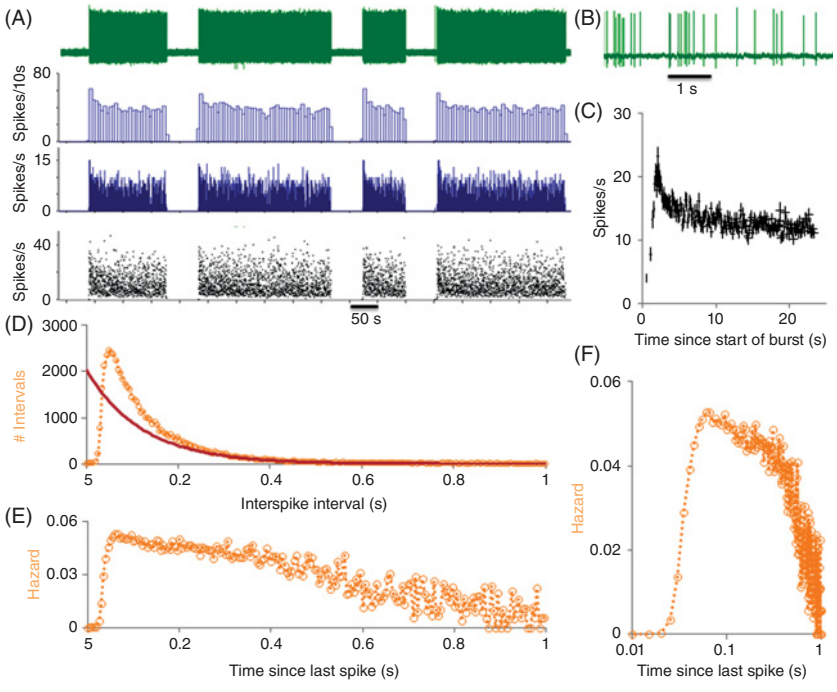

Figure 1.4 Phasic cells in the supraoptic nucleus. (A) Top, in green, the raw voltage trace of an extracellular recording of a phasically firing neuron. Below, in blue, the rate records in 10-s bins and in 1-s bins, and the instantaneous firing rate record, in black, which plots the reciprocals of the interspike intervals. Note that the apparent regularity of discharge in the 10-s bin ratemeter record is belied by the considerable variability of the instantaneous firing rate. (B) An expansion of the raw voltage trace displaying this irregularity. (C) Despite the irregularity of discharge on a short timescale, the phasic bursts have a very consistent structure, shown by the average shape of the start of bursts from the cell in B. Sixty-five successive bursts were analyzed, and the data show the mean arrival times of the first 200 spikes of each burst measured from the first spike in each burst, plotted against the mean instantaneous frequency (the crosses are the standard errors). (D) The interspike interval distribution for this cell; the red line is a single negative exponential fitted to data for intervals >300 ms (r^2 (0.95); note that shorter intervals lie above this line. (E) The corresponding hazard function: the hazard rises to a maximum at 60 ms and declines thereafter. This shape suggests that spike activity within a burst is strongly influenced by a sequence of spike-dependent hyperpolarizations and depolarizations, as expected from the superimposed influences of a large, transient HAP and a small, slower DAP. In addition, as shown in (F), where the hazard function is plotted on a log scale, there is a precipitous decline in hazard for intervals >500 ms. This reflects the fact that generally, phasic bursts contain few or no intervals exceeding 500 ms.

intermingling of magnocellular neurons and other non-neuroendocrine neurons. In hypothalamic slice preparations, the outline of the supraoptic nucleus is clearly visible: but reference to a brain atlas will make it clear that, in a 400-μm-thick coronal brain slice, it is difficult to find any orthogonal path for a recording electrode that will ensure that it encounters only magnocellular neurons. With the benefit of knowledge accumulated by many workers over many years, it is easy to find published examples of "supraoptic" neurons recorded *in vitro* that are, in hindsight, almost certainly not magnocellular neurons. Techniques for electrophysiological identification of magnocellular neurons *in vitro* are now more refined, and identification can be confirmed *post hoc* by filling cells and using immunohistochemistry. Thus far, intracellular recording of magnocellular neurons *in vivo* has proved so difficult that few have attempted it. There are approaches that enable extracellularly recorded neurons to be individually filled with dye (juxtacellular labeling)—but so far nothing has been published from these approaches for magnocellular neurons.

Accordingly, contemporaneous identification of magnocellular neurons *in vivo* requires precise placement of a stimulating electrode on the neural stalk. There are two ways of achieving this reliably. The first is that taken by Wakerley and Lincoln: they recorded from lactating rats, and in this preparation, a stimulating electrode sterotactically placed on the neural stalk from a dorsal approach will trigger secretion of a bolus of oxytocin that causes a sharp rise in intramammary pressure. Such confirmation of accurate placement is essential, but it restricts studies to studies of neurons during lactation. The alternative is to directly expose the neural stalk and supraoptic nucleus by transpharyngeal (ventral) surgery. Using this approach, we can record from a single identified cell for several hours, allowing repeated testing with drugs applied either intravenously (i.v.) or i.c.v. to either the third ventricle or (with a cannula implanted dorsally) to a lateral ventricle. It also is compatible with simultaneous microdialysis of the nucleus, which allows one to administer drugs directly to the nucleus or to collect samples for measurement of dendritic peptide release, and can be combined with electrical stimulation of afferent pathways. The ventral approach, however, is not compatible with the milk-ejection reflex.

1.3 The milk-ejection reflex

When pups suckle, magnocellular oxytocin cells in the maternal hypothalamus discharge a burst of spikes every 5–10 min (Figure 1.1), resulting in an abrupt milk let-down. Each burst lasts just 1–3 s, and every oxytocin cell will burst within about 500 ms of one another. Peak instantaneous firing rates are attained within a few spikes from the onset, and can

briefly reach 200 Hz (instantaneous frequency; bursts typically contain 50–100 spikes in 0.5–1.5 s), with interspike intervals of 5–10 ms. Between bursts, interspike intervals of <30 ms are almost never seen, marking out the burst discharge as wholly exceptional. After the burst, the cells typically fall silent for several seconds before they resume normal levels of spontaneous activity (Figure 1.3). The mechanisms underlying the generation and synchronization of bursts are now reasonably well understood, and involve dendro-dendritic communication between the magnocellular neurons.

Suckling releases oxytocin from the magnocellular neuron dendrites (Figure 1.1), this depolarizes the oxytocin cells and triggers Ca^{2+} release from intracellular stores, and these effects stimulate even more oxytocin release. After i.c.v. injection of as little as 1 ng of oxytocin, the bursts are more frequent and more intense, while i.c.v. injections of oxytocin antagonists, or microinjection into just one supraoptic nucleus, have the opposite effect—they block the reflex. Thus, the reflex depends upon endogenous oxytocin release from magnocellular neuron dendrites, making this the clearest known example of an essential role for a neuropeptide, and one of the few neurobiological examples of positive feedback.

1.3.1 Vasopressin cells and phasic firing

In response to dehydration or osmotic stimuli, and as a consequence of both of their intrinsic osmosensitivity and increased synaptic input, many vasopressin cells fire in a distinctive phasic pattern, with bursts mostly lasting 15–60 s and separated by silent periods mostly of 15–40 s, and with an intraburst firing rate of typically 4–10 Hz (Figure 1.4). Not all vasopressin cells fire phasically—in many conditions, most fire continuously, but it seems that many (and possibly all) can and do exhibit this mode of firing in some circumstances, such as dehydration. This pattern has attracted considerable attention; many neurons fire in bursts of spikes, but few have a bursting period as long as that of vasopressin cells. Much of what we know about the mechanisms underlying phasic firing has come from studies *in vitro*, but it is important to note that while phasic firing is observable *in vitro*, there are important differences between *in vitro* and *in vivo* phasic firing, differences that apparently arise from the fact that *in vitro* preparations are largely deafferented (Sabatier *et al.*, 2004). The loss of synaptic input has several consequences: many vasopressin cells in slice preparations (see Armstrong *et al.*, 2010) are silent unless the cells are exposed to extrinsic depolarization, and their input resistance is much higher because fewer ligand-gated ion channels are open. The elevated input resistance alters passive membrane properties, and exaggerates voltage changes that arise in response to imposed conductance changes; voltage-dependent conductances are likely larger and slower *in vitro* than *in vivo*. However, the

basic burst generating mechanisms are intact. If vasopressin cells *in vitro* are maintained at a resting potential close to spike threshold, then spikes will be followed by a depolarizing afterpotential (DAP) that triggers further spikes—thus spiking is regenerative *in vitro* (in slice preparations), and sustains a plateau potential, with bursts characterized by relatively regular spike discharge. As the burst progresses, the DAP is inactivated, terminating the burst. However, *in vivo*, spiking is not regenerative, but depends on afferent input. Fluctuations in excitatory input trigger spikes randomly, and the post-spike DAP enhances the probability of excitatory postsynaptic potentials (EPSPs) triggering spikes, and accordingly, spiking within bursts is very stochastic.

A curious feature of phasic cells is that they act as bistable oscillators—they have two stable states, an active state and a quiescent state, and small perturbations can flip a cell from either state into the other. Thus the same transient stimulus can either activate a phasic cell if it is silent or inhibit it if it is active (Figure 1.5). The significance of phasic firing thus lies not in the

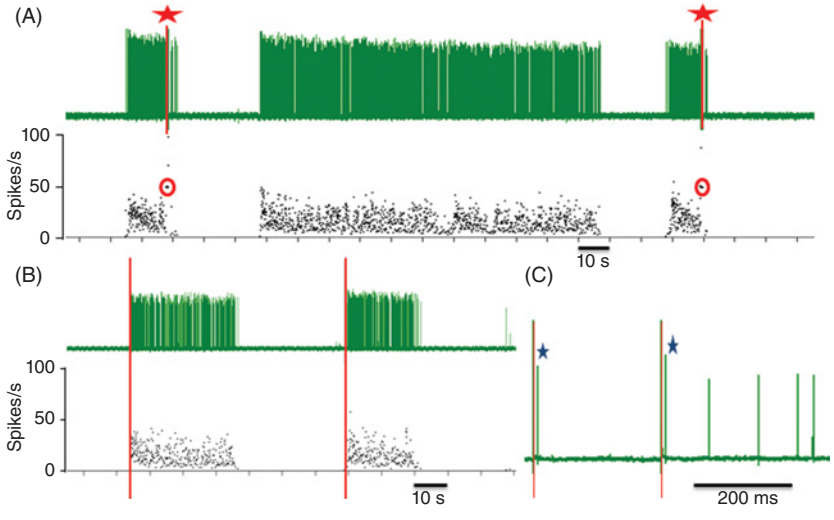

Figure 1.5 Vasopressin cells as bistable oscillators. Extracellular recording of a phasic neuron from the supraoptic nucleus of a urethane-anesthetized rat: voltage traces are shown in green above instantaneous frequency plots. Stimuli applied to the neural stalk evoke antidromic spikes that invade the cell bodies of the magnocellular supraoptic neurons. In (A), antidromic stimuli were applied during the bursts (red stars, lines and circles). Short trains of stimuli at 50 Hz were applied—note how the bursts are arrested, after a brief delay. In (B), stimuli were applied during the silent periods between bursts (red lines)—note how just two stimuli trigger bursts of activity. (C) Expansion of the record of the first episode of stimulation shown in (B); the blue stars mark the antidromic spikes evoked by each of two stimulus pulses, the artifacts from which are overlain by the red lines.

phasic patterning of information—this patterning is lost in the output from the pituitary gland because phasic cells discharge asynchronously. Instead it appears that phasic firing optimizes the efficiency of stimulus–secretion coupling at the nerve terminals.

1.4 Osmotic responses

Both oxytocin and vasopressin cells are excited by increased plasma osmotic pressure (and equally strongly, so this alone cannot distinguish vasopressin cells from oxytocin cells). An early study of Brimble and Dyball (1977) used intraperitoneal (i.p.) injection of hypertonic saline (1 mL of 1.5 M NaCl) as a standard stimulus, which raises plasma osmotic pressure by ~12 mOsm/kg over about 20 min; as the kidneys are nonfunctional under urethane anesthesia, this is a maintained increase. A problem is that i.p. injections can activate pain pathways, and there is often an initial transient response to i.p. injections that seems to be independent of plasma osmotic pressure. A better alternative is therefore slow i.v. infusion. When hypertonic saline is given in this way, there is an initial step rise in plasma sodium concentration depending on the concentration and rate of infusion, and thereafter, plasma sodium concentration rises linearly while the infusion continues. Thus in Leng *et al.* (2001), after infusion of 4.3 mL of 1 M NaCl over 60 min in urethane-anesthetized rats, plasma $[Na^+]$ increased from 146 to 165 mM, and plasma osmolality from 296 to 334 mOsm/L. Plasma $[K^+]$ also rose (from 3.3 to 4 mM), consistent with extensive cell shrinkage and passage of intracellular electrolytes into the extracellular fluid compartment. Hematocrit fell from 44.5% to 40%, consistent with an 11% increase in plasma volume.

1.5 Responses to other stimuli

While both oxytocin and vasopressin cells are activated by osmotic stimuli, two other stimuli have been used extensively to discriminate between them *in vivo*:

1 If 10 μg phenylephrine is injected i.v. (in 0.1 mL physiological saline), it produces a large (40–60 mm Hg) and abrupt transient increase in blood pressure. This will interrupt the firing of a phasic cell if applied in mid-burst, but has little effect upon most continuously firing cells (Harris *et al.*, 1975). In some continuously active cells, however, baroreceptor stimulation will interrupt the activity for ~20 s, and activity resumes with the abrupt onset typical of phasic bursts. This has led some authors to use baroreceptor stimulation for discriminating oxytocin cells from

vasopressin cells, but this stimulus has not been systematically studied in cells identified by the milk-ejection reflex. Furthermore, in the rat, vasopressin cells are relatively insensitive to baroreceptor stimulation. A large rise in blood pressure (over 50 mm Hg) must be induced, and even for phasic cells, the response may be unreliable: if baroreceptor stimulation is applied at the beginning of a burst it may fail to interrupt the burst. Nonetheless, baroreceptor stimulation may be useful in recognizing some vasopressin cells that are active but which do not exhibit phasic firing. This baroreceptor pathway is thought to involve GABA as a final inhibitory transmitter from neurons in the perinuclear zone dorsolateral to the supraoptic nucleus, and to involve a projection from the caudal brainstem to the diagonal band of Broca.

2 If cholecystokinin (CCK) is injected i.v. in rats (25 μg/kg in 0.1 mL physiological saline), it will produce a modest increase in the firing rate of oxytocin cells that lasts for 10–15 min (Renaud *et al.*, 1987; Figure 1.6). This increase is accompanied by an increased secretion of oxytocin. Injections of CCK inhibit most vasopressin cells for a similar duration (accompanied by reduced vasopressin secretion). Systemically injected CCK acts at CCK-1 receptors on afferent vagal neurons that innervate the stomach and duodenum, these in turn activate noradrenergic neurons of the A2 cell group in the nucleus tractus solitarii that project directly to magnocellular oxytocin neurons. How the inhibition of vasopressin neurons is mediated is not known.

Both of these stimuli can be given repeatedly with consistent effects. Experience with these stimuli and functional identification with the milk-ejection reflex has led to refined ways of distinguishing between the cell types based on statistical features of their discharge patterning.

Many other stimuli are known to activate magnocellular neurons under urethane anesthesia—but are not so helpful in discriminating between oxytocin cells and vasopressin cells. Secretin for example activates both cell types when injected at very low doses (as little as 1 μg injected i.v.; Figure 1.6). The physiological significance of this is as yet unknown. The response to CCK seems likely to be associated either with the appetite-suppressing effects of centrally released oxytocin, or with the natriuretic effects of peripherally secreted oxytocin: CCK is secreted from the duodenum in response to food intake and triggers satiety.

The ability to identify oxytocin and vasopressin neurons *in vivo* has allowed systematic analysis of the role of afferent pathways in controlling their activity. In the case of the activation of oxytocin neurons by CCK, for example, we now know that this stimulus begins as activation of CCK-1 receptors on afferent vagal nerve endings that innervate the gastrointestinal tract. This pathway relays in the nucleus tractus solitarii of the caudal brainstem, from where noradrenergic neurons of the A2 cell group

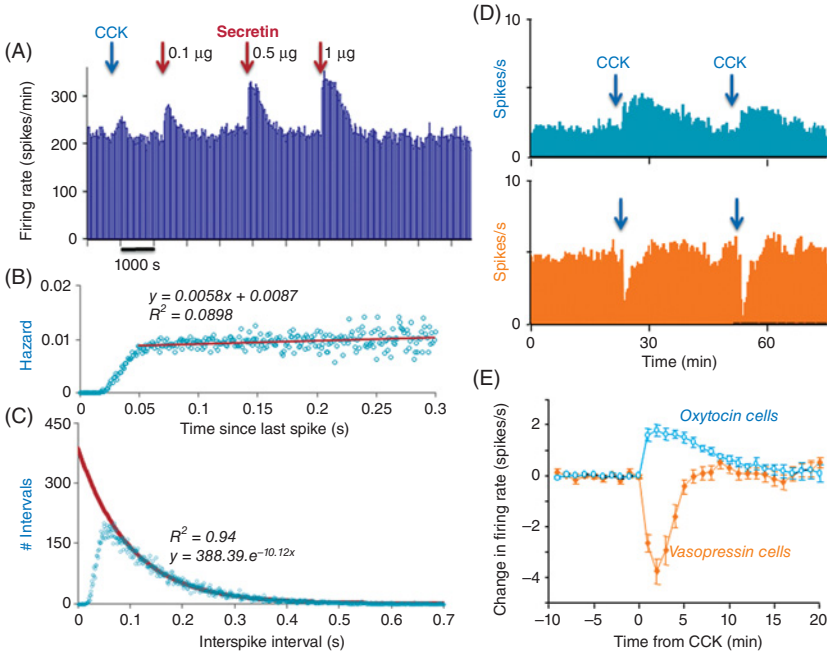

Figure 1.6 Responses of supraoptic neurons to gut-related peptides given i.v. (A) Responses of an oxytocin cell in a urethane-anesthetized rat to oxytocin and secretin given i.v. (from Velmurugan *et al.*, 2010). The excitatory response to cholecystokinin (CCK) is typically small (0.5–1 spikes/s)—larger responses are evoked by secretin, but secretin injections also activate vasopressin cells. (B) The hazard function from the cell shown in A: the function has the shape that is characteristic of oxytocin cells, rising slowly to a relatively constant plateau level after about 50 ms. The plateau level is shown in red as the line of best fit to hazard data from 50 ms onward. (C) The corresponding interspike interval distribution; in this case, the red line represents a single negative exponential fit to intervals >50 ms. (D) Data from a simultaneously recorded oxytocin neuron (in blue) and a continuously active vasopressin neuron (in orange). Two sequential injections of CCK elicited repeatable excitation of the oxytocin cell and inhibition of the vasopressin cell. (E) Averaged responses to CCK of oxytocin cells and continuously active vasopressin cells. Modified from Sabatier *et al.* (2004).

that co-express prolactin-releasing peptide, (and other peptides including enkephalin) project directly to magnocellular oxytocin neurons. This projection is modulated by opioids: μ-opioid receptors are present presynaptically, and retrodialysis of μ-agonists onto the supraoptic nucleus blocks CCK-evoked noradrenaline release at that site (Onaka *et al.*, 1995).

The projections from the caudal brainstem thus mediate gastric-related stimuli as well as cardiovascular stimuli and stimuli arising from the reproductive tract. Projections from anterior regions (the subfornical organ, organum vasculosum of the lamina terminalis (OVLT) and the nucleus