Review

Beyond the wiring diagram: signalling through complex neuromodulator networks

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During the computations performed by the nervous system, its 'wiring diagram'—the map of its neurons and synaptic connections—is dynamically modified and supplemented by multiple actions of neuromodulators that can be so complex that they can be thought of as constituting a biochemical network that combines with the neuronal network to perform the computation. Thus, the neuronal wiring diagram alone is not sufficient to specify, and permit us to understand, the computation that underlies behaviour. Here I review how such modulatory networks operate, the problems that their existence poses for the experimental study and conceptual understanding of the computations performed by the nervous system, and how these problems may perhaps be solved and the computations understood by considering the structural and functional 'logic' of the modulatory networks.

Keywords: neurotransmitter; neuromodulator; neuropeptide; biological system architecture; neural computation; computational modelling

1. BEYOND THE WIRING DIAGRAM

Ideas about how the brain works have always looked for inspiration to the current technology of the era. After the pneumatic, hydraulic and mechanical analogies of earlier centuries, in the twentieth century came electrical and electronic analogies such as the telephone exchange and the computer (Churchland 1986; Kirkland 2002). Fundamental to these latter analogies is the concept of a network of interconnected components, and this remains the standard paradigm for thinking about the nervous system today. The nervous system is seen as composed of interconnected subsystems, which themselves are composed of lower-level subsystems, until at the lowest level there are components, such as individual neurons, that are (from the top-down perspective of the network) relatively simple and stereotyped, but there are very many of them and they are interconnected in a rich, structured way to make a very large, complex network. In such a network, given some understanding of the capabilities of the individual components, the key fact is how the components are connected together— the wiring diagram. The wiring diagram specifies and organizes the computation performed by the network that, in the case of the nervous system, emerges ultimately in the behaviour of the animal. Naturally, this paradigm has motivated an intensive effort to determine the wiring diagrams of nervous systems. We now have high-level wiring diagrams for many parts of the vertebrate, including human, brain (Sporns *et al.* 2004; Grillner & Graybiel 2006; Bullmore &

Sporns 2009), wiring diagrams down to the level of individual neurons for some simple circuits, such as the crustacean stomatogastric ganglion (STG; Harris-Warrick *et al.* 1992*a*), and even the complete wiring diagram for one entire nervous system, that of the nematode worm *Caenorhabditis elegans* (White *et al.* 1986; http://www.wormatlas.org).

In this article, I will not argue that knowledge of the wiring diagram is not required to understand how a nervous system works. I will argue, however, that it is not sufficient—at least, if the wiring diagram is thought of, as it often is, as a relatively static, hard-wired set of neuronal connections. In this article, I will focus on one reason for this insufficiency: the fact that the neuronal wiring diagram is not static, but rather is dynamically modified by multiple actions of neuromodulators that indeed can be so complex that they can be thought of as constituting a biochemical network that combines with the neuronal network to perform the computation. Thus, the neuronal wiring diagram alone is not sufficient to specify, and permit us to understand, the computation that underlies behaviour. Ironically, it is often in the nervous systems whose neuronal wiring diagrams are best known and that therefore might be thought to be closest to being 'solved', such as in the STG, *C. elegans*, and *Drosophila* whose nervous system is being proposed as ripe for definitive 'circuit-cracking' (Olsen & Wilson 2008), where the greatest multiplicity of neuromodulators and their effects has been identified (Harris-Warrick *et al.* 1992*b*; Brownlee & Fairweather 1999; Orchard *et al.* 2001; Skiebe 2001; Nässel 2002; Taghert & Veenstra 2003; Husson *et al.* 2007; Ma *et al.* 2009; Stein 2009). Figure 1, for example, shows, in the now classic diagram of Marder and colleagues, the multiplicity of
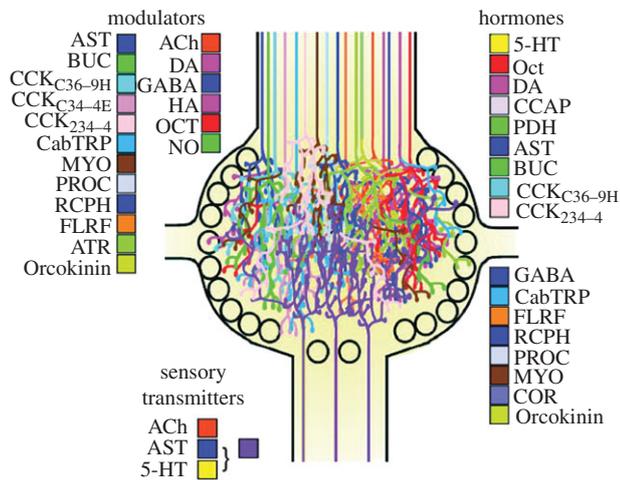
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Figure 1. Multiplicity of neuromodulators identified in the crustacean stomatogastric ganglion. Reproduced with permission from Marder & Bucher (2001).

modulators—probably still a substantial underestimate of the true number (Fu *et al.* 2005; Ma *et al.* 2009)—identified in the STG. That the complexity of the modulatory networks appears greatest in these simple systems may, however, merely reflect the fact that these systems are the most completely characterized genetically, biochemically and physiologically: there are already many indications that similar modulatory complexity exists also in the vertebrate brain (Vizi & Lábos 1991; McCormick 1992; Herbert 1993; Hasselmo 1995; Hökfelt *et al.* 2000: Merighi 2002; Baraban & Tallent 2004; Salio *et al.* 2006; Abbracchio *et al.* 2008).

In this article, I will review how such modulatory networks operate, the problems that their existence poses for the experimental study and conceptual understanding of the computations performed by the nervous system, and how these problems may perhaps be solved and the computations understood by considering the structural and functional 'logic' of the modulatory networks. I will be able to present a sketch only; space limitations force me to refer to reviews rather than the vast primary literature and to omit many details and even substantial subsidiary topics, but with the hope that, if the sketch is clear, the reader will readily see how to complete the picture.

2. MULTIPLICITY OF NEUROMODULATORS

What is a neuromodulator? Neurochemical signalling agents have traditionally been classified primarily by their chemical identity, distinguishing for example between the classical small-molecule neurotransmitters, biogenic amines and neuropeptides. From the systems-level point of view emphasized in this article, however, it is more relevant to distinguish primarily between transmitter and modulator *functions*. Whether a signalling agent functions as a transmitter or a modulator depends ultimately on the spatial and temporal scale on which the agent acts. If the agent acts so locally in both space and time that it merely communicates the current activity of one component of the neuronal network to another component—for example, the fact that a spike has just occurred in a

presynaptic neuron to a postsynaptic neuron—then it is acting as a transmitter that merely implements one of the connections of the neuronal wiring diagram and so, for the purposes of this article, can be subsumed into that diagram. Indeed, such signalling has been termed 'wiring transmission' (Agnati *et al.* 1995; Zoli & Agnati 1996). A modulator, in contrast, acts globally on multiple components, over longer times, or usually both, so that its effects are not directly correlated with the current activity of any particular component or connection of the neuronal network: the modulator does not mediate that activity but rather modifies or *modulates* it. It is this spatial and temporal dissociation from the activity of the neuronal network that is responsible for many of the problems posed by neuromodulation as well as for its computational power.

There is of course considerable correspondence between the traditional chemical classes of signalling agents and this functional classification. In particular, the transmitter and modulator roles are played by certain classes of molecules that are adapted for these roles by the cell biology of their synthesis, storage and release. In the nervous system, the local transmitter role that subserves wiring transmission is played predominantly by classical small-molecule neurotransmitters such as ACh, GABA and glutamate, which signal locally because they are released from neurons by rapid spike-activated presynaptic mechanisms, and then rapidly open postsynaptic ligand-gated ion channels, at discrete, highly organized synapses that are 'closed' in that the transmitter remains confined within them (Zoli & Agnati 1996). The global modulator role, on the other hand, is played predominantly by biogenic amines such as dopamine, norepinephrine, serotonin and histamine, by adenosine and adenine nucleotides (Abbracchio *et al.* 2008; Gourine *et al.* 2009), by neuropeptides (discussed in more detail below), and probably by a number of other classes of molecules (Zoli *et al.* 1999; Barañano *et al.* 2001; Hofer & Lefkimmiatis 2007), the best established of which are gases such as nitric oxide (NO; Barañano *et al.* 2001; Guix *et al.* 2005). These agents signal globally because they are released, from both neurons and glia, more slowly at less organized, 'open' synapses or indeed non-synaptic sites from which they are then able to diffuse for long times over long distances through the intercellular space, or travel even longer distances as neurohormones through the circulatory system, in what has been termed 'volume transmission' (Agnati *et al.* 1995; Stjärne & Stjärne 1995; Zoli & Agnati 1996; Zoli *et al.* 1999). These agents then furthermore exert inherently slow actions through metabotropic receptors coupled to second-messenger cascades and other slow intracellular signalling processes. Perhaps the most extreme case of such global volume signalling is that of the gaseous NO, which passes through membranes and so can diffuse from the interior of one cell to directly modulate intracellular processes throughout the entire volume of surrounding tissue, largely irrespective of cellular boundaries.

The accumulating evidence has, however, also revealed considerable overlap in all these properties.
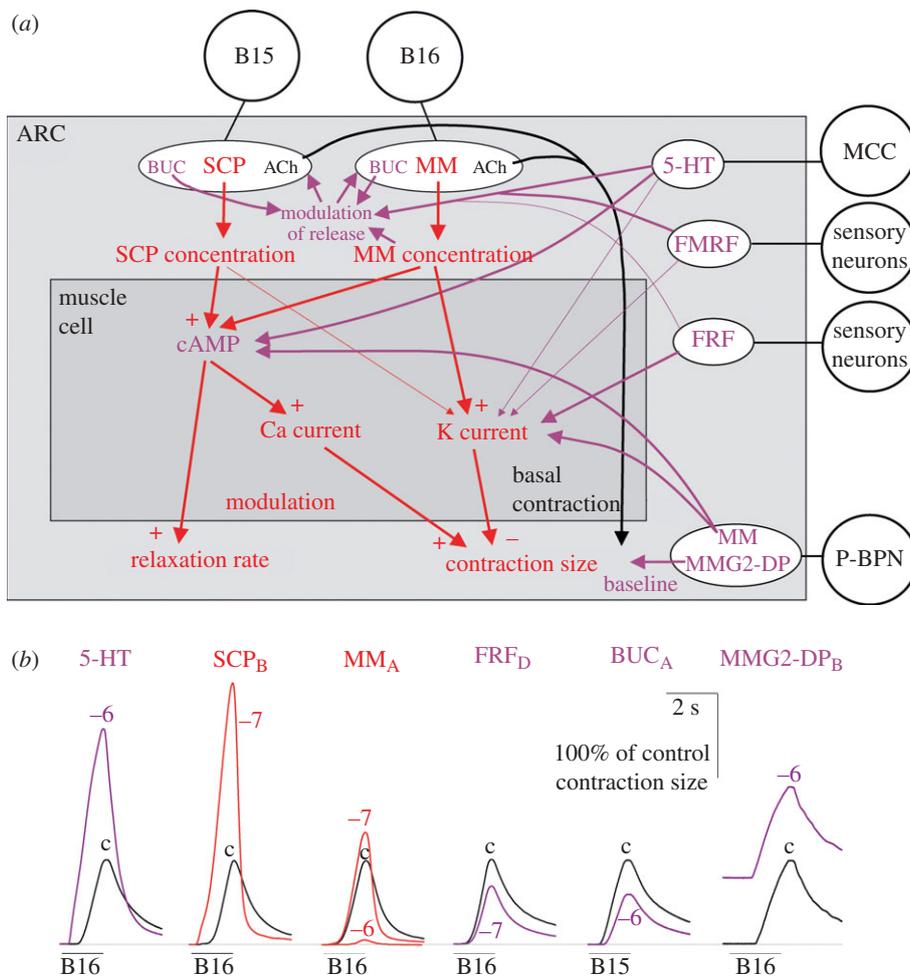
Figure 2. Network of modulatory actions that shapes the contractions of the ARC muscle of *Aplysia*. (*a*) Schema of the ARC muscle (grey box), its two motorneurons B15 and B16 (circles at top) that release ACh to elicit the basal contractions of the muscle (black arrow) but also modulatory neuropeptides belonging to the SCP, MM and buccalin (BUC) families, other neurons (circles down right-hand side) that release additional modulators, and the network of the principal modulatory actions (coloured arrows). Thick arrows denote strong actions, thin arrows weaker actions. The subnetwork analysed in figure 3 is shown in red, the rest of the modulatory network in purple. Modified from Brezina *et al.* (2003*a*) and based in the first instance on the body of previous work summarized there, then brought up to date with the more recent findings of Orekhova *et al.* (2003), Proekt *et al.* (2005) and Vilim *et al.* (2010). (*b*) The diversity of ARC muscle contraction shapes produced by the various modulators. Representative contractions elicited *in vitro* by firing of either motor neuron B15 or B16 under control conditions ('c') and after exogenous application of 10^{-7} or 10^{-6} M ('− 7', '− 6') of the modulators. Modified from Hooper *et al.* (1999), with the MMG2-DP traces taken from an experiment of Proekt *et al.* (2005). Abbreviations: MMG2-DP, myomodulin gene 2-derived peptides; FMRF, FMRFamide peptides; FRF, FRFamide peptides; 5-HT, serotonin; MCC, metacerebral cells; P-BPN, pedal–buccal projecting neurons.

In addition to acting through metabotropic receptors, serotonin, the adenine nucleotides, and even some neuropeptides (Lingueglia *et al.* 1995) open ligand-gated channels. ATP often has the hallmarks of a local transmitter, even being coreleased from the same synaptic vesicles as a classical transmitter such as ACh or GABA (Abbracchio *et al.* 2008; Zimmermann 2008). In many invertebrates, dopamine, histamine and serotonin function prominently as local transmitters (Gerschenfeld 1973). Conversely, there is abundant evidence that, upon release even at 'closed' synapses, the classical transmitters not only rapidly open ligand-gated channels but also exert parallel but slower modulatory actions through metabotropic receptors (see numerous examples in Katz 1999). Furthermore, 'closed' synapses can become 'open' through transmitter spillover at times of high release or reduced strength of the mechanisms that

confine the transmitter within the synapse, so that the released transmitter acquires a very different spatio-temporal profile and can reach additional receptors that may mediate additional, modulatory actions (Agnati *et al.* 1995; Zoli & Agnati 1996). The overall picture emerges not of an absolute differentiation between transmitters and modulators but rather of a continuum between the transmitter and modulator functions, along which the signalling agents occupy various, often multiple, positions that can shift dynamically depending on the circumstances. Furthermore, as will be seen in the rest of this article, the transmitter and modulator functions are intimately intertwined at the points of contact between the neuronal and modulatory networks.

The neuropeptides (Strand 1999; Hökfelt *et al.* 2000; Skiebe 2001; Nässel 2002, 2009; Baraban & Tallent 2004; Landgraf & Neumann 2004; Salio

et al. 2006) have a special place in this account because, in addition to being quintessential modulators in the sense just described, they are so diverse. Whereas there are only a few molecular species of the other modulators, there is a seemingly infinite variety of neuropeptide amino acid sequences (e.g. Kobayashi & Muneoka 1990; Schoofs *et al.* 1997; Brownlee & Fairweather 1999; Orchard *et al.* 2001; Taghert & Veenstra 2003; Boonen *et al.* 2009). The combinatorial nature of the neuropeptide structure and its linear encoding in the genome, then operated on by the evolutionary processes of coding-sequence and gene duplication followed by divergence under relaxed evolutionary pressure (Niall 1982; Strand 1999), has led to the encoding of multiple variant neuropeptide sequences in most neuropeptide genes, and multiple genes encoding neuropeptides that are closely as well as more distantly related in the genome. The complexity of the actual neuropeptidergic signalling is (fortunately) somewhat reduced by the fact that all of the neuropeptides synthesized by a neuron are usually copackaged into the same exocytotic vesicles and coreleased. Selective sorting of the neuropeptides for selective release from different processes of the same neuron is known, however (Sossin & Scheller 1991; Perone *et al.* 1997). Furthermore, different neurons release different complements of the neuropeptides, thanks to such mechanisms as alternative splicing of the neuropeptide mRNAs (Buck *et al.* 1987; Benjamin & Burke 1994; Perone *et al.* 1997) and, of course, the transcription of different subsets of the neuropeptide genes. It is now clear that very many neurons—in some nervous systems, seemingly all neurons—release some complement of neuropeptides as cotransmitters alongside their 'primary' classical transmitter (Kupfermann 1991; Maggi 1995; Lundberg 1996; Hökfelt *et al.* 2000; Merighi 2002). Strikingly illustrating the cell-biological basis of the transmitter–modulator distinction, the large dense-core vesicles that contain the neuropeptides are typically segregated, even within the same synaptic terminals, away from the small clear vesicles that contain the classical transmitter (Merighi 2002; Salio *et al.* 2006), to sites from which the neuropeptides are released more diffusely by smaller, slower elevations of intracellular Ca^{2+} (Verhage *et al.* 1994), so that the release of the neuropeptides and the classical transmitter can occur to some degree independently, for example in response to different firing patterns of the neuron. Altogether, it is the diversity of the neuropeptides that accounts for much of the modulator multiplicity in a system such as the crustacean STG (figure 1). The most recent study of a representative crustacean 'neuropeptidome' has identified 142 neuropeptides belonging to 17 different families (Ma *et al.* 2009).

3. MULTIPLICITY OF NEUROMODULATORY ACTIONS

An immense amount of data has accumulated to show what the modulators can do. The standard experimental paradigm to gather such data is to exogenously apply a particular modulator and observe the

perturbation of a particular measured variable in the system. It is then assumed that the modulator, if released endogenously within the system, is at least capable of exerting the same action. A fair summary of the data gathered over the past several decades with this approach is that modulators are capable of modulating essentially every variable, every process, at all levels of organization of the nervous system. Perturbation can be observed of the activities of the molecules and pathways of gene expression, intermediary metabolism and intracellular signal transduction in both neurons and glial cells, of essentially every type of membrane ion channel, transmission at many synapses, and the electrical behaviour of neurons and neuronal circuits of all kinds (numerous examples can be found in Lundberg 1996; Katz 1999; Marder & Thirumalai 2002; Dickinson 2006; Abbracchio *et al.* 2008). Moreover, the nervous system is very *dense* with these modulatory actions. Examining just a single synapse, for instance, we observe modulation of the propagation of action potentials into the presynaptic terminal, of multiple presynaptic ion channels, of the presynaptic Ca^{2+} concentration, of the molecular machinery of transmitter release and the actual release of the classical transmitter as well as any modulatory cotransmitters, then of multiple postsynaptic ion channels and the postsynaptic electrical properties that govern the generation of a postsynaptic action potential, and so, overall, of virtually every aspect of the transmission of information through the synapse (Vizi & Lábos 1991; Lundberg 1996; Marder & Thirumalai 2002).

One experimental system in which the issues raised in this article have been particularly well investigated, and which, along with the crustacean STG, I shall discuss as an example, is the feeding system of the sea slug *Aplysia* (Kupfermann 1974; Weiss *et al.* 1993; Hooper *et al.* 1999). In this system, although much is now known also about the modulation of the central pattern generator (CPG) that drives the rhythmic feeding movements (e.g. Fujisawa *et al.* 1999; Furukawa *et al.* 2001; Sweedler *et al.* 2002; Proekt *et al.* 2005; Vilim *et al.* 2010), the best understood modulation is in the periphery of the neuromuscular plant that executes the movements. Figure 2a shows a schema of the modulation in a representative muscle, the accessory radula closer (ARC) muscle. Numerous modulators, in particular neuropeptides belonging to the small cardioactive peptide (SCP), myomodulin (MM), buccalin, FMRFamide and other families, are released as cotransmitters from the muscle's two motorneurons, B15 and B16, and from additional modulatory and even sensory neurons (for details and references see the figure legend). These modulators then act presynaptically to modulate the release of the motorneurons' primary, contraction-inducing transmitter, ACh, as well as of the modulatory cotransmitters—in some cases, therefore, of themselves through a feedback loop—and postsynaptically on the muscle cells to modulate their intracellular second-messenger systems, membrane ion channels and ultimately multiple parameters—baseline, latency, amplitude, relaxation rate—of the shape of the muscle's contractions

(figure 2a,b). This example illustrates furthermore how the modulatory events in the nervous system join seamlessly with, and for the purposes of this article are no different from, those in the other tissues with which the nervous system interacts and indeed most other tissues of the body (see also Furness *et al.* 1992; Maggi 1995; Stjärne & Stjärne 1995; Lundberg 1996; Hooper *et al.* 1999; Gourine *et al.* 2009).

4. THE NEUROMODULATORY NETWORK

Figure 2a exhibits a very characteristic feature of modulatory pathways: their extensive divergence and convergence (Brezina & Weiss 1997). Each modulator exerts multiple actions, and the same action is exerted by multiple modulators, and similarly at all levels of the modulatory system so that it forms a single, richly interconnected network, often with multiple feedback loops. As a result, a perturbation at any point in the network—the release of a single modulator, for example—will ramify throughout the network to perturb many of its components.

The modulatory network therefore has a structure, with its own 'wiring diagram', that is no less complex than that of the neuronal network in the same region of nervous tissue. In important respects, it is more complex. Whereas the discrete nodes and arrows of the network diagram constitute a natural representation of the discrete neurons and synaptic connections of the actual neuronal network, they constitute only a very abstract representation of the actual events in the modulatory network, which are inescapably continuous in three-dimensional space as well as time. More realistically, we should picture cocktails of multiple modulators released from many neurons, diffusing through the extracellular space over different distances to create spatio-temporal concentration profiles that are highly dependent on the dynamics of the release and the particular geometry of the tissue, and then exerting their many actions on a multiplicity of time-scales, all this dynamically changing with different patterns of neuronal firing and the state of the nervous system (Furness *et al.* 1992; Stjärne & Stjärne 1995; Zoli & Agnati 1996; Zoli *et al.* 1999; Gourine *et al.* 2009; Nässel 2009).

The neuronal and modulatory networks, moreover, are intimately coupled through their myriad interactions so that information flows repeatedly from one network to the other and back again. Thus, the neuronal firing that constitutes the activity of the neuronal network typically leads to the release of the neurons' modulatory cotransmitters—these are therefore *intrinsic* modulators in the terminology of Katz & Frost (1996)—that modulate, in turn, that firing. At synapses, even phenomena such as classical synaptic plasticity (Fisher *et al.* 1997; Zucker & Regehr 2002) that normally are interpreted as inherent to the release of the classical transmitter itself, and so part of the intrinsic dynamics of the neuronal network, may actually be due in some cases to slow modulatory actions of coreleased neuropeptides (Fox & Lloyd 2001; Koh & Weiss 2005). In the crustacean STG, modulatory neurons projecting from other ganglia (coloured lines leading to the STG in figure 1) release modulators that initiate and maintain the rhythmic motor patterns that are produced by the neuronal STG network, and those patterns, in turn, impose a rhythmicity also on the modulator release from the projection neuron terminals (Nusbaum *et al.* 2001; Nusbaum 2002). Altogether, it is through this intimate coupling of the neuronal and modulatory networks that the application of a modulator can affect seemingly any component not only of the modulatory but also the neuronal network, as is observed.

5. PROBLEMS POSED BY NEUROMODULATION

The existence of the modulatory network and its coupling to the neuronal network poses a number of problems for our attempts to investigate and understand how the nervous system performs its computations, indeed what computations it performs.

First, we have very imperfect knowledge of what modulators are contained in the cocktail of modulators that acts at any point in the nervous system. With the neuropeptides in particular, when each neuron can express its own individual complement of multiple neuropeptides, to map this expression comprehensively is a heroic task. Even in such a well-explored system as the *Aplysia* feeding system, entire new neuropeptide families continue to be discovered (Fujisawa *et al.* 1999; Furukawa *et al.* 2001; Sweedler *et al.* 2002; Proekt *et al.* 2005; Vilim *et al.* 2010). The issue is complicated by the fact that, like all biological systems, the modulatory system is a contingently evolved system that in some respects is bound to be far from optimized, and, as already mentioned, the neuropeptides in particular pay little penalty for multiplication and structural diversification. Thus, it is not surprising that some neuropeptides that are expressed and released into the cocktail appear to be redundant, or more properly degenerate (Edelman & Gally 2001), variants (e.g. Brezina *et al.* 1995; Hewes *et al.* 1998) or even appear inactive (Bowers 1994). It is difficult to be sure that they really are so, of course, when the modulatory actions, too, are only imperfectly understood.

The modulatory actions are imperfectly understood because not only the modulators but also the rest of the modulatory network is known only in part, with many additional network nodes remaining unidentified and many potential connections untested. However, it is also because the standard experimental paradigm, mentioned above, that uses exogenous modulator application to identify the modulatory actions is grossly inadequate. Typically, only one modulator, at a series of concentrations, is applied at a time. Mixtures of even just two or three modulators—when the real physiological cocktail may well contain dozens!—have been systematically tested only in a handful of studies (Wood 1995; Brezina *et al.* 1996; Dickinson *et al.* 1997; Djokaj *et al.* 2001; Mesce *et al.* 2001; Svensson *et al.* 2001). Mixtures of more than three modulators have not been studied because, even when the identities of more modulators are known, their physiologically relevant concentrations are not known, and systematic testing of all possible combinations of concentrations presents an experimentally insuperable combinatorial problem.
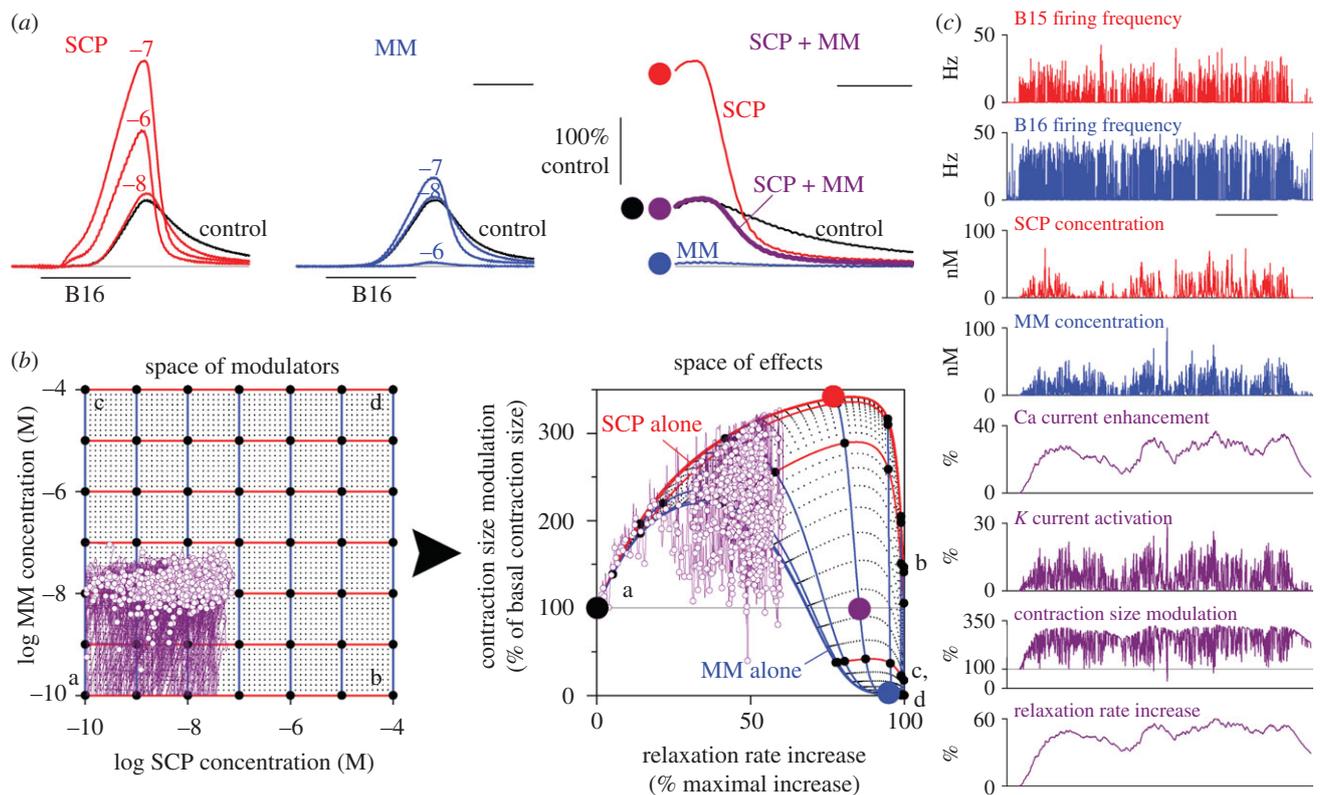
Figure 3. Analysis of the actions of modulator combinations in the *Aplysia* ARC muscle system. This partial analysis considers the combinatorial action of just two modulators, SCP released from motor neuron B15 and MM released from motor neuron B16, through the subnetwork of actions shown in red in figure 2*a*, on just two parameters, the size and the relaxation rate, of the ARC muscle contractions. (*a*) Representative ARC muscle contraction shapes, recorded as in figure 2*b*, produced by various exogenously applied steady concentrations of SCP alone (red), MM alone (blue) and both SCP and MM applied together (purple; the peak and relaxation phase of just one contraction is shown; scale bar 1 s, 500 ms). (*b*) Mapping from the space of all combinations of SCP and MM concentrations (over the range from 10^{-10} to 10^{-4} M) to the space of all combinations of the effects on contraction size and relaxation rate. The grid of small dots in the two spaces shows the steady-state mapping generated by the computational model of Brezina *et al.* (1996, 2003*a*). The larger black dots and blue and red curves show how the steady-state mapping is traversed as the SCP concentration varies in the presence of constant MM concentration (red curves) or the converse (blue curves); the letters 'a'–'d' identify the mapping of the four corners of the modulator space for orientation. The large black, red, blue and purple dots mark the approximate locations of the contraction shapes in (*a*), right. The purple overlay then shows the dynamical trajectory through the modulator and effect spaces of the entire meal modelled in (*c*), in the modulator space plotting against each other the SCP and MM concentration waveforms shown in (*c*) and in the effect space the contraction size and relaxation rate waveforms, plotting either the entire continuous waveforms (thin purple curve) or just their values at the end of the retraction phase of each feeding cycle (small purple circles). (*c*) Modelled waveforms of the principal variables during an entire meal eaten by a real *Aplysia in vivo*, comprising 749 feeding cycles over approximately 2.5 h, during which the firing frequencies of the motorneurons B15 and B16 (top two waveforms) were recorded with chronically implanted electrodes by Horn *et al.* (2004) and were then used by Brezina *et al.* (2005) to drive the computational model of Brezina *et al.* (2003*a*). Scale bar, 30 min. (*a*,*b*) modified from Brezina *et al.* (2003*a*) and (*c*) from Brezina *et al.* (2005).

However, those few studies that have tested two modulators have invariably found not only strong concentration-dependence of their actions but strong nonlinearities in their interactions, so that the actions of either modulator in the presence of the other were often very different from its actions alone (see further below). This implies that adding another modulator to the studied mixture, or perhaps in the future discovering a novel modulator in the system, may change all of the results obtained so far using this paradigm. The finding that a modulator *can* exert a certain action in such experiments does not mean that it *does* exert that action in the real system.

Yet a further problem with the standard paradigm is that the exogenous modulator application, even at the real *average* concentration, does not reproduce

the complex real spatio-temporal concentration profile that can be critical for the proper expression of the modulator actions (Brezina *et al.* 1997). Rhythmic oscillations of concentration, for example, are required in the crustacean STG (Nusbaum 2002) and for the actions of a number of vertebrate neuropeptide hormones (Strand 1999). Similarly in the spatial domain, the precise distribution of the modulator in the tissue can have profound functional and computational consequences (Stjärne & Stjärne 1995; Nässel 2009), as has been modelled for NO signalling in different geometries of NO sources and targets (Ott *et al.* 2007) and reported, once more, in the STG where the sculpting of concentration profiles by extracellular peptidases so as to restrict the released modulatory neuropeptides to only one or another

part of the ganglion appears to play a significant functional role (Nusbaum 2002; Wood & Nusbaum 2002; Stein 2009).

Finally, by modifying the electrical properties of the neurons in the neuronal network and their synaptic connections, neuromodulators can profoundly change the functional—as opposed to the anatomical—connectivity of the neuronal network (Dickinson 2006). The first and still the best example is again provided by the crustacean STG, where the different modulators can variously reconfigure the STG neurons into different functional assemblies that generate different motor patterns (Dickinson & Moulins 1992; Nusbaum *et al.* 2001; Scholz *et al.* 2001; Skiebe 2001; Stein 2009). Thus, until the modulation is understood, even the basic neuronal wiring diagram, apparently so hard-wired and well defined, becomes uncertain.

6. COMPUTATION PERFORMED BY THE NEUROMODULATORY NETWORK

Why is the neuromodulatory system so complex? A more tractable subsidiary question that has often been asked (Segal 1983; Bloom 1984; Kupfermann 1991; Marder *et al.* 1995; Brezina & Weiss 1997) is, why are there so many neuromodulators? The answer has usually been sought by assuming that the structure of the modulatory system has a functional logic that aims, fundamentally, to provide a separate, unique addressability and controllability of each of the systems' 'outputs'—the various modulator actions, or sometimes the neurons of the coupled neuronal system—by the 'input' modulators. Since there are many outputs, and, in contrast to the anatomically labelled lines of the neuronal wiring diagram, the modulators are all mixed together in the modulator cocktail, there must then be at least as many input modulators as output modulator actions (Brezina & Weiss 1997). Such a system could in principle consist of chemically labelled 'signalling wires' (Weng *et al.* 1999) of one-to-one mapping of modulators to actions; in such a system, we could speak of *the* function of each modulator. As already discussed, however, all real modulatory systems are divergent–convergent networks in which each modulator exerts many actions, and each action is exerted by many modulators. Unique addressability and controllability can still be achieved, nevertheless, in a combinatorial manner, no longer by single modulators but by the cocktail of all of the modulators controlling simultaneously the ensemble of all of the actions. It is then no longer meaningful to attribute any specific function to any modulator alone (Brezina & Weiss 1997; Olypher & Calabrese 2007; Boonen *et al.* 2009).

Figure 3 summarizes an analysis along these lines of the core of the peripheral modulatory network in the *Aplysia* feeding system, specifically of the subnetwork shown in red in figure 2, of just two of the modulators, SCP and MM, released from the motorneurons B15 and B16, respectively, then each modulating both the amplitude and the relaxation rate of the ARC muscle's contractions. Figure 3b shows the mapping, generated by a realistic mathematical model of the subnetwork,

of the space of all possible combinations of the concentrations of SCP and MM to the space of all possible magnitudes of the two effects. Note how in combination, although not either one alone, SCP and MM uniquely address and control a large region of the effect space. Note also the nonlinearity of the mapping and how the addition of SCP changes the effect of MM and vice versa (Brezina *et al.* 1996). As already noted, of course, this implies that the entire mapping might be changed by the addition of yet a third modulator, several candidates for which certainly exist in the system (figure 2a). Figure 3a shows experimental confirmation of the mathematical analysis that indeed combinations of the effects on contraction amplitude and relaxation rate can be achieved by the combinations of SCP and MM that cannot be achieved by SCP or MM alone. Generalizing to higher dimensions, it is tempting to speculate that the additional modulators in the system offer the possibility of simultaneously controlling even more of the features of the contractions (figure 2b).

Similar combinatorial thinking, although overlaid by many details, informs also the work on the crustacean STG, where the different modulatory neuropeptides released from different projection neurons both diverge and converge to act on partially overlapping complements of ion currents, synaptic connections and STG neurons so as to sculpt a number of multidimensional motor patterns (Nusbaum *et al.* 2001; Marder & Thirumalai 2002; Stein 2009).

The multidimensional combinatorial mechanism just sketched out automatically operates in modulatory networks simply in consequence of their network structure. However, the structure appears unlikely to have evolved solely to implement this rather logically severe mechanism. Indeed, recent work in systems biology strongly suggests an alternative set of primary design criteria. It is striking to what extent the complex network structure of the modulatory system resembles the structures that have now been well mapped out for intracellular biochemical networks of various kinds—protein–protein interaction networks, G-protein, second-messenger and other signal-transduction networks, and genetic networks (Hille 1992; Bray 1995; Weng *et al.* 1999; Jordan *et al.* 2000; Alon 2007)—into which the modulatory network indeed transforms at the interface of the cell membrane (Boonen *et al.* 2009). Similar questions concerning the functional logic of divergence, convergence, pleiotropy and specificity have been asked and answered rather similarly in these networks (Ross 1989; Gudermann *et al.* 1996; Jordan *et al.* 2000). It is therefore tempting to extend to the modulatory network also the more general design principles that have been postulated for these networks.

These principles are of two kinds. First, the nonlinearities that are generated by the properties of the nodes and connections at various sites within the network perform various local computations. In modulatory networks, more than sufficient scope for very complex nonlinearities, and so complex computations, appears to exist, for example, in the interactions between multiple modulators at the synapses where

they modulate each other's release (Vizi & Lábos 1991; Stjärne & Stjärne 1995; Lundberg 1996; Salio *et al.* 2006)—sometimes even differentially the release of just a specific subset of the total modulator complement (DeLong *et al.* 2009)—and in their modulation, through intracellular signal-transduction pathways, of each other's receptors and so actions (Sebastião & Ribeiro 2000). Rooted in such inter-actions are then higher-order functional phenomena such as metamodulation (Katz & Edwards 1999; Stein 2009), the gating of the actions of one modulator by another (Dickinson *et al.* 1997; Svensson *et al.* 2001), complex dependence of the expression of the modulator actions on the state and activity of the underlying neuronal network (Ito & Schuman 2008; Sakurai & Katz 2009), and ultimately, it has been proposed, even such top-level functionalities of the vertebrate nervous system as the representations of memory, salience, reward, expectation and uncertainty (Hasselmo 1995; Yu & Dayan 2005; Cragg 2006). Very complex computations are inherent also in the processing of the released modulators—in particular, the purines and the neuropeptides—by the extracellu-lar processing enzymes that transform the cocktail of active modulators into another cocktail with different activities, with dynamics that themselves can be modu-lated (Dale & Gilday 1996; Konkoy & Davis 1996; Dale 2002; Nusbaum 2002; Huxtable *et al.* 2009). In more abstract terms, the computations can be mod-elled as mathematical operations (Kupfermann 1991) or logical operations such as associativity (Dale 2002), coincidence detection (Bourne & Nicoll 1993; Offermanns & Schultz 1994) and decision-making (Helikar *et al.* 2008). Altogether, the modulatory network thus offers a powerful example of the idea that computation can be performed by bio-chemical networks just as well as it can be performed by traditional neuronal networks (see Bray 1995, 2009; Katz & Clemens 2001; Koch 1999, ch. 20).

In most nervous systems, as I have already argued, it is actually not the modulatory network alone but rather an intimate combination of the modulatory and neuronal networks that performs the compu-tations. There are, however, some systems where the computation is truly mostly modulatory. This may be the case in the enteric 'brain' of the vertebrate gut (Furness *et al.* 1992; Strand 1999), the vertebrate hypothalamic system (Leng & Ludwig 2006), and other systems where the dynamics of release, diffusion, and feedback action of the released modulators can give rise to such dynamic phenomena as oscillations and travelling waves of modulatory signalling (Přibyl *et al.* 2003).

In this respect, the *Aplysia* feeding system again pre-sents an instructive case study, since, in the neuromuscular periphery, essentially all of the system's complexity reflects the operation of the modulatory network. Specifically, an extended analysis (Brezina *et al.* 2003*a*,*b*, 2005) has revealed how particular dynamics of the modulatory processes can help endow the modulatory network with a truly independ-ent computational role. Figure 3*c*, for example, plots the principal variables of the mathematical model of the system when the model was driven by firing

patterns of the motorneurons B15 and B16 that were previously recorded with chronically implanted electrodes during an approximately 2.5 h meal consumed by a real animal. The purple trajectories in figure 3*b* show the corresponding motions through the modulator and effect spaces. The key point to note is that whereas some of the modulatory variables have fast dynamics, others—in particular, the modulation of the calcium current and of the relax-ation rate—are much slower than the characteristic time-scale of the motorneuronal firing input. These slow dynamics act to decouple the modulatory net-work from the instantaneous motorneuronal input, endowing the network with its own autonomous memory of past activity that filters the current motor-neuronal input in a history-dependent manner. At the same time, there is reason to believe that the combin-ation of the fast and slow dynamics of the different variables endows the network with a multidimensional robustness whereby the modulated muscle contractions and movement are, at least on average, always optimal even as they vary greatly in response to a highly variable feeding environment (Brezina *et al.* 2005).

Such conclusions lead directly to the second design principle that the example of other networks also suggests for modulatory networks: that, quite apart from the specific identities of any particular nodes and connections and their local computations, the complex network structure as a whole is an end in itself. It provides multiple compensatory pathways of information and activity flow and feedback loops that allow autoregulation and stability of outputs (Beckskei & Serrano 2000), indeed usually multiple outputs from the same multifunctional network (Bhalla & Iyengar 1999; Jordan *et al.* 2000). Concomi-tantly, the structure provides what is arguably the most important global attribute of a biological system selected for by evolution (Kitano 2004), robustness with respect to parameter variation and stochastic noise as well as resistance to and graceful degradation upon damage (Barkai & Leibler 1997; Alon *et al.* 1999; Gonze *et al.* 2002; Kitano 2004; Li *et al.* 2004).

If we attribute such features to the modulatory net-works as well, their study becomes a branch of systems biology and can be pursued by its methods (Boonen *et al.* 2009). We may, for instance, expect to find in modulatory networks such features of the intracellular biochemical networks as modularity (Qi & Ge 2006), perhaps involving the repetition of simple structural motifs that perform elementary computational oper-ations (Bourne & Nicoll 1993; Milo *et al.* 2002; Ma'ayan *et al.* 2005; Prill *et al.* 2005; Brandman & Meyer 2008). Given the complexity of modulatory networks, the methods for their study are increasingly likely to involve mathematical modelling (Hasselmo 1995; Brezina *et al.* 1996, 2003*a*,*b*; Fellous & Linster 1998), analytical methods such as multidimensional sensitivity analysis, multivariable regression, and other methods that examine the global structure of the network (Olypher & Calabrese 2007; Weaver & Wearne 2008), and the application of engineering methods and principles to 'reverse-engineer' the bio-logical system to understand simultaneously its computational mechanisms and the computational

problems to which those mechanisms provide the solution (Hartwell *et al.* 1999; Csete & Doyle 2002; El-Samad *et al.* 2005; Tomlin & Axelrod 2005; Reeves & Fraser 2009).

REFERENCES

Abbracchio, M. P., Burnstock, G., Verkhratsky, A. & Zimmermann, H. 2008 Purinergic signalling in the nervous system: an overview. *Trends Neurosci.* **32**, 19–29. (doi:10.1016/j.tins.2008.10.001)

Agnati, L. F., Zoli, M., Strömberg, I. & Fuxe, K. 1995 Intercellular communication in the brain: wiring versus volume transmission. *Neuroscience* **69**, 711–726. (doi:10.1016/0306-4522(95)00308-6)

Alon, U. 2007 *An introduction to systems biology: design principles of biological circuits.* Boca Raton, FL: Chapman & Hall/CRC.

Alon, U., Surette, M. G., Barkai, N. & Leibler, S. 1999 Robustness in bacterial chemotaxis. *Nature* **397**, 168–171. (doi:10.1038/16483)

Baraban, S. C. & Tallent, M. K. 2004 Interneuronal neuropeptides—endogenous regulators of neuronal excitability. *Trends Neurosci.* **27**, 135–142. (doi:10.1016/j.tins.2004.01.008)

Barañano, D. E., Ferris, C. D. & Snyder, S. H. 2001 Atypical neural messengers. *Trends Neurosci.* **24**, 99–106. (doi:10.1016/S0166-2236(00)01716-1)

Barkai, N. & Leibler, S. 1997 Robustness in simple biochemical networks. *Nature* **387**, 913–917. (doi:10.1038/43199)

Beckskei, A. & Serrano, L. 2000 Engineering stability in gene networks by autoregulation. *Nature* **405**, 590–593. (doi:10.1038/35014651)

Benjamin, P. R. & Burke, J. F. 1994 Alternative mRNA splicing of the FMRFamide gene and its role in neuropeptidergic signalling in a defined neural network. *BioEssays* **16**, 335–342. (doi:10.1002/bies.950160508)

Bhalla, U. S. & Iyengar, R. 1999 Emergent properties of networks of biological signaling pathways. *Science* **283**, 381–387. (doi:10.1126/science.283.5400.381)

Bloom, F. E. 1984 The functional significance of neurotransmitter diversity. *Am. J. Physiol.* (*Cell Physiol.*) **246**, C184–C194.

Boonen, K., Creemers, J. W. & Schoofs, L. 2009 Bioactive peptides, networks and systems biology. *BioEssays* **31**, 300–314. (doi:10.1002/bies.200800055)

Bourne, H. R. & Nicoll, R. 1993 Molecular machines integrate coincident synaptic signals. *Cell 72/Neuron* **10**, 65–75.

Bowers, C. W. 1994 Superfluous neurotransmitters? *Trends Neurosci.* **17**, 315–320. (doi:10.1016/0166-2236(94)90168-6)

Brandman, O. & Meyer, T. 2008 Feedback loops shape cellular signals in space and time. *Science* **322**, 390–395. (doi:10.1126/science.1160617)

Bray, D. 1995 Protein molecules as computational elements in living cells. *Nature* **376**, 307–312. (doi:10.1038/376307a0)

Bray, D. 2009 *Wetware: a computer in every living cell.* New Haven, CT: Yale University Press.

Brezina, V. & Weiss, K. R. 1997 Analyzing the functional consequences of transmitter complexity. *Trends Neurosci.* **20**, 538–543. (doi:10.1016/S0166-2236(97)01120-X)

Brezina, V., Bank, B., Cropper, E. C., Rosen, S., Vilim, F. S., Kupfermann, I. & Weiss, K. R. 1995 Nine members of the myomodulin family of peptide cotransmitters at the B16-ARC neuromuscular junction of *Aplysia.* *J. Neurophysiol.* **74**, 54–72.

Brezina, V., Orekhova, I. V. & Weiss, K. R. 1996 Functional uncoupling of linked neurotransmitter effects by combinatorial convergence. *Science* **273**, 806–810. (doi:10.1126/science.273.5276.806)

Brezina, V., Orekhova, I. V. & Weiss, K. R. 1997 Control of time-dependent biological processes by temporally patterned input. *Proc. Natl Acad. Sci. USA* **94**, 10 444–10 449. (doi:10.1073/pnas.94.19.10444)

Brezina, V., Orekhova, I. V. & Weiss, K. R. 2003a Neuromuscular modulation in *Aplysia.* I. Dynamic model. *J. Neurophysiol.* **90**, 2592–2612. (doi:10.1152/jn.01091.2002)

Brezina, V., Orekhova, I. V. & Weiss, K. R. 2003b Neuromuscular modulation in *Aplysia.* II. Modulation of the neuromuscular transform in behavior. *J. Neurophysiol.* **90**, 2613–2628. (doi:10.1152/jn.01093.2002)

Brezina, V., Horn, C. C. & Weiss, K. R. 2005 Modeling neuromuscular modulation in *Aplysia.* III. Interaction of central motor commands and peripheral modulatory state for optimal behavior. *J. Neurophysiol.* **93**, 1523–1556. (doi:10.1152/jn.00475.2004)

Brownlee, D. J. A. & Fairweather, I. 1999 Exploring the neurotransmitter labyrinth in nematodes. *Trends Neurosci.* **22**, 16–24. (doi:10.1016/S0166-2236(98)01281-8)

Buck, L. B., Bigelow, J. M. & Axel, R. 1987 Alternative splicing in individual *Aplysia* neurons generates neuropeptide diversity. *Cell* **51**, 127–133. (doi:10.1016/0092-8674(87)90017-1)

Bullmore, E. & Sporns, O. 2009 Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* **10**, 186–198. (doi:10.1038/nrn2575)

Churchland, P. S. 1986 *Neurophilosophy: toward a unified science of the mind/brain.* Cambridge, MA: MIT Press.

Cragg, S. J. 2006 Meaningful silences: how dopamine listens to the ACh pause. *Trends Neurosci.* **29**, 125–131. (doi:10.1016/j.tins.2006.01.003)

Csete, M. E. & Doyle, J. C. 2002 Reverse engineering of biological complexity. *Science* **295**, 1664–1669. (doi:10.1126/science.1069981)

Dale, N. 2002 Resetting intrinsic purinergic modulation of neural activity: an associative mechanism? *J. Neurosci.* **22**, 10 461–10 469.

Dale, N. & Gilday, D. 1996 Regulation of rhythmic movements by purinergic neurotransmitters in frog embryos. *Nature* **383**, 259–263. (doi:10.1038/383259a0)

DeLong, N. D., Beenhakker, M. P. & Nusbaum, M. P. 2009 Presynaptic inhibition selectively weakens peptidergic cotransmission in a small motor system. *J. Neurophysiol.* **102**, 3492–3504. (doi:10.1152/jn.00833.2009)

Dickinson, P. S. 2006 Neuromodulation of central pattern generators in invertebrates and vertebrates. *Curr. Opin. Neurobiol.* **16**, 604–614. (doi:10.1016/j.conb.2006.10.007)

Dickinson, P. S. & Moulins, M. 1992 Interactions and combinations between different networks in the stomatogastric nervous system. In *Dynamic biological networks: the stomatogastric nervous system* (eds R. M. Harris-Warrick, E. Marder, A. I. Selverston & M. Moulins), pp. 139–160. Cambridge, MA: MIT Press.

Dickinson, P. S., Fairfield, W. P., Hetling, J. R. & Hauptman, J. 1997 Neurotransmitter interactions in the stomatogastric system of the spiny lobster: one peptide alters the response of a central pattern generator to a second peptide. *J. Neurophysiol.* **77**, 599–610.

Djokaj, S., Cooper, R. L. & Rathmayer, W. 2001 Presynaptic effects of octopamine, serotonin, and cocktails of the two modulators on neuromuscular transmission in

crustaceans. *J. Comp. Physiol. A* **187**, 145–154. (doi:10.1007/s003590100187)

Edelman, G. M. & Gally, J. A. 2001 Degeneracy and complexity in biological systems. *Proc. Natl Acad. Sci. USA* **98**, 13 763–13 768. (doi:10.1073/pnas.231499798)

El-Samad, H., Kurata, H., Doyle, J. C., Gross, C. A. & Khammash, M. 2005 Surviving heat shock: control strategies for robustness and performance. *Proc. Natl Acad. Sci. USA* **102**, 2736–2741. (doi:10.1073/pnas.0403510102)

Fellous, J.-M. & Linster, C. 1998 Computational models of neuromodulation. *Neural Comput.* **10**, 771–805. (doi:10.1162/089976698300017476)

Fisher, S. A., Fischer, T. M. & Carew, T. J. 1997 Multiple overlapping processes underlying short-term synaptic enhancement. *Trends Neurosci.* **20**, 170–177. (doi:10.1016/S0166-2236(96)01001-6)

Fox, L. E. & Lloyd, P. E. 2001 Evidence that post-tetanic potentiation is mediated by neuropeptide release in *Aplysia*. *J. Neurophysiol.* **86**, 2845–2855.

Fu, Q., Kutz, K. K., Schmidt, J. J., Hsu, Y.-W. A., Messinger, D. I., Cain, S. D., de la Iglesia, H. O., Christie, A. E. & Li, L. 2005 Hormone complement of the *Cancer productus* sinus gland and pericardial organ: an anatomical and mass spectrometric investigation. *J. Comp. Neurol.* **493**, 607–626. (doi:10.1002/cne.20773)

Fujisawa, Y. *et al.* 1999 The *Aplysia Mytilus* inhibitory peptide-related peptides: identification, cloning, processing, distribution, and action. *J. Neurosci.* **19**, 9618–9634.

Furness, J. B., Bornstein, J. C., Murphy, R. & Pompolo, S. 1992 Role of peptides in transmission in the enteric nervous system. *Trends Neurosci.* **15**, 66–71. (doi:10.1016/0166-2236(92)90029-8)

Furukawa, Y. *et al.* 2001 The enterins: a novel family of neuropeptides isolated from the enteric nervous system and CNS of *Aplysia*. *J. Neurosci.* **21**, 8247–8261.

Gerschenfeld, H. M. 1973 Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. *Physiol. Rev.* **53**, 1–119.

Gonze, D., Halloy, J. & Goldbeter, A. 2002 Robustness of circadian rhythms with respect to molecular noise. *Proc. Natl Acad. Sci. USA* **99**, 673–678. (doi:10.1073/pnas.022628299)

Gourine, A. V., Wood, J. D. & Burnstock, G. 2009 Purinergic signalling in autonomic control. *Trends Neurosci.* **32**, 241–248. (doi:10.1016/j.tins.2009.03.002)

Grillner, S. & Graybiel, A. M. 2006 *Microcircuits: the interface between neurons and global brain function.* Cambridge, MA: MIT Press.

Gudermann, T., Kalkbrenner, F. & Schultz, G. 1996 Diversity and selectivity of receptor–G protein interaction. *Annu. Rev. Pharmacol. Toxicol.* **36**, 429–459.

Guix, F. X., Uribesalgo, I., Coma, M. & Munoz, F. J. 2005 The physiology and pathophysiology of nitric oxide in the brain. *Prog. Neurobiol.* **76**, 126–152. (doi:10.1016/j.pneurobio.2005.06.001)

Harris-Warrick, R. M., Marder, E., Selverston, A. I. & Moulins, M. 1992a *Dynamic biological networks: the stomatogastric nervous system.* Cambridge, MA: MIT Press.

Harris-Warrick, R. M., Nagy, F. & Nusbaum, M. P. 1992b Neuromodulation of stomatogastric networks by identified neurons and transmitters. In *Dynamic biological networks: the stomatogastric nervous system* (eds R. M. Harris-Warrick, E. Marder, A. I. Selverston & M. Moulins), pp. 87–137. Cambridge, MA: MIT Press.

Hartwell, L. H., Hopfield, J. J., Leibler, S. & Murray, A. W. 1999 From molecular to modular cell biology. *Nature* **402**, C47–C52. (doi:10.1038/35011540)

Hasselmo, M. E. 1995 Neuromodulation and cortical function: modeling the physiological basis of behavior. *Behav. Brain Res.* **67**, 1–27. (doi:10.1016/0166-4328(94)00113-T)

Helikar, T., Konvalina, J., Heidel, J. & Rogers, J. A. 2008 Emergent decision-making in biological signal transduction networks. *Proc. Natl Acad. Sci. USA* **105**, 1913–1918. (doi:10.1073/pnas.0705088105)

Herbert, J. 1993 Peptides in the limbic system: neurochemical codes for co-ordinated adaptive responses to behavioural and physiological demand. *Prog. Neurobiol.* **41**, 723–791. (doi:10.1016/0301-0082(93)90033-O)

Hewes, R. S., Snowdeal, E. C. & Taghert, P. H. 1998 Functional redundancy of FMRFamide-related peptides at the *Drosophila* larval neuromuscular junction. *J. Neurosci.* **18**, 7138–7151.

Hille, B. 1992 G protein-coupled mechanisms and nervous signaling. *Neuron* **9**, 187–195. (doi:10.1016/0896-6273(92)90158-A)

Hofer, A. M. & Lefkimmiatis, K. 2007 Extracellular calcium and cAMP: second messengers as 'third messengers'? *Physiology* **22**, 320–327. (doi:10.1152/physiol.00019.2007)

Hökfelt, T., Broberger, C., Xu, Z.-Q. D., Sergeyev, V., Ubink, R. & Diez, M. 2000 Neuropeptides—an overview. *Neuropharmacology* **39**, 1337–1356. (doi:10.1016/S0028-3908(00)00010-1)

Hooper, S. L., Brezina, V., Cropper, E. C. & Weiss, K. R. 1999 Flexibility of muscle control by modulation of muscle properties. In *Beyond neurotransmission: neuromodulation and its importance for information processing* (ed. P. S. Katz), pp. 241–274. Oxford, UK: Oxford University Press.

Horn, C. C., Zhurov, Y., Orekhova, I. V., Proekt, A., Kupfermann, I., Weiss, K. R. & Brezina, V. 2004 Cycle-to-cycle variability of neuromuscular activity in *Aplysia* feeding behavior. *J. Neurophysiol.* **92**, 157–180. (doi:10.1152/jn.01190.2003)

Husson, S. J., Mertens, I., Janssen, T., Lindemans, M. & Schoofs, L. 2007 Neuropeptide signaling in the nematode *Caenorhabditis elegans*. *Prog. Neurobiol.* **82**, 33–55. (doi:10.1016/j.pneurobio.2007.01.006)

Huxtable, A. G., Zwicker, J. D., Poon, B. Y., Pagliardini, S., Vrouwe, S. Q., Greer, J. J. & Funk, G. D. 2009 Tripartite purinergic modulation of central respiratory networks during perinatal development: the influence of ATP, ectonucleotidases, and ATP metabolites. *J. Neurosci.* **29**, 14 713–14 725. (doi:10.1523/JNEUROSCI.2660-09.2009)

Ito, H. T. & Schuman, E. M. 2008 Frequency-dependent signal transmission and modulation by neuromodulators. *Front. Neurosci.* **2**, 138–144. (doi:10.3389/neuro.01.027.2008)

Jordan, J. D., Landau, E. M. & Iyengar, R. 2000 Signaling networks: the origins of cellular multitasking. *Cell* **103**, 193–200. (doi:10.1016/S0092-8674(00)00112-4)

Katz, P. S. 1999 *Beyond neurotransmission: neuromodulation and its importance for information processing.* Oxford, UK: Oxford University Press.

Katz, P. S. & Clemens, S. 2001 Biochemical networks in nervous systems: expanding neuronal information capacity beyond voltage signals. *Trends Neurosci.* **24**, 18–25. (doi:10.1016/S0166-2236(00)01686-6)

Katz, P. S. & Edwards, D. H. 1999 Metamodulation: the control and modulation of neuromodulation. In *Beyond neurotransmission: neuromodulation and its importance for information processing* (ed. P. S. Katz), pp. 349–381. Oxford, UK: Oxford University Press.

Katz, P. S. & Frost, W. N. 1996 Intrinsic neuromodulation: altering neuronal circuits from within. *Trends Neurosci.* **19**, 54–61. (doi:10.1016/0166-2236(96)89621-4)

Kirkland, K. L. 2002 High-tech brains. *Persp. Biol. Med.* **45**, 212–223. (doi:10.1353/pbm.2002.0033)

Kitano, H. 2004 Biological robustness. *Nat. Rev. Genet.* **5**, 826–837. (doi:10.1038/nrg1471)

Kobayashi, M. & Muneoka, Y. 1990 Structure and action of molluscan neuropeptides. *Zool. Sci.* **7**, 801–814.

Koch, C. 1999 *Biophysics of computation: information processing in single neurons.* New York, NY: Oxford University Press.

Koh, H.-Y. & Weiss, K. R. 2005 Peptidergic contribution to posttetanic potentiation at a central synapse of *Aplysia.* *J. Neurophysiol.* **94**, 1281–1286. (doi:10.1152/jn.00073.2005)

Konkoy, C. S. & Davis, T. P. 1996 Ectoenzymes as sites of peptide regulation. *Trends Pharmacol. Sci.* **17**, 288–294. (doi:10.1016/0165-6147(96)10036-5)

Kupfermann, I. 1974 Feeding behavior in *Aplysia*: a simple system for the study of motivation. *Behav. Biol.* **10**, 1–26. (doi:10.1016/S0091-6773(74)91644-7)

Kupfermann, I. 1991 Functional studies of cotransmission. *Physiol. Rev.* **71**, 683–732.

Landgraf, R. & Neumann, I. D. 2004 Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. *Front. Endocrinol.* **25**, 150–176. (doi:10.1016/j.yfrne.2004.05.001)

Leng, G. & Ludwig, M. 2006 Information processing in the hypothalamus: peptides and analogue computation. *J. Neuroendocrinol.* **18**, 379–392. (doi:10.1111/j.1365-2826.2006.01428.x)

Li, F., Long, T., Lu, Y., Ouyang, Q. & Tang, C. 2004 The yeast cell-cycle network is robustly designed. *Proc. Natl Acad. Sci. USA* **101**, 4781–4786. (doi:10.1073/pnas.0305937101)

Lingueglia, E., Champigny, G., Lazdunski, M. & Barbry, P. 1995 Cloning of the amiloride-sensitive FMRFamide-gated sodium channel. *Nature* **378**, 730–733. (doi:10.1038/378730a0)

Lundberg, J. M. 1996 Pharmacology of cotransmission in the autonomic nervous system: integrative aspects of amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. *Pharmacol. Rev.* **48**, 113–178.

Ma, M., Wang, J., Chen, R. & Li, L. 2009 Expanding the crustacean neuropeptidome using a multifaceted mass spectrometric approach. *J. Proteome Res.* **8**, 2426–2437. (doi:10.1021/pr801047v)

Ma'ayan, A. *et al.* 2005 Formation of regulatory patterns during signal propagation in a mammalian cellular network. *Science* **309**, 1078–1083. (doi:10.1126/science.1108876)

Maggi, C. A. 1995 Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. *Prog. Neurobiol.* **45**, 1–98. (doi:10.1016/0301-0082(94)E0017-B)

Marder, E. & Bucher, D. 2001 Central pattern generators and the control of rhythmic movements. *Curr. Biol.* **11**, R986–R996. (doi:10.1016/S0960-9822(01)00581-4)

Marder, E. & Thirumalai, V. 2002 Cellular, synaptic and network effects of neuromodulation. *Neural Netw.* **15**, 479–493. (doi:10.1016/S0893-6080(02)00043-6)

Marder, E., Christie, A. E. & Kilman, V. L. 1995 Functional organization of cotransmission systems: lessons from small nervous systems. *Invert. Neurosci.* **1**, 105–112. (doi:10.1007/BF02331908)

McCormick, D. A. 1992 Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog. Neurobiol.* **39**, 337–388. (doi:10.1016/0301-0082(92)90012-4)

Merighi, A. 2002 Costorage and coexistence of neuropeptides in the mammalian CNS. *Prog. Neurobiol.* **66**, 161–190. (doi:10.1016/S0301-0082(01)00031-4)

Mesce, K. A., Crisp, K. M. & Gilchrist, L. S. 2001 Mixtures of octopamine and serotonin have nonadditive effects on the CNS of the medicinal leech. *J. Neurophysiol.* **85**, 2039–2046.

Milo, R., Shen-Orr, S., Itzkovitz, S., Kashtan, N., Chklovskii, D. & Alon, U. 2002 Network motifs: simple building blocks of complex networks. *Science* **298**, 824–827. (doi:10.1126/science.298.5594.824)

Nässel, D. R. 2002 Neuropeptides in the nervous system of *Drosophila* and other insects: multiple roles as neuromodulators and neurohormones. *Prog. Neurobiol.* **68**, 1–84. (doi:10.1016/S0301-0082(02)00057-6)

Nässel, D. R. 2009 Neuropeptide signaling near and far: how localized and timed is the action of neuropeptides in brain circuits? *Invert. Neurosci.* (doi:10.1007/s10158-009-0090-1)

Niall, H. D. 1982 The evolution of peptide hormones. *Annu. Rev. Physiol.* **44**, 615–624. (doi:10.1146/annurev.ph.44.030182.003151)

Nusbaum, M. P. 2002 Regulating peptidergic modulation of rhythmically active neural circuits. *Brain Behav. Evol.* **60**, 378–387. (doi:10.1159/000067791)

Nusbaum, M. P., Blitz, D. M., Swensen, A. M., Wood, D. & Marder, E. 2001 The roles of co-transmission in neural network modulation. *Trends Neurosci.* **24**, 146–154. (doi:10.1016/S0166-2236(00)01723-9)

Offermanns, S. & Schultz, G. 1994 Complex information processing by the transmembrane signaling system involving G proteins. *Naunyn-Schmied Arch. Pharmacol.* **350**, 329–338.

Olsen, S. R. & Wilson, R. I. 2008 Cracking neural circuits in a tiny brain: new approaches for understanding the neural circuitry of *Drosophila*. *Trends Neurosci.* **31**, 512–520. (doi:10.1016/j.tins.2008.07.006)

Olypher, A. V. & Calabrese, R. L. 2007 Using constraints on neuronal activity to reveal compensatory changes in neuronal parameters. *J. Neurophysiol.* **98**, 3749–3758. (doi:10.1152/jn.00842.2007)

Orchard, I., Lange, A. B. & Bendena, W. G. 2001 FMRF-amide-related peptides: a multifunctional family of structurally related neuropeptides in insects. *Adv. Insect. Physiol.* **28**, 268–329.

Orekhova, I. V., Alexeeva, V., Church, P. J., Weiss, K. R. & Brezina, V. 2003 Multiple presynaptic and postsynaptic sites of inhibitory modulation by myomodulin at ARC neuromuscular junctions of *Aplysia*. *J. Neurophysiol.* **89**, 1488–1502. (doi:10.1152/jn.00140.2002)

Ott, S. R., Philippides, A., Elphick, M. R. & O'Shea, M. 2007 Enhanced fidelity of diffusive nitric oxide signalling by the spatial segregation of source and target neurones in the memory centre of an insect brain. *Eur. J. Neurosci.* **25**, 181–190.

Perone, M. J., Windeatt, S. & Castro, M. G. 1997 Intracellular trafficking of prohormones and proneuropeptides: cell type-specific sorting and targeting. *Exp. Physiol.* **82**, 609–628.

Přibyl, M., Muratov, C. B. & Shvartsman, S. Y. 2003 Long-range signal transmission in autocrine relays. *Biophys. J.* **84**, 883–896. (doi:10.1016/S0006-3495(03)74906-6)

Prill, R. J., Iglesias, P. A. & Levchenko, A. 2005 Dynamic properties of network motifs contribute to biological network organization. *PLoS Biol.* **3**, 1881–1892.

Proekt, A. *et al.* 2005 Identification of a new neuropeptide precursor reveals a novel source of extrinsic modulation in the feeding system of *Aplysia*. *J. Neurosci.* **25**, 9637–9648. (doi:10.1523/JNEUROSCI.2932-05.2005)

Qi, Y. & Ge, H. 2006 Modularity and dynamics of cellular networks. *PLoS Comput. Biol.* **2**, 1502–1510.

Reeves, G. T. & Fraser, S. E. 2009 Biological systems from an engineer's point of view. *PLoS Biol.* **7**, e1000021.

Ross, E. M. 1989 Signal sorting and amplification through G protein-coupled receptors. *Neuron* **3**, 141–152. (doi:10.1016/0896-6273(89)90027-5)

Sakurai, A. & Katz, P. S. 2009 State-, timing-, and pattern-dependent neuromodulation of synaptic strength by a serotonergic neuron. *J. Neurosci.* **29**, 268–279. (doi:10.1523/JNEUROSCI.4456-08.2009)

Salio, C., Lossi, L., Ferrini, F. & Merighi, A. 2006 Neuropeptides as synaptic transmitters. *Cell Tissue Rev.* **326**, 583–598. (doi:10.1007/s00441-006-0268-3)

Scholz, N. L., de Vente, J., Truman, J. W. & Graubard, K. 2001 Neural network partitioning by NO and cGMP. *J. Neurosci.* **21**, 1610–1618.

Schoofs, L., Veelaert, D., Vanden Broeck, J. & De Loof, A. 1997 Peptides in the locusts, *Locusta migratoria* and *Schistocerca gregaria*. *Peptides* **18**, 145–156. (doi:10.1016/S0196-9781(96)00236-7)

Sebastião, A. M. & Ribeiro, J. A. 2000 Fine-tuning neuromodulation by adenosine. *Trends Pharmacol. Sci.* **21**, 341–346. (doi:10.1016/S0165-6147(00)01517-0)

Segal, M. M. 1983 Specification of synaptic action. *Trends Neurosci.* **6**, 118–121. (doi:10.1016/0166-2236(83)90061-9)

Skiebe, P. 2001 Neuropeptides are ubiquitous chemical mediators: using the stomatogastric nervous system as a model system. *J. Exp. Biol.* **204**, 2035–2048.

Sossin, W. S. & Scheller, R. H. 1991 Biosynthesis and sorting of neuropeptides. *Curr. Opin. Neurobiol.* **1**, 79–83. (doi:10.1016/0959-4388(91)90013-W)

Sporns, O., Chialvo, D. R., Kaiser, M. & Hilgetag, C. C. 2004 Organization, development and function of complex brain networks. *Trends Cogn. Sci.* **8**, 418–425. (doi:10.1016/j.tics.2004.07.008)

Stein, W. 2009 Modulation of stomatogastric rhythms. *J. Comp. Physiol. A* **195**, 989–1009. (doi:10.1007/s00359-009-0483-y)

Stjärne, L. & Stjärne, E. 1995 Geometry, kinetics and plasticity of release and clearance of ATP and noradrenaline as sympathetic cotransmitters: roles for the neurogenic contraction. *Prog. Neurobiol.* **47**, 45–94. (doi:10.1016/0301-0082(95)00018-Q)

Strand, F. L. 1999 *Neuropeptides: regulators of physiological processes*. Cambridge, MA: MIT Press.

Svensson, E., Grillner, S. & Parker, D. 2001 Gating and braking of short- and long-term modulatory effects by interactions between colocalized neuromodulators. *J. Neurosci.* **21**, 5984–5992.

Sweedler, J. V., Li, L., Rubakhin, S. S., Alexeeva, V., Dembrow, N. C., Dowling, O., Jing, J., Weiss, K. R. & Vilim, F. S. 2002 Identification and characterization of the feeding circuit-activating peptides, a novel neuropeptide family of *Aplysia*. *J. Neurosci.* **22**, 7797–7808.

Taghert, P. H. & Veenstra, J. A. 2003 *Drosophila* neuropeptide signaling. *Adv. Genet.* **49**, 1–65. (doi:10.1016/S0065-2660(03)01001-0)

Tomlin, C. J. & Axelrod, J. D. 2005 Understanding biology by reverse engineering the control. *Proc. Natl Acad. Sci. USA* **102**, 4219–4220. (doi:10.1073/pnas.0500276102)

Verhage, M., Ghijsen, W. E. & Lopes da Silva, F. H. 1994 Presynaptic plasticity: the regulation of Ca^{2+}-dependent transmitter release. *Prog. Neurobiol.* **42**, 539–574. (doi:10.1016/0301-0082(94)90050-7)

Vilim, F. S. *et al.* 2010 Distinct mechanisms produce functionally complementary actions of neuropeptides that are structurally related but derived from different precursors. *J. Neurosci.* **30**, 131–147. (doi:10.1523/JNEUROSCI.3282-09.2010)

Vizi, E. S. & Lábos, E. 1991 Non-synaptic interactions at presynaptic level. *Prog. Neurobiol.* **37**, 145–163. (doi:10.1016/0301-0082(91)90025-V)

Weaver, C. M. & Wearne, S. L. 2008 Neuronal firing sensitivity to morphologic and active membrane parameters. *PLoS Comput. Biol.* **4**, 130–150.

Weiss, K. R., Brezina, V., Cropper, E. C., Heierhorst, J., Hooper, S. L., Probst, W. C., Rosen, S. C., Vilim, F. S. & Kupfermann, I. 1993 Physiology and biochemistry of peptidergic cotransmission in *Aplysia*. *J. Physiol.* (Paris) **87**, 141–151. (doi:10.1016/0928-4257(93)90025-O)

Weng, G., Bhalla, U. S. & Iyengar, R. 1999 Complexity in biological signaling systems. *Science* **284**, 92–96. (doi:10.1126/science.284.5411.92)

White, J. G., Southgate, E., Thomson, J. N. & Brenner, S. 1986 The structure of the nervous system of the nematode *C. elegans*. *Phil. Trans. R. Soc. Lond. B* **314**, 1–340. (doi:10.1098/rstb.1986.0056)

Wood, D. E. 1995 Neuromodulation of rhythmic motor patterns in the blue crab *Callinectes sapidus* by amines and the peptide proctolin. *J. Comp. Physiol. A* **177**, 335–349.

Wood, D. E. & Nusbaum, M. P. 2002 Extracellular peptidase activity tunes motor pattern modulation. *J. Neurosci.* **22**, 4185–4195.

Yu, A. J. & Dayan, P. 2005 Uncertainty, neuromodulation, and attention. *Neuron* **46**, 681–692. (doi:10.1016/j.neuron.2005.04.026)

Zimmermann, H. 2008 ATP and acetylcholine, equal brethren. *Neurochem. Int.* **52**, 634–648. (doi:10.1016/j.neuint.2007.09.004)

Zoli, M. & Agnati, L. F. 1996 Wiring and volume transmission in the central nervous system: the concept of closed and open synapses. *Prog. Neurobiol.* **49**, 363–380.

Zoli, M., Jansson, A., Syková, E., Agnati, L. F. & Fuxe, K. 1999 Volume transmission in the CNS and its relevance for neuropsychopharmacology. *Trends Pharmacol. Sci.* **20**, 142–150. (doi:10.1016/S0165-6147(99)01343-7)

Zucker, R. S. & Regehr, W. G. 2002 Short-term synaptic plasticity. *Annu. Rev. Physiol.* **64**, 355–405. (doi:10.1146/annurev.physiol.64.092501.114547)