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Review

Complexities and uncertainties of neuronal network function

David Parker*

Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

The nervous system generates behaviours through the activity in groups of neurons assembled into networks. Understanding these networks is thus essential to our understanding of nervous system function.

Understanding a network requires information on its component cells, their interactions and their functional properties. Few networks come close to providing complete information on these aspects. However, even if complete information were available it would still only provide limited insight into network function. This is because the functional and structural properties of a network are not fixed but are plastic and can change over time. The number of interacting network components, their (variable) functional properties, and various plasticity mechanisms endows networks with considerable flexibility, but these features inevitably complicate network analyses.

This review will initially discuss the general approaches and problems of network analyses. It will then examine the success of these analyses in a model spinal cord locomotor network in the lamprey, to determine to what extent in this relatively simple vertebrate system it is possible to claim detailed understanding of network function and plasticity.

Keywords: neuronal network; plasticity; spinal cord; lamprey; neuromodulation; complexity

1. INTRODUCTION

Understanding the brain is one of our greatest challenges. Bertrand Russell (1935) remarked, 'the sciences have developed in the reverse of what might have been expected. What was most remote from ourselves was first brought under the domain of law… and last of all (as yet very imperfectly) the human mind'. In case its complexity is not appreciated, the brain consists of hundreds of billions of neurons, each of which can connect to thousands of other neurons. Networks of neurons influence all behaviours, including perception, movement, memory and language. If each neuron in the brain was in one of two states, resting or active (there is actually a range of functional states), the number of potential configurations would exceed the number of elementary particles in the universe (Sagan 1977).

To a large extent practical applications of ideas in neurobiology leave science on the sidelines. Lawyers submit brain scans as evidence of their clients' lack of responsibility and governments plan to scan the brains of employees, despite the lack of evidence that the scans predict behaviour; children are given amphetamines to correct disruptive behaviour, despite the lack of evidence for disturbances in brain chemistry; while children with no obvious learning disabilities take cognitive 'enhancing' smart drugs ('Viagra for the brain'), with little evidence of any beneficial effects (Caplan 2002; Rose 2002). The sophistication implied by these approaches presumably reflects the desire for simple answers to complex problems. More traditional approaches to the brain are also dogged by uncertainty. Psychiatric and neurological treatments often lack insight into their mechanisms of action. For example, deep brain stimulation is used as a treatment for several disorders, including Parkinson's disease, but it is unclear how it alleviates symptoms (Greenberg 2002; McIntyre *et al.* 2004), and the potential benefits and underlying mechanisms of electroshock therapy, psychosurgery and psychopharmacology are uncertain at best (Breggin 1993; Schloss & Henn 2004). Detailed insight into normal and pathological function in the nervous system is essential if we are to claim to understand, let alone cure the nervous system.

The 1990s were declared the decade of the brain by the US congress. The Dana Alliance, an organization of neuroscientists, listed 10 objectives to be attained during the decade. These were: identifying the genes defective in Alzheimer's disease, Huntington's disease, hereditary blindness, deafness and manic depression; developing strategies for reducing nerve cell death and promoting regeneration after injury; developing drugs to alleviate chronic pain, multiple sclerosis, Alzheimers's disease, motor neuron disease, Parkinson's disease and epilepsy; developing treatments for manic depression, anxiety and schizophrenia; and understanding the mechanisms of addiction, learning and memory. A Dana Alliance report in 2000 stated that our knowledge of the nervous system during the 1990s had 'doubled', and that with the genetic knowledge obtained 'will come, very quickly, new targeted drugs and preventative measures… (to an extent that) faulty

*djp27@cam.ac.uk

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genes are replaced and whole families can be relieved of the curse of genetic disease' (Blakemore 2000). This optimism is unlikely to be shared by people affected by the disorders listed above, and is not shared by all neuroscientists. Torsten Wiesel, who won the Nobel prize for his work on visual cortex, claimed that 'we need a century, maybe a millennium' to comprehend the brain, and that beyond understanding a few simple mechanisms 'we are at a very early stage of brain science' (cited in Horgan 1999). Caution about our potential for understanding were earlier raised by the Nobel prize-winning neurophysiologist Charles Sherrington who said that 'physiology has not enough to offer about the brain in relation to mind to lend the psychiatrist much help' (cited in Horgan 1999), and by Stent (1969) who suggested that 'searching for a molecular explanation of consciousness is a waste of time since the physiological processes responsible… (will) degenerate into seemingly ordinary reactions no more and no less fascinating than those occurring in the liver'. Wolpert (1993) summed the primitive state of our knowledge, by saying that it is not yet possible to do an experiment 'at the level of brain function or neurophysiology, which would contradict psychoanalytic theory'.

These more cautious views are evidenced by the lack of an increase in citations to basic neuroscience during the decade of the brain in four major psychological journals (Robins *et al.* 1998). This illustrates that while we know a lot about the components of the nervous system, we have little insight into how these components are used to enable us to think, remember, or behave, or why these functions go wrong. There is thus an explanatory gap (Levine 1983) between our understanding of elemental components and the emergent outputs that they produce. An early example of this gap is provided by Plato, who in Phaedo described how in the last hours of his life Socrates ridiculed the idea that his behaviour could be explained in mechanistic terms, saying that someone holding this belief would explain his posture in terms of muscles pulling on bones and his speech to the properties of sound, air and hearing, forgetting the true cause of his behaviour; that he had been sentenced to death and had chosen to stay. To say, as Crick (1994) does, that all joys, sorrows, memories and ambitions are no more than the behaviour of vast numbers of neurons and associated molecules may be true, but it does not advance our understanding of joy, sorrow, memory or ambition.

There are obvious reasons for wanting to understand the brain: to understand our thoughts and behaviours, questions that have been the focus of philosophical discussions since antiquity; to correct the effects of injury or disease; and finally, given that even very simple nervous systems outperform the most sophisticated machines, the opportunity to apply insight obtained on the nervous system to technology.

2. BEHAVIOUR REFLECTS THE ACTIVITY IN NEURONAL NETWORKS

Two core assumptions underlie analyses of the nervous system: that behaviour reflects activity in the nervous system; and that understanding the nervous system will lead to the understanding of normal or abnormal behaviour. Specific behaviours result from the activity in assemblies of interconnected nerve cells ('neuronal networks'). Neuronal networks process sensory inputs, perform cognitive functions, and programme motor outputs. These networks assemble interacting groups of neurons that act together to generate behaviours, making the network the interface between the physiological (cellular) and behavioural levels. Understanding these networks is thus an essential component to our understanding of normal and abnormal behaviour. Invertebrate, lower vertebrate, or developmentally immature systems have been used to facilitate analyses of basic network properties (Marder 2002). Analyses have also focused on rhythmically active networks that control movements like walking, swimming, breathing, or chewing, as these networks generate a basic repeating pattern of activity that is easier to examine than networks that generate non-repetitive behaviours (i.e. behaviours that continuously change in response to internal or external conditions). The extent to which we can understand these relatively simple networks sets a benchmark for the understanding of more complicated systems and functions. The initial hope was that a particular function (e.g. rhythmic motor outputs) would be reflected in common network properties, and that similar networks would generate similar outputs. However, while many rhythmically active networks generate outputs with several features in common (Arshavsky *et al.* 1993), the underlying network mechanisms can vary markedly, networks with similar organizations can generate different outputs, and similar outputs can arise from different network organizations (Getting 1988).

This review will initially outline the general features, approaches, and difficulties associated with analysing neuronal network function. It will then use the lamprey, a lower vertebrate system, to illustrate the actual extent to which network function is understood in this relatively simple vertebrate model system.

3. NETWORK FUNCTION

Neuronal network activity reflects the functional and structural properties of the network (figure 1*a*). Some basic network properties initially need to be defined. Networks consist of interacting collections of individual cells ('neurons'). The inside of a neuron has a negative potential at rest (usually between −60 and −70 mV). A neuron transmits a signal ('an action potential') when the membrane potential is depolarized to a threshold level (figure 1*b*(i)). Action potentials reflect the rapid entry of sodium ions through pores in the cells membrane ('voltage-dependent sodium channels') that makes the inside of the cell transiently positive. Potassium ions leave the cell through voltage-dependent potassium channels to restore the cell to its resting membrane potential. The presence of different types of channels with different properties can influence the net output of a cell (figure 1*b*(ii,iii)).

Nerve cells communicate at specific regions called synapses (figure 1*c*). Calcium entry through voltage-dependent calcium channels at the synapse causes the release of a chemical neurotransmitter from the

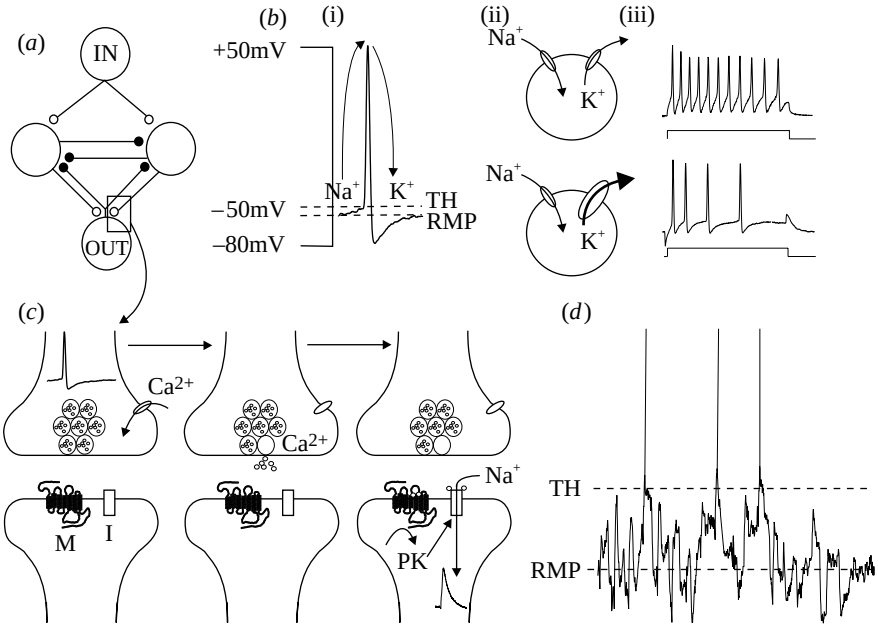

Figure 1. Basic nervous system properties. (*a*) Example of a neuronal network containing input, intermediate, and output elements, and feedback and feed-forward connections. The large circles indicate single types of cells or cell populations. The small circles indicate connections between cells (open circles are excitatory, filled circles inhibitory). (*b*(i)) Neurons send signals by generating action potentials. The entry of positively charged sodium ions makes the inside of the cell positive. The effect is transient due to the exit of positively charged potassium ions. (ii and iii) The relative contribution of sodium and potassium ions can alter the functional properties of cells by changing the number or frequency of action potentials. (*c*) Schematic diagram of synaptic transmission. An action potential in the presynaptic cell results in the opening of voltage-activated calcium channels in the synaptic terminal. This results in the release of neurotransmitters from synaptic vesicles. The transmitter diffuses to the postsynaptic cell where it binds to ionotropic receptors (I) that result in the direct entry or exit of ions, or metabotropic receptors (M) that activate intracellular pathways (PK, protein kinase). (*d*) The membrane potential of a cell during network activity reflects the integration of excitatory and inhibitory inputs (RMP, the resting potential of cell). If the membrane potential is depolarized above the threshold level (TH) an action potential is generated.

presynaptic neuron that binds to specific receptors in one or more postsynaptic neurons. Excitatory (depolarizing) postsynaptic potentials (EPSPs) are usually associated with the entry of sodium or calcium ions, while inhibitory (hyperpolarizing) potentials (IPSPs) are usually associated with the entry of chloride ions. These receptors allow the direct entry of ions into or out of the cell and are called ionotropic receptors. Another class of receptor (metabotropic receptors) do not cause direct movements of ions across the cells membrane, but instead activate intracellular pathways that in turn alter cellular or synaptic properties ('neuromodulation'; see Katz 1999).

The electrical properties of individual nerve cells can be examined by placing fine glass electrodes in the cell ('intracellular recording'). Alternatively, relatively large tipped electrodes can be placed on a region of the cell ('patch clamp'). Suction applied to the patch electrode results in a tight seal that allows single ion channels under the electrode to be recorded, or the membrane inside the electrode can be ruptured to monitor whole cell properties. Synaptic analyses require simultaneous recordings from two or more connected cells: stimulation of the presynaptic cell causes neurotransmitter release, which can be monitored in the postsynaptic cell (figure 1*c*). The integration of inhibitory and excitatory inputs determines the net change in membrane potential of the postsynaptic cell (figure 1*d*). If the postsynaptic cell is depolarized beyond the spike threshold an action potential is generated and the cell will signal to other cells in the network. Action potentials do not usually result from single synaptic inputs, but instead usually require that EPSPs from either a single neuron or from several presynaptic neurons are summed together (temporal and spatial summation, respectively).

The cellular and synaptic properties of a neuron reflect the types of voltage- and neurotransmitter-gated ion channels it contains. The complement of voltage-gated sodium and potassium channels will influence the number, duration, and pattern of action potentials generated by the cell (figure 1*b*(ii,iii)); different types of calcium channels at the synapse will influence transmitter release; and the type of transmitter released and the type of ionotropic or metabotropic receptors that it binds to will determine the synaptic response. Voltage and transmitter-gated ion channels are formed from one or more subunits, and the molecular properties of these subunits in turn influence the function of the channels. Molecular properties thus influence cellular and synaptic properties; cellular and synaptic properties influence network activity; and network activity influences behaviour.

4. NETWORK ACTIVITY REFLECTS THE FUNCTIONAL AND STRUCTURAL PROPERTIES OF THE NETWORK

The molecular and behavioural levels form the two extreme levels from which network function can be

examined: analyses can either work down from the behaviour (top-down analysis) or up from the molecular level (bottom-up analysis). Bottom-up analyses assume that the function of a system can be understood by reducing it into its component parts (reductionism). Reductionism was encouraged by the development of organic chemistry in the mid-nineteenth century, which showed that organic molecules could be synthesized and studied *in vitro*, and thus that there was no vital element associated with living systems. The contemporary version of this is exemplified by the Human Genome Project, which has claimed that once the sequence of the human genome was completed 'the outstanding problems in human biology…. will all be illuminated in a strong and steady light' (Willis 1991). The reductionist approach assumes that a system (S) containing different components $A_1, \ldots, A_n, B_1, \ldots, B_n, C_1, \ldots, C_n$ in relation (R) to each other, $R(A_1, \ldots, A_n, B_1, \ldots, B_n, C_1, \ldots, C_n)$, results in behaviour ($B$), and that this behaviour can be explained from the analysis of the individual component parts in isolation or in various combinations. The opposite, or emergent view states that it is impossible to understand the behaviour of a system from examining the components in isolation or in combinations that are not in the actual relation $R(A_1, \ldots, A_n, B_1, \ldots, B_n, C_1, \ldots, C_n)$.

Partly in response to the rise of organic chemistry, Claude Barnard wrote that understanding the environment in which function occurred was as important as understanding the function itself. So while neurons and synapses are elements of the nervous system, they are components of larger networks, just as DNA is a component of (and thus dependent on) a functioning cell (Lewontin 1991). Cellular or synaptic properties thus must also be understood in the context of ongoing network activity and behaviour.

While analyses usually are top-down or bottom-up, neither type of analysis is sufficient on its own. A bottom-up analysis would need considerable computing power if the interactions between all molecular, cellular, and synaptic components of a network were to be examined. This may eventually be possible, but any bottom-up analysis would still require insight into higher-level function if effects were to be placed in the appropriate functional context. Top-down approaches suffer from the problem that any network or behavioural output could be compatible with several lower-level processes. Without insight into the lower levels (e.g. the region of the central nervous system responsible), it would be impossible to understand higher-level function. Brenner (1999) suggested that a middle-out approach, where the analysis starts at the middle and works out to the higher and lower levels, can overcome the problems of top-down and bottom-up analyses. However, this approach will probably combine the problems of top-down and bottom-up analyses: by definition knowing the middle requires information on the upper and lower levels. Whatever starting point is chosen, analyses must take into account integrated effects at several levels.

Several criteria must be met if we are to claim to understand a network. As network activity is influenced by the types of cells it contains, network neurons must be identified. While this is a basic requirement, it is not straightforward: networks can consist of large numbers of cells; these may not be anatomically segregated (i.e. neurons belonging to one network may be in regions containing neurons belonging to other networks), and individual neurons can even switch between networks (Hooper and Moulins 1988). Two criteria are used to identify network neurons: they are active when the network is active; and their activity influences the network output. However, neither criterion unequivocally identifies a network neuron. Where networks consist of populations of specific cell types, not all members of a population will necessarily be active during a network output. For example, not all motor neurons to a muscle are active when the muscle contracts, but they are instead recruited to be used during different movements (e.g. fast or slow contractions). Conversely, single cells will not influence ongoing network activity if several cells in a population need to be activated simultaneously. Negative effects thus cannot rule out a network component. Positive effects may also not unequivocally identify a network component. For example, sensory inputs to spinal cord networks influence network activity and locomotion, but as network activity can be generated in their absence these inputs are not assumed to be components of the locomotor network (Grillner 1985). This has been a long-standing contentious issue, which in itself illustrates the conceptual difficulties of network analyses. Finally, network components may not be limited to neurons, but may also include glial cells. These have traditionally been viewed as supporting cells, but recent studies suggest that they are functional network components (Fields & Stevens-Graham 2002).

While identifying network neurons can be difficult, particularly if the networks contains large numbers of small cells, this is only the first step: the connections between network neurons must also be determined. This ideally requires simultaneous recordings from pairs of identified network neurons. The ultimate aim is to generate a circuit diagram that describes the connectivity of the network. This analysis can be complicated by the presence of large numbers of cells. Getting (1988) calculated that there were 132 possible connections between the 12 neurons in the network that controls escape swimming in the sea snail *Tritonia*. Given this sort of combinatorial complexity, it would clearly be impractical to try to deduce the actual connectivity of most networks, even if all individual network neurons were identified. Analyses are usually simplified by characterizing the connectivity between cell populations rather than between all individual cells, but this relies on the unlikely assumption that all members of a population will make the same patterns of synaptic connections.

The identification of network neurons and synaptic connections gives the structure of the network, or network architecture. While this is a significant achievement, the network architecture is simply descriptive and provides little insight into function. The architecture may identify what a network potentially can and cannot do, just as a blueprint allows a warehouse to be distinguished from an office block. But in the same way that a blueprint cannot

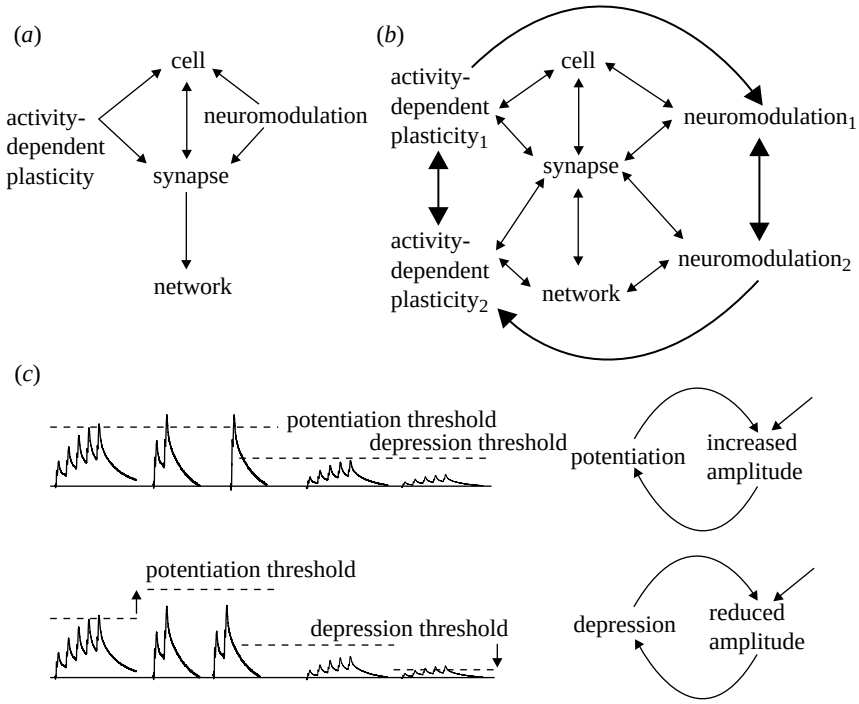

Figure 2. (*a*) Traditional mechanisms of network plasticity. Activity-dependent and neuromodulator-evoked changes act on cells and synapses to alter the network output. (*b*) Metaplastic effects. Interactions can occur between activity-dependent plasticity (metaplasticity) and between neuromodulators (metamodulation). Neuromodulators can also influence activity-dependent plasticity, and activity can influence neuromodulation. These individual and interactive effects act on, or are altered by ongoing cellular, synaptic, and network activity. (*c*) Adaptive changes that ensure that plasticity does not alter ongoing function. If the synaptic input exceeds a threshold level the synapse is potentiated. With a fixed potentiation threshold this will make it more likely that subsequent inputs will exceed the potentiation threshold, resulting in further potentiation. Conversely, synapses can be depressed if the input falls below a depression threshold. This will make it more likely that subsequent inputs fall below the threshold, and the synapse will be successively weakened. The potentiation and depression threshold could instead be altered to prevent disruption of network activity.

identify the work done in an office, the network architecture cannot predict the output of a network, let alone how it is generated. This instead requires information on the functional properties of network neurons and synapses.

A wide range of properties can influence cellular and synaptic function (Getting 1989; Marder & Calabrese 1996). Cellular mechanisms include the spike threshold (voltage at which an action potential is generated), excitability (the number or frequency of action potentials evoked by an input), spike frequency adaptation (the reduction in excitability during repetitive spiking), post-inhibitory rebound (increased excitability after inhibition is removed), and plateau potentials (sustained spiking that outlasts the input that triggers it). Synaptic mechanisms include the sign of the input (excitatory or inhibitory), its amplitude, time-course, and activity-dependent properties (change in amplitude when the synapse is activated repetitively), and the presence of gap junctions that allow voltage changes in one cell to spread to other cells. These cellular and synaptic properties are in turn influenced by the molecular properties of voltage and transmitter-gated ion channels. Insight into functional properties thus requires analyses at the molecular, the cellular, and the synaptic level, which of course requires that network cells and synapses have been identified and are amenable to detailed analyses.

5. NETWORK PLASTICITY REFLECTS CHANGES IN FUNCTIONAL AND STRUCTURAL PROPERTIES

The characterization of network organization and function is thus difficult. There is an added complication in that functional and structural properties are not fixed, but are plastic and can change over time. Thus even complete information on a network would not allow the output to predicted from one occasion to another. Plasticity is an important component to network function, as a network with fixed properties could only generate a limited range of outputs. Plasticity can endow each network component with a range of functional and structural properties, allowing a single network to generate a range of outputs. While flexibility is a huge adaptive advantage, it complicates network analyses because examining properties under one condition only provides a snapshot of the network. Insight is thus also needed into when and how plasticity is triggered and how it can alter network function.

Short or long-term plasticity can result from two general mechanisms: activity-dependent plasticity, where functional and structural changes in cellular or synaptic properties occur as a result of changes in the pattern of activity in network neurons or synapses (Feldman *et al*. 1999; Zucker & Regehr 2002); or through neuromodulation caused by neurotransmitter-mediated changes in cellular or synaptic properties (Katz 1999; figure 2*a*). Neuromodulation differs to conventional fast (millisecond) synaptic transmission

because its effects are relatively slow (seconds to hours), and it may have no direct effect but instead alters the effects of other inputs to the cell. This reflects differences in the mechanisms underlying the two types of transmission: fast transmission occurs through the direct movement of ions across the cells membrane ('ionotropic'), whereas neuromodulation reflects the activation of intracellular pathways ('metabotropic'; figure 1*c*). These different effects are not necessarily related to different types of transmitters, but depend instead on the type of transmitter receptors they activate.

While activity-dependent plasticity and neuromodulation were traditionally considered as separate phenomena, recent studies suggest that interactions can occur between these effects (figure 2*b*). For example, neuromodulation can be altered when it is evoked after previous modulation, and activity-dependent plasticity can be altered by previous activity. Neuromodulation can also alter activity-dependent plasticity, and activity can influence the release of different types of transmitters that may trigger neuromodulatory effects (Verhage *et al.* 1991). These interactions have been termed metaplasticity (Abraham & Bear 1996) or metamodulation (Katz & Edwards 1999). The prefix 'meta' signifies higher-order effects, that activity-dependent plasticity is plastic and that neuromodulation can be modulated. The presence of these effects will result in interactive cycles of plasticity, the functional state of a cell, synapse, or network reflecting the point at which an equilibrium has been reached between these interacting effects. The range of activity patterns and the large number of neurotransmitters and transmitter receptors in the nervous system means that these meta interactions are likely to be widespread, resulting in the potential for a bewildering range of plasticity effects, and thus of functional network states.

While all nervous systems are plastic, it is vital that plasticity does not disrupt ongoing function. The nervous system thus has to both 'be and become' (Rose 1997). For example, an infant must gradually develop the ability to chew, but the mechanisms that cause this developmental change cannot occur at the expense of sucking. This requires that there are adaptive changes that ensure that plasticity effects are integrated with ongoing function. These adaptive effects have been termed 'homeostatic' plasticity (Turrigiano 1999). Homeostasis suggests stability through constancy, but in adaptive plasticity properties are instead altered to compensate for changes to keep function within an optimal level, an example of 'allostasis' (Sterling 2004). A cellular example of how plasticity must be regulated is provided by long-term potentiation, where synapses are potentiated when the postsynaptic input exceeds a threshold level (Malenka & Nicoll 1999; figure 2*c*). This potentiation will make it more likely that subsequent synaptic inputs will exceed the potentiation threshold, resulting in a potential positive feedback loop of continual potentiation that will prevent any meaningful processing of synaptic inputs. Conversely, synapses can be depressed if the postsynaptic input falls below a threshold level. This will make it more likely that subsequent inputs will fall below the depression threshold, leading the synapse to depress to nothing. Bienenstock *et al.* (1982) suggested that these effects are avoided by adaptive changes that modify the plasticity threshold to prevent disruption.

Functional properties and different forms of activity and neuromodulator-dependent plasticity, as well as meta effects and adaptive changes could all interact to influence the current state of a network. For example, a change in the activity (spiking) of a neuron could alter the activity-dependent plasticity of its output synapses; this will in turn alter the synaptically evoked activity of postsynaptic cells, and thus change the activity-dependent plasticity of their output synapses, subsequently altering the activity of the neurons to which these cells connect. Activity can also influence the release of different types of amino acids, amines, and neuropeptides (Verhage *et al.* 1991), which may trigger modulatory, metamodulatory or metaplastic effects. This will alter cellular and synaptic properties, resulting in a further wave of effects. Effects of this sort mean that changes at one site could spread throughout the network ('transsynaptic plasticity'; Fitzsimonds *et al.* 1997), the resulting functional configuration reflecting a dynamic equilibrium between different types of plasticity mechanisms. This is a useful property, as it means that networks could self-organize the integrated changes needed to form new stable functional states.

Understanding network function thus requires the identification of individual network cells, their interactions, their functional properties, and the effects of plasticity and plasticity interactions. Complexity is added at each level: the difficulty of identifying network neurons complicates the analysis of the network architecture; uncertainty over the network architecture complicates the analysis of functional properties and plasticity; plasticity complicates the understanding of network structure and function; and plasticity interactions complicate the understanding of plasticity. The reductionist approach continues to be successful in identifying many components that could contribute to network function. However, a network is not the linear sum of its parts, but instead reflects the spatial and temporal interaction of nonlinear properties. Network function thus does not simply reduce to the sum of its cellular or synaptic properties, and cellular and synaptic function does not simply reduce to molecular properties. Properties emerge at each level from nonlinear interactions, so that individual component parts both influence and are influenced by the network output.

Technological advances often claim to overcome the limitations of previous techniques, and thus the difficulties associated with network analyses. While new techniques have facilitated higher level or lower level analysis, no technique has been developed that allows detailed experimental analyses of multiple interacting network components. For example, the development of neural imaging techniques (e.g. positron emission tomography, magnetic resonance imaging) allows regions of the nervous system activated during behaviours to be identified. While these techniques add a technological sophistication to top-down analyses, the limited resolution of these techniques means that they can currently only say where

increased activity is occurring, not what the increased activity means or how it is generated. Bottom-up analyses of molecular and cellular components are facilitated by techniques such as patch clamp, polymerase chain reaction, and microarrays. Molecular and genetic techniques are already playing a major role in network analyses, and this is likely to grow in the future. The manipulation of specific network components can facilitate the analysis of network organization and function by removing, activating, or inactivating potential network neurons (Watanabe *et al*. 1998; Suster & Bate 2002; Wulff & Wisden 2005). However, as outlined above, positive or negative effects cannot necessarily prove that a type of cell is or is not part of the network. In addition, adaptive changes may alter the functional effects of any manipulation and thus complicate its interpretation (Routenberg 1995; Watanabe *et al*. 1998; Greenspan 2001), although the possibility of adaptive changes could be reduced by using inducible promoter systems that allow the rapid activation or termination of effects. Molecular approaches could also facilitate the mapping of neuronal connections by expressing tracers that spread to presynaptic or postsynaptic cells (retrograde and anterograde labelling, respectively). This, however, depends on the expression of the tracer being sufficient to allow connected cells to be visualized, that the tracer does not have toxic effects that cause cells to degenerate, and that it does not move both retrogradely and anterogradely, thus preventing the determination of which cell was pre or postsynaptic. Even if the labelling was perfect, as outlined above, the identification of the network architecture may say nothing about functional properties.

Another technological aspect that has increased considerably is computing power. Computer simulations of neuronal networks can be carried out routinely, and often offer the only way in which the relevance of cellular and synaptic interactions between even small populations of cells can be determined. Simulations can be used to test the relevance of experimentally identified properties. If a simulation cannot approximate the actual network output then some descriptive or conceptual insight must be lacking, thus motivating further experimental analyses; if it can simulate the network output then some degree of understanding can be claimed, and the model can be used to predict target effects for future experimental analyses. However, to be useful simulations must be based on an appropriate network organization and functional properties. The difficulties of network analyses mean that gaps in this knowledge will be inevitable. These can be filled by assuming properties or extrapolating them from other systems, but the validity and power of the simulation will be reduced as the number of assumptions increases, so that simulated effects may no longer reflect function in the actual system. There is also the danger of reifying assumed network properties in successful simulations, giving the impression that a network has been characterized. This could ultimately slow progress by reducing the motivation for further experimental analyses. Models cannot be criticized for simplifying, this is their function, but properties that have not been verified experimentally must be highlighted, even if the simulated output mimics the real network. Without this, modelling simply becomes an exercise in itself.

Advances in molecular and computational techniques will certainly facilitate network analyses, but they are not a panacea; the application of both approaches depends on more traditional network analyses. Molecular approaches depend on specific markers that target specific cells, but these are usually lacking in mammalian systems (Sharma & Peng 2001; Sapir *et al*. 2004). Information on the network will be needed to identify specific markers, and to allow the effects of any manipulation on network function to be investigated. Computer simulations also do not overcome the difficulties of network analyses: experimental data is needed to build realistic simulations, and the relevance of any simulated effects must ultimately be tested on the actual network.

6. THE LAMPREY SPINAL CORD AS A MODEL SYSTEM

I examine network function and plasticity using the lamprey spinal cord locomotor network. It is claimed that this network is the best understood vertebrate network, and that network activity can be explained in terms of its underlying molecular, cellular, and synaptic properties (Grillner 2003; Grillner *et al*. 2005). It thus offers a useful model system for evaluating the degree to which we can claim to understand network function and plasticity.

The use of the lamprey as a model system was pioneered by Carl Rovainen in the mid 1960s. The lamprey swims using undulatory movements of its eel like body. As in other vertebrates, these movements are generated by a neuronal network in the spinal cord that coordinates muscle activity, in this case alternating contractions of muscles on the left and right sides of the body. The spinal cord does not contain a single network: instead the network is repeated in each of approximately 100 spinal cord segments along the body. The activity in these segments is coordinated, so that each network is activated with a delay from the one preceding it. This travelling wave of activity along the body pushes the animal through the water. There are thus two components to swimming: the coordination of muscle activity in each segment, and the intersegmental coordination of the segmental activity along the body. This discussion will only focus on the segmental network.

The lamprey has a number of features that facilitate network analyses. Firstly, the spinal cord is thin (approximately 250 μm thick) and lacks a blood supply; it is instead oxygenated from the surrounding cerebrospinal fluid. While this may seem unimportant when examining the nervous system, it is advantageous as it allows the intact spinal cord to be isolated and kept in oxygenated Ringer *in vitro*. This facilitates cellular analyses by offering greater stability and access to the nervous system. The need to oxygenate tissue in higher vertebrates means that *in vitro* analyses either use enzymatically dissociated single cells or thin tissue slices: these approaches will obviously disrupt the

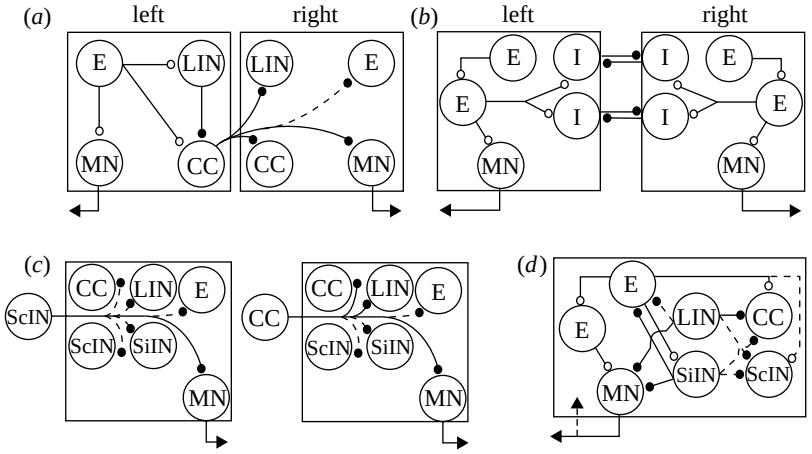

Figure 3. Models of the lamprey segmental locomotor network. (*a*) The initial putative network scheme proposed by Buchanan & Grillner 1987). The model consists of hemisegmental networks on the left and right sides of the spinal cord that coordinate muscle activity on the left and right sides of the body. Connections within one hemisegment are shown on the left, crossing connections between hemisegments on the right. The dashed line indicates a connection that had not been identified. This model is assumed to generate a rhythmic output in the following way. Given that there is a tonic excitatory input, EIN on one side (e.g. assume the left hemisegment) will become activated. The left EINs in turn activate left motor neurons, to cause muscle contraction on the left side of the body, and also activate the left CC interneurons, which inhibit neurons in the right hemisegment: to ensure that motor neuron and muscle activity only occurs on the left side. A number of cellular or synaptic mechanisms could contribute to the termination of activity on the left. When this happens EINs on the right side are relieved of inhibition and become active. This activates motor neurons on the right side, and right side CC interneurons to inhibit the left hemisegment. Right-side activity will then terminate, and the left side again becomes active. Given a constant excitatory input, this model could generate a rhythmic motor pattern. (*b*) Recent locomotor network scheme proposed by Grillner (2003). Crossing inputs are not specified in this diagram, but they actually reflect the CC interneurons and the smaller crossing interneurons (ScIN), and both are claimed to inhibit all cells in the opposite hemisegment. It is also assumed that the EINs excite all neurons within a hemisegment. Neither assumption is based on experimental analyses. In (*c*), the actual experimentally identified connectivity of the inhibitory ScINs and CC interneurons is shown, dashed lines showing the connections that have not been identified experimentally (there are also excitatory CC interneurons and ScINs, and the experimental information available on the connectivity of these cells is the same as shown in *c*). (*d*) The information available about the connectivity of neurons within one hemisegment is shown. The dashed lines again show connections that have not been verified experimentally. E, excitatory glutamatergic interneuron; MN, motor neuron; LIN, glycinergic lateral inhibitory interneuron; CC, glycinergic crossed caudal inhibitory interneuron; ScIN, small crossing inhibitory interneuron; SiIN, small ipsilateral inhibitory interneuron. Inhibitory crossing neurons are not specified in *b*, and are referred to as I. In all diagrams large open circles reflect cell bodies, small open circles excitatory synaptic connections, small filled circles inhibitory synapses.

network, and may also affect functional properties (e.g. Kuenzi *et al.* 2000).

While it is experimentally beneficial, the ability to work *in vitro* is only of use if the isolated spinal cord can generate a meaningful output. This is where the second feature of the lamprey comes in: network activity can be evoked in the isolated spinal cord by bath applying the neurotransmitter glutamate or glutamate receptor agonists. This results in alternating bursts of activity on the left and right sides of the body that can be monitored by recording from the ventral nerve roots that carry motor neuron axons to the muscles. Ventral root activity thus provides a measure of the locomotor network output (because there is no actual movement this activity is called 'fictive locomotion'). To be useful for network analyses this activity must be behaviourally relevant. Wallèn & Williams (1984) concluded that fictive locomotion corresponded to the regular pattern of muscle activity during swimming in intact animals, and thus that activity in the isolated network corresponded to the activity in a behaving animal. However, in an earlier study Ayers *et al.* (1983) concluded that fictive activity did *not* resemble activity during swimming in intact animals, but instead represents a general undulatory pattern that can be modified to produce different types of locomotor behaviour. The discrepancy between these studies may relate to the fact that Wallèn and Williams state that they only analysed activity that was regular. Fictive activity in lamprey and other spinal systems can often be irregular or disrupted (Wallèn & Williams 1984; Parker *et al.* 1998; Pearlstein *et al.* 2005), which does not correlate with swimming in intact animals. Differences in regularity may reflect the absence of the sensory or descending inputs that influence locomotor activity in the intact animal. *In vitro* fictive activity thus probably does not represent behaviour *per se*, but will be the spinal network contribution to locomotion (Ayers *et al.* 1983).

The third feature that makes the lamprey a useful model system is that it is simple compared to mammalian systems and contains relatively few neurons (approximately 1000 in each spinal segment; Rovainen 1979), some of which are relatively large (40–80 μm). However, it is important to recognize that this simplicity is relative: 1000 neurons is still a large number, and many of these neurons are small (10–20 μm). Nevertheless, single or paired recordings can be made from identified neurons (Buchanan 2001). This led to the publication of a putative locomotor network scheme (Buchanan & Grillner 1987; figure 3*a*). Variants of this network have subsequently been produced (see figure 3*b*; Hellgren

et al. 1992; Grillner 2003) that have been claimed to provide an experimentally characterized network (Grillner 2003; Kiehn & Kullander 2004; but see figure 3*c*,*d*). All schemes assume that each spinal segment consists of two identical half or hemisegmental networks, which control muscle activity on the left and right sides of the body. Within each hemisegment there are populations of motor neurons that generate muscle activity; excitatory interneurons (EIN) that provide the excitatory drive to other neurons within the hemisegment (including other EINs); reciprocal inhibitory interneurons that inhibit neurons in the opposite hemisegment (these could contribute to the generation of alternating muscle activity during swimming by ensuring that when one side of the body is active, the other side is silent); and, depending on the model, inhibitory neurons that provide inhibition within the hemisegment (Buchanan 1999*a*,*b*).

7. NETWORK ACTIVITY REFLECTS THE FUNCTIONAL AND STRUCTURAL PROPERTIES OF THE NETWORK

Schematic models of the lamprey locomotor network have been incorporated into computer simulations (Grillner *et al.* 1988; Buchanan 1992; Hellgren *et al.* 1992). Simulated networks can generate locomotor-like outputs, which supported the proposed network organization. However, uncertainties remain in both the initial (Buchanan 1999*a*) and more recent network schemes (Grillner 2003). In the more recent scheme (figure 3*b*), the crossing inhibitory inputs are not named, but the neurons indicated as I neurons include the larger crossed caudal (CC) interneurons that have relatively long axons that project over many spinal segments, and smaller inhibitory cells (small crossing inhibitory interneurons, IScIN) that have relatively short axonal projections. While the CC interneurons were included in the original scheme (Buchanan & Grillner 1987), a number of features suggest that they probably do not mediate segmental reciprocal inhibition (see Rovainen 1983): they have long axons that are not consistent with a segmental role (but could suggest an intersegmental role; Buchanan 1999*b*); they do not influence ongoing segmental network activity when stimulated; and they cannot account for the inhibition in the opposite hemisegmental network during ongoing activity. In addition, while the CC interneurons are claimed to inhibit all contralateral cells (Grillner 2003), there is no experimental evidence from paired recordings that they inhibit EINs in the opposite hemisegment (Buchanan 1999*a*; Parker & Grillner 1999; figure 3*b*). Labelling of the IScINs by applying tracers to the caudal region of the spinal cord shows that they have short axonal projections, and electrophysiological analyses suggest that the functional properties of these cells are consistent with a role in segmental reciprocal inhibition (Buchanan 1999*b*). However, the segmental role of the IScINs has not been proven using electrophysiological techniques. If these cells do mediate segmental reciprocal inhibition then many uncertainties are introduced into the network scheme: although they are claimed to receive inputs from the EINs and to inhibit all cells in the opposite hemisegment (Grillner 2003; figure 3*b*), EIN inputs to these cells have not been identified, and only connections to motor neurons in the opposite hemisegment have been shown. In reality, the connectivity of these cells is almost completely unknown (see Parker 2000; figure 3*c*(i)). As these cells form 50% of the neurons in each hemisegment (Ohta *et al.* 1991), this leaves a major gap in our knowledge of the network organization, and should prevent any claims to a characterized network architecture.

In addition to the lack of information on assumed components included in the network, uncertainty is also introduced by experimentally identified components that are omitted from the network scheme. The latest scheme (Grillner 2003) does not include any ipsilateral inhibitory interneurons (i.e. those contained within the hemisegment; figure 3*b*). These inputs were initially removed because computer simulations suggested they were not needed to generate a simulated rhythmic network output (Hellgren *et al.* 1992). This, however, does not mean that they do not contribute to the patterning of network activity under some conditions, and their removal in the simulations did in fact alter the frequency of the simulated network output. Further motivation for removing these cells from the segmental network scheme was that their inclusion prevented the modulatory effects of 5-HT on the network (a reduction of the burst frequency; see below) from being simulated (Hellgren *et al.* 1992). The logic was that the effects of 5-HT were understood; thus, if the simulations do not mimic its effects then we must change the simulation, rather than examine 5-HT effects in more detail. However, the simulations relied on assumptions over the mechanisms underlying the effects of 5-HT. These assumptions lacked experimental support, and have subsequently been shown to be wrong (see below), thus reducing the justification for removing ipsilateral inhibition.

As with the crossed inhibitory neurons there are two classes of ipsilateral inhibitory cells: larger lateral inhibitory interneurons (LIN), and the smaller ipsilateral inhibitory interneurons (SiIN; Buchanan 1999*a*). The LINs inhibit CC interneurons, and were thought to contribute to the patterning of alternating network activity by removing the inhibition of the opposite hemisegment, thus allowing the opposite side to become active (Wallén *et al.* 1992). However, the LINs, like the CC interneurons, are probably not components of the segmental network: anatomical studies showed that they have long axonal projections that are inconsistent with a segmental role, they do not affect ongoing network activity when stimulated by current injection through an intracellular electrode, and segmental network activity can be evoked in segments, where the LINs are absent (see Rovainen 1983). Unlike the LINs, the SiINs have short axonal projections, suggestive of a segmental role, they influence ongoing segmental network activity, and they are found along the length of the spinal cord (Buchanan & Grillner 1988; Parker 2003*a*). However, these cells have not been included in segmental network schemes. Paired intracellular recordings from the SiINs and the EINs have shown that the SiINs receive functionally powerful inputs from the EINs,

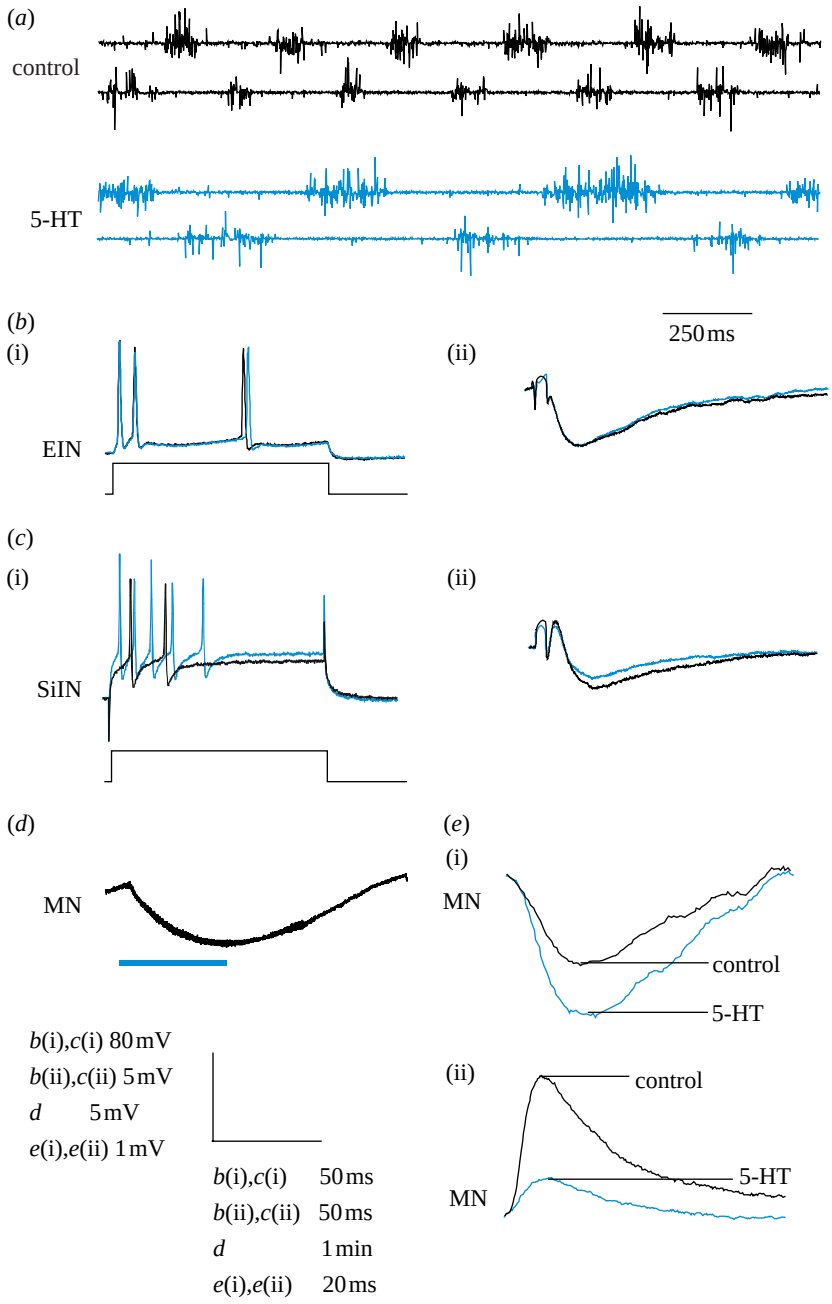

Figure 4. Effects of 5-HT on the lamprey locomotor network. (*a*) 5-HT (1 μM) slows the frequency of network activity evoked by bath application of the glutamate receptor antagonist NMDA. The traces show activity recorded extracellularly in ventral roots on the left and right sides of the body in control and 10 min after 5-HT application (the effects of 5-HT are shown in blue in all traces). (*b*(i)) 5-HT (1 μM) does not usually affect the spiking in response to a depolarizing current pulse or the sAHP in the EINs (ii). (*c*(i)) 5-HT (1 μM) increases the number of spikes in response to depolarizing current injection in an SiIN and reduces the slow afterhyperpolarization (sAHP) following the action potential (ii). (*d*) 5-HT (1 μM) can hyperpolarize the membrane potential of motor neurons. The bar shows the onset and duration of 5-HT application. (*e*(i)) 5-HT potentiates inhibitory synaptic inputs from CC interneurons, but reduces the amplitude of glutamatergic synaptic inputs from EINs (ii).

and in turn provide feedback excitation onto the EINs (Parker 2003*a*). They could thus contribute to ongoing network activity under some conditions. The connectivity of the SiINs has also not been characterized completely using electrophysiological techniques, but they are known to receive inputs from the EINs, and to inhibit EINs, CC interneurons, and motor neurons.

A further omission is that both the initial and more recent network schemes do not consider the role of excitatory crossing interneurons (excitatory CC and ScINs; Buchanan 2001). Inhibitory crossing inputs have been assumed in network models because these provide the simplest mechanism to account for alternating activity on the left and right sides of the spinal cord. This basic arrangement was first proposed in the early twentieth century (Brown 1911), and has subsequently been assumed in all spinal networks. It claims support from the fact that blocking inhibition with strychnine abolishes alternating segmental network activity. However, the use of strychnine cannot separate between the direct crossing inhibition commonly assumed, and crossing excitation that activates inhibitory interneurons within the opposite hemisegment. A study in the neonatal rat suggested that alternating activity may in fact be mediated by reciprocal excitatory inputs between hemisegmental

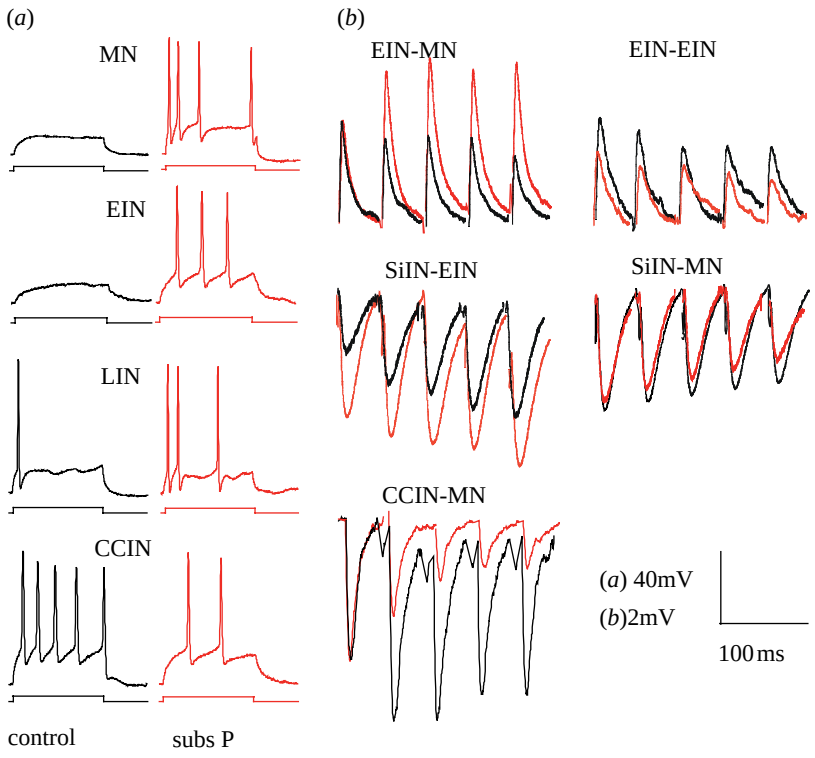

Figure 5. Substance P (1 μM) has neuron and synapse-specific effects on cellular and synaptic properties. (*a*) Effects of substance P on spiking in different types of network neurons evoked by depolarizing current injection (all effects of substance P are shown in red). (*b*) The effects of substance P on synaptic inputs from different types of network neurons. The synaptic input is evoked by a train of presynaptic action potentials evoked at 20 Hz.

networks, which terminate activity in the opposite hemisegment by activating ipsilateral inhibitory interneurons (Kjaerulff and Kiehn 1997). In addition, while the presence of reciprocal inhibitory inputs is assumed to generate alternating activity, this may not be the case: reciprocal inhibition can evoke synchronous activity when the inhibitory input is relatively long-lasting (Elson *et al.* 2002).

Finally, motor neurons were assumed to only be output elements of the locomotor network, and not to make connections back into the network as they do in other vertebrates (e.g. Roberts *et al.* 1998). This conclusion was based on the failure of ventral root stimulation, which will activate motor neurons (antidromic activation), to alter ongoing network activity (Wallén & Lansner 1984). However, this experiment cannot rule motor neurons out of the network, as antidromic activation may not evoke normal postsynaptic responses (El Manira *et al.* 1991). Recent detailed electrophysiological analyses suggest that motor neurons do in fact provide feedback inputs to the network, and they could thus contribute to the patterning of network activity (Buchanan 1999*a*; Quinlan *et al.* 2004). The role of motor neuron feedback to the network thus also needs to be considered, an aspect that could significantly alter the organization of the network.

There is a lot of information on lamprey spinal cord neurons and their organization (Buchanan 2001), but the locomotor network cannot claim to be characterized in detail: assuming that all classes of neurons have been identified there is still the uncertainty over which inhibitory neurons mediate segmental inhibition (CC and LIN or SiIN and ScIN), the relative relevance of ipsilateral inhibition and crossing excitation, the role of motor neuron feedback to the network, and a large number of assumed connections that have not been verified experimentally (approximately 50% of the connections shown in Grillner 2003 have actually been verified experimentally; figure 3*c*,*d*). This does not detract from the insight obtained, but these uncertainties must not be lost in attempts to over simplify the network, or claim a degree of understanding that is not supported by data. This will ultimately reduce the motivation to examine the uncertainties, and the reification of assumed properties that are incorrect will ultimately make it more difficult to explain and to analyse the network.

Network activity is not simply dependent on the network organization, but also on functional cellular and synaptic properties. Several functional properties have been suggested to be important in generating segmental network activity in lamprey (Grillner 2003). The principal mechanisms claimed are NMDA-dependent cellular oscillations, which could contribute to the excitatory drive underlying network activity, and the slow afterhyperpolarization (sAHP) following an action potential, which could contribute to the termination of spiking in individual cells. The actual functional relevance of these effects, however, is not certain. Functional analyses of cellular and synaptic effects should focus on recordings from identified types of network neurons and synapses (Buchanan 1993). Thus, the uncertainty over the network architecture necessarily reduces the extent to which any understanding of network functional properties can be claimed. Where cellular properties have been examined electrophysiologically it is usually in motor neurons, large interneurons, or unidentified cells (e.g. NMDA

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oscillations, Wallén & Grillner 1987; sAHP, Wallèn *et al.* 1989). Synaptic properties are often examined using extracellular stimulation, which will activate an unknown number of unidentified presynaptic cells. Cells are also often stimulated using single low frequency pulses, while network neurons generate trains of up to five action potentials at frequencies of 5–30 Hz (figure 5*b*). The input during a train cannot be assumed from low frequency inputs, but can instead depress or facilitate. This activity-dependent plasticity could have a significant effect on network function, and as it is specific to individual synapses it must be examined by making paired recordings from identified cells. Recordings from unidentified or large cells and the use of extracellular stimulation offers the advantage of speed, and overcomes the difficulties of making stable recordings from small interneurons. This can be useful in preliminary analyses, but these approaches do not provide the necessary detail needed to claim understanding of the functional relevance of any effect, or how network activity is generated (see below).

The lack of experimentally verified details, and the uncertainties over identified components in this relatively simple vertebrate system are or course a reflection of the general difficulties of examining network organization. These difficulties are common, and are not limited to vertebrate networks. A number of invertebrate networks that were claimed to be characterized in the 1970s were subsequently shown to be wrong or not as simple as claimed (see Selverston 1980). For example, the molecular, cellular, and synaptic basis of learning in the sea slug *Aplysia* has been explained by presynaptic changes in transmitter release at sensory neuron to motor neuron synapses (Kandel 2001). However, the locus of the change is not exclusively presynaptic (see Glanzman 1995), and large populations of interneurons are involved in the behaviour (Zecevic *et al.* 1989); connections between these interneurons may be crucial sites for the behavioural plasticity (Trudeau & Castellucci 1993).

8. NETWORK PLASTICITY REFLECTS CHANGES IN NETWORK FUNCTIONAL AND STRUCTURAL PROPERTIES

If the lack of a characterized network architecture complicates the analysis of network functional properties, the lack of information on both the network architecture and functional properties will obviously complicate the analysis of network plasticity. Two examples will be used to illustrate the extent to which network plasticity can claim to be understood in the lamprey, the effects of the neuropeptide substance P and of the amine 5-HT.

5-HT slows the frequency of locomotor network activity (Harris-Warrick & Cohen 1985; figure 4*a*). It also reduces the sAHP following action potentials (Van Dongen *et al.* 1986). These two effects were subsequently linked (Grillner & Matsushima 1992; Grillner & Wallèn 1999) with the support of computer simulations (Hellgren *et al.* 1992). The properties of the sAHP were used to explain certain features of network activity. The sAHP sums during a train of action potentials to move the cell below spike threshold and terminate spiking. The reduced sAHP by 5-HT will reduce the summed sAHP amplitude and slow the termination of spiking in motor neurons and network interneurons. As the locomotor burst frequency reflects the number of bursts in a given time, the increase in burst length resulting from the slower burst termination could reduce the frequency of network activity. However, the link between the cellular and network effects of 5-HT relied on two untested assumptions: that 5-HT affected the sAHP in *all* types of network neuron; and that the reduction of the sAHP was the *only* functional effect of 5-HT. The first assumption may be wrong as 5-HT does not usually affect the sAHP in EINs (a reduction occurs in two of 15 cells; figure 4*b*(i,ii)) or excitatory ScINs (a reduction occurs in three of 11 cells), although it does usually reduce the sAHP in SiINs and inhibitory ScINs (a reduction occurring in 11 of 14 and nine of 12 cells, respectively; figure 4*c*(i,ii)). The second assumption was always unlikely, given that an earlier analysis of the effects of 5-HT suggested that it hyperpolarized the membrane potential of some cells (Harris-Warrick & Cohen 1985; figure 4*d*). The validity of the second assumption was further weakened by evidence that showed that 5-HT potentiated inhibitory synaptic inputs but reduced excitatory synaptic inputs to motor neurons (see Parker 2000; figure 4*e*(i,ii)). The net effect of the changes in synaptic inputs will be to reduce the excitatory drive to the network. Experimental and computational analyses suggest that a reduction in excitatory drive can reduce the network burst frequency (Brodin *et al.* 1985; Hellgren *et al.* 1992).

Thus, several mechanisms either individually or in combination could potentially account for the network effects of 5-HT. The relative influence of these effects is unknown, but a recent experimental analysis has further weakened the original assumption over the mechanisms of 5-HT by showing that its network effects depend on the modulation of glutamatergic synaptic transmission, and not the modulation of the sAHP (Schwartz *et al.* 2005).

Understanding the mechanisms underlying the network effects of 5-HT (or any modulator) ultimately requires the analysis of identified functional effects in identified classes of network neurons and synapses.

In contrast to 5-HT, bath application of the tachykinin neuropeptide substance P or an increase in endogenous tachykinin levels increases the frequency of network activity (see figure 6*a*; Parker 2000). Unlike 5-HT, substance P results in a protein synthesis-dependent change that is maintained at least 30 h after substance P was applied (Parker 2000). The mechanisms that trigger and maintain these effects thus need to be examined.

Substance P has a wide range of cellular and synaptic effects (Parker 2000). It can increase or decrease the excitability of specific classes of spinal cord neurons (figure 5*a*), and increase, decrease, or have no effect on glutamatergic or glycinergic synaptic transmission (figure 5*b*); paired recordings from identified types of neuron shows that the type of effect seen depends on the type of cell or synapse examined. This shows that modulatory effects cannot be generalized between cells and synapses. This is not unique to substance P or the

lamprey, but is instead a general property of neuromodulation (Harris-Warrick *et al.* 1998). These distributed neuron and synapse-specific effects emphasize the necessity of identifying cells and synapses if the mechanisms underlying network function and plasticity are to be understood.

Potential mechanisms underlying the induction of the network effect of substance P were identified relatively quickly. Network, cellular, and synaptic analyses suggested that the induction required the potentiation of glutamatergic inputs from the EINs through the protein kinase C-mediated potentiation of NMDA responses, and increased calcium levels in network neurons (Parker *et al.* 1998; figure 6*b*). However, it was only after several years that even a potential mechanism for the protein synthesis-dependent maintenance of the network modulation was identified (Bevan & Parker 2004). Substance P transiently (less than 1 h) potentiates single EIN-evoked EPSPs (figure 6*c*(i)), but it results in a long-lasting metaplastic effect on EIN-evoked inputs to motor neurons that converts the synapse from depressing into facilitating (figure 6*c*(ii)). The metaplastic facilitation reflects changes in transmitter release properties (figure 6*c*(iii); Bevan & Parker 2004), and provides an example of the metaplastic interactions between neuromodulator and activity-dependent effects.

The short-term induction mechanisms and the long-term metaplastic facilitation have the same properties as the long-term network modulation, which suggests that they contribute to the long-term network plasticity. This feature could be used to claim that the cellular and synaptic mechanisms underlying the network effects of substance P are understood. There are, however, several aspects that prevent such a claim from being made. The first reflects the fact that the neuron and synapse-specific effects of neuromodulators (figure 5*a*,*b*) require that identified cells and synapses involved in patterning network activity must be examined. However, the effects of substance P have only been examined in detail on connections to motor neurons (figure 6*d*; Parker 2000; Bevan & Parker 2004). Although recent work (Buchanan 1999*a*,*b*; Quinlan *et al.* 2004) suggests that motor neurons could contribute to the patterning of network activity, and thus that the plasticity of inputs to motor neurons could influence the network output, connections between network interneurons must also be involved in patterning network activity. As effects cannot be extrapolated between connections, synapses between these interneurons must be examined directly. These analyses are complicated by the uncertainty over the network architecture (basically, which connections should be examined). Connections between the large LIN and CC interneurons have been examined to a limited extent, but as outlined above the relevance of these synapses to segmental network is uncertain at best. The effects of substance P have been examined on connections between the smaller (segmental?) interneurons (EIN, ScIN and SiIN). However, the technical difficulties of making paired recordings from small interneurons have prevented the detailed analyses needed to characterize the mechanisms underlying the changes at these connections. Information on these mechanisms is needed to link any cellular and synaptic effects to the changes in the network output. The analysis has thus focused on the experimentally tractable connections, not necessarily the connections that are most relevant to understanding the changes in the network output.

Secondly, the effects of substance P are examined by applying it to the spinal cord. This removes the spatial and temporal features of endogenous substance P release, and could alter its neuromodulatory effects (Teshiba *et al.* 2001). Modulator effects should be examined under conditions of natural release, but there are few systems in which this is possible (see Katz 1999).

A third potential problem reflects the possibility that the long-term plasticity requires a change in activity-dependent synaptic properties (metaplasticity; figure 6*c*(ii)). Activity-dependent synaptic plasticity could be one of the basic mechanisms for patterning network activity. For example, depression of excitatory inputs between the EINs or the facilitation of inhibitory inputs could act as burst terminating factors within a hemisegment. Activity-dependent effects have been examined on several types of connection within the hemisegment. These activity-dependent and metaplastic effects have been reviewed (Grillner *et al.* 2005; Silberberg *et al.* 2005), but several of the details in these reviews are in error: changes in activity-dependent synaptic properties by a neuromodulator are actually called metaplasticity (Parker & Grillner 1999), not metamodulation as stated (which is a change caused by interactions between neuromodulators; Katz & Edwards 1999); 5-HT usually reduces, not increases as stated, the net excitatory drive from the EINs during spike trains even though it facilitates the input, because facilitation develops from a marked reduction in the initial EPSP amplitude (Parker & Grillner 1999); all, not some, of the examined connections between EINs depress (Parker & Grillner 1999; although it is possible that in the future some connections within this population will be found that have different properties); it is stated that the connectivity ratio between the EINs has been estimated to be *ca* 10%, but no ratio can yet be claimed from the experimental data (Parker & Grillner 1999; Parker 2003*a*); and finally, it is stated that the IScINs facilitate during spike trains, when they are instead usually unchanged (Parker & Grillner 1999). The confusion generated by these errors only complicate what is already a complicated picture. It is also claimed that 'the clear importance of this property (activity-dependent plasticity) within networks has been established' (my brackets); in reality the relevance of activity-dependent properties in the lamprey is far from understood. It is also claimed that substance P facilitates ipsilateral excitation, and that computer simulations that incorporate this feature can account for the network effects of substance P (Kozlov *et al.* 2001). However, this again assumes that effects on motor neurons can be extrapolated to other types of connection. Once again, the data shows that this assumption is not valid. If anything substance P reduces ipsilateral excitation by reducing the strength

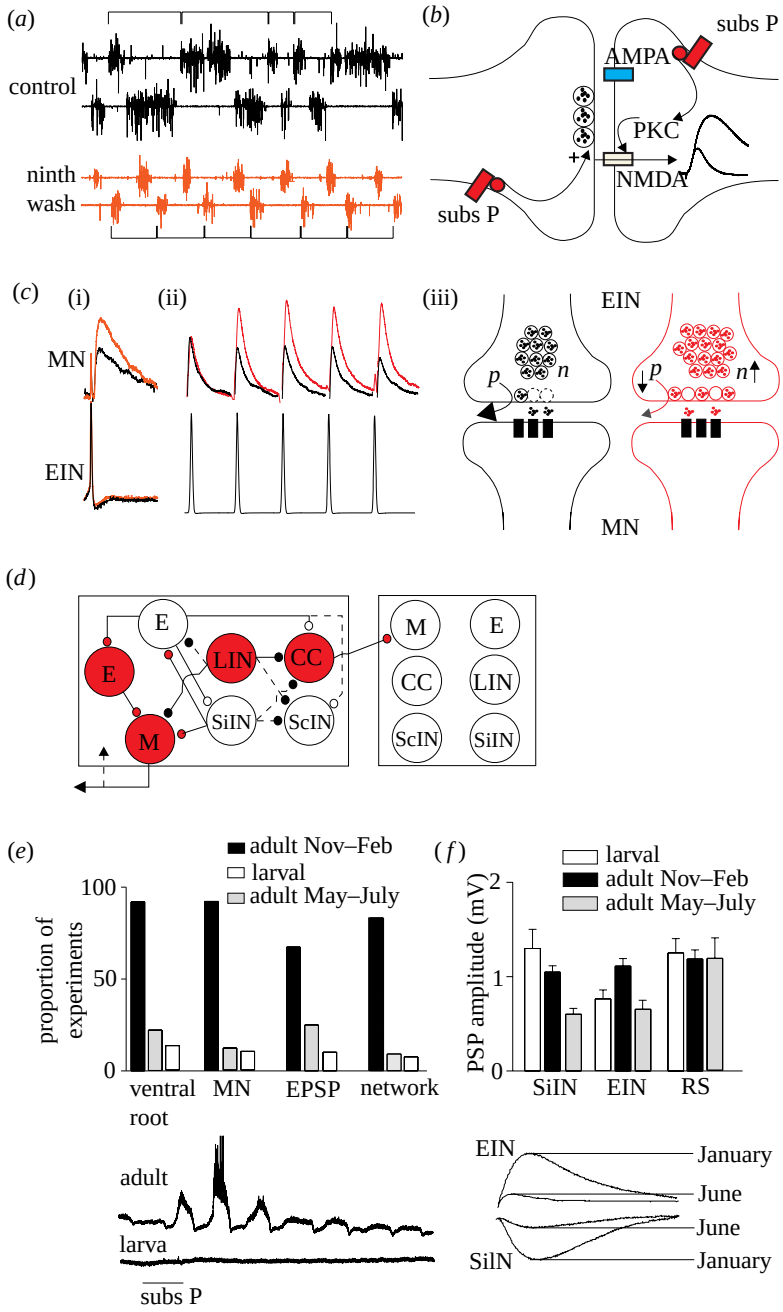

Figure 6. Effects of substance P (1 μM for 10 min) on the locomotor network. (*a*) Substance P increases the frequency of NMDA-evoked network activity. The bars above and below the traces show the interval between successive bursts in a single ventral root in control, and 9 h after the start of substance P wash-off. The reduced variability of the interval between bursts reflects the improved regularity of network activity by substance P. (*b*) Schematic diagram showing the effects of substance P on presynaptic and postsynaptic properties that induce the network plasticity. Substance P acts through an unidentified mechanism to potentiate transmitter release from the presynaptic cell, and through protein kinase C to potentiate the NMDA component of the postsynaptic glutamatergic input. (*c*) (i) Substance P results in short-term (less than 1 h) potentiation of glutamatergic inputs from the EINs to motor neurons, but results in the long-term conversion of the activity-dependent depression of EIN inputs to motor neurons during spike trains into facilitation (ii), providing an example of metaplasticity. (iii) Summary diagram showing the effects that are assumed to trigger the metaplastic facilitation. Substance P increases the number of neurotransmitter containing vesicles, but reduces the probability of releasing these vesicles. The net effect is that the initial EPSP amplitude stays constant, but the input facilitates during the spike train (Zucker & Regehr 2002). (*d*) Summary diagram of the cells and synapses in which the effects of substance P have been examined (shown in red). (*e*) Graph showing the developmental differences in substance P effects in larval and adult animals. The traces below show the significant potentiating effect of substance P in an adult motor neuron, but the lack of effect in a larval motor neuron. (*f*). Graph showing developmental differences in the amplitude of network inhibitory (SiIN), network excitatory (EIN), and descending inputs to the spinal cord (RS). The traces below show examples of differences in synaptic properties over time in adults.

of connections between the EINs, and by facilitating inhibitory feedback inputs from the SiINs (figure 5*b*).

A further complication is that as synaptic properties can change when the network is active (Spira *et al.* 1976; Fitzsimonds *et al.* 1997), activity-dependent synaptic properties should be analysed during network activity. This, however, cannot easily be done, as the barrage of synaptic inputs that single neurons receive

during network activity (see figure 1*d*) prevents individual synapses from being examined. The way around this problem is to try to reduce the amount of synaptic input that the postsynaptic cell receives, but this will alter network function and mean that synapses are not studied under normal network conditions. Activity-dependent changes at individual synapses can thus be measured with precision when the network is inactive, or imprecisely (at best) when it is active (i.e. precision decreases as physiological relevance increases, a biological form of the uncertainty principle?). The analysis of substance P effects has again been based on what can be done, rather than what should ideally be done.

A final aspect that complicates analyses of network plasticity is that cellular and synaptic properties can vary across a wide range. Variability has not traditionally been examined in the nervous system; studies have instead focused on mean values. Variability, however, may be a necessary feature of any adaptive system; healthy physiological systems are intrinsically variable, with highly regular activity being associated with pathological states (Buchman 2002; Sterling 2004). It is increasingly being recognized that variability is a characteristic feature of cellular, synaptic, and network properties, and that changes in this variability can have significant functional effects (Aradi & Soltesz 2002). This variability means that large sample sizes are needed to examine cellular or synaptic effects, but technical difficulties in recording from small neurons can make this difficult.

Variability is seen in the lamprey at the cellular, synaptic and network levels (Buchanan 1993; Parker 2003*b*). Network activity is usually only analysed when a regular network output is generated (e.g. Wallèn & Williams 1984), but as in other motor systems (Horn *et al*. 2004; Pearlstein *et al*. 2005), the activity pattern is often irregular (Wallèn & Williams 1984; Parker *et al*. 1998; Zhang and Grillner 2000). Substance P can improve the regularity of network activity in the lamprey and neonatal rat (Barthe & Clarac 1997; Parker *et al*. 1998; figure 6*a*). This is a separate network effect to the burst frequency modulation, and if anything is the dominant effect of substance P in lamprey in that it occurs in a higher proportion of experiments. The considerable variability in cellular, synaptic, and network properties in the lamprey may influence the variability of substance P effects, which occur in 50–80% of experiments (figure 6*e*). Some of the variability in functional properties and substance P plasticity is also related to developmental effects. The lamprey has a larval stage, a transformer stage (where larvae develop into adults), a parasitic adult stage, an adult migratory phase (where animals swim long distances to freshwater streams), and finally a sexually mature stage where animals mate and then die. The effects of substance P described above only occur in migratory adult animals, not in larvae, transformers, or sexually mature adults (see figure 6*e*; sexually maturity here refers to animals that have been kept in captivity for several months; they have features associated with sexual maturity, but it is possible that the differences reflect the influence of animals being kept in captivity over this time). The lack of effect of substance P in larval and sexually mature animals is associated with developmental changes in synaptic properties (figure 6*f*). Given the multiple factors that have already been identified which may have to act together to influence the effects of substance P (activation of at least two second messenger pathways; multiple cell and synapse-specific changes; two phases of protein synthesis; multiple changes in transmitter release mechanisms and synaptic ultrastructure; inhibition of substance P effects by 5-HT; see Parker 2000; Bevan & Parker 2004), developmental or other differences that cause variability in functional properties could result in state-dependent influences on the mechanisms that evoke the long-term effects of substance P, as has been suggested to occur in other forms of long-term plasticity (Edwards 1995). Variability in the triggering of an effect, and in particular variable effects resulting from different treatments, also complicate the analysis and explanation of the underlying plasticity mechanisms.

Rather than bridging the explanatory gap, studies of 5-HT and substance P in lamprey illustrate how wide the gap between cellular, synaptic, and network levels is: the requirement of examining identified cells and synapses that generate network activity, and understanding how the integrated molecular, cellular, and synaptic properties generate actual network outputs. The analysis of the effects of substance P and 5-HT also illustrate the uncertainties of using computer simulations to explain network function. Simulations of the effects of the network effects of 5-HT depended on the assumptions about the sAHP outlined above, while simulations of the substance P-evoked network modulation assumed that the effects on EIN inputs to motor neurons could be extrapolated to other connections (Kozlov *et al*. 2001). Neither assumption was supported by experimental analyses (see figure 5*b*), but this is partly the reason for using simulations, to test ideas and assumptions. However, of potential concern is that despite the inclusion of cellular and synaptic properties that differ to the actual physiological effects, the simulations were able to mimic the network effects of 5-HT and substance P. The insight obtained from the assumption of properties or the extrapolation from experimentally amenable components in computer simulations generates very useful hypotheses of how network activity *could* be generated and modified, but cannot be used to say that this is *how* an effect occurs.

The analysis of the effects of substance P has largely focused on single or paired intracellular recordings from network neurons. Due to the intrinsic variability of cellular and synaptic properties, the analysis of the properties and modulation of connections between the small network interneurons will require a sample size of at least 50 connections between three interneurons (EIN, SiIN, IscIN; i.e. a total sample of 450 connections). At the current rate of 1 stable connection a week this will require almost 9 years. Clearly, a new approach is needed that will facilitate these analyses, in the same way that the recent development of a new preparation of the *Xenopus* tadpole is providing significant insight into spinal network organization and function by facilitating detailed analyses of anatomical, cellular, and synaptic properties (Li *et al*. 2002).

9. SPINAL INJURY

Finally, a practical reason for understanding spinal cord locomotor networks, is to facilitate locomotor recovery after injury (Dietz 2003). Analyses of functional recovery have focused on reconnecting the spinal cord by encouraging axonal regeneration across lesion sites (Fawcett 2002). This effect on its own, however, does not necessarily account for functional recovery where it occurs. For example, adult mice gradually regain locomotor function after spinal lesions (Leblond *et al.* 2003), but this is not simply due to the axonal regeneration as the functional recovery persists after the spinal cord is re-lesioned.

To make appropriate interventions after injury requires insight into the normal function of the system, and how this function has been disturbed; this in turn depends on insight into network function. In mammalian systems, there is evidence that spinal injury triggers plastic effects in the spinal cord (Edgerton *et al.* 2001; Dietz 2003). This includes changes in the number, size and distribution of synapses (Tai *et al.* 1997), in the properties of neurotransmitter systems (Edgerton *et al.* 2001), and in cellular and synaptic properties (Hochman and McRea 1994; Tillakaratne *et al.* 2002; Li *et al.* 2004). These changes may reflect adaptive plasticity mechanisms (see above) that attempt to compensate for the effects of injury.

Locomotor networks below lesion sites persist after injury. The ability to activate or modulate spinal locomotor networks pharmacologically (i.e. fictive locomotion) prompted the investigation of drug approaches for improving function after spinal injuries (Rossignol *et al.* 2001). Drugs that act on endogenous glutamatergic and aminergic transmitter systems can improve some aspects of locomotor function. However, there are few general effects: the influence of different transmitter systems depends on the nature of the spinal lesion (complete or incomplete), the system studied, and the time after injury that specific drugs are applied. Training can also improve the ability of lesioned animals to step or stand (Edgerton *et al.* 2001). These abilities are acquired at the expense of each other: step-training results in poor stand performance, and vice versa. Training effects also occur in humans (Dietz 2003). Animal studies suggest that step training reflects changes in inhibitory transmitter systems in the spinal cord (Edgerton *et al.* 2001), making these systems useful targets for pharmacological interventions that aim to improve function after injury.

Any intervention after injury needs to be integrated with ongoing network function, which requires information on the organization and functional properties of the network. The lack of detailed information on network properties will make it difficult to ensure that any drug or training intervention will be optimal. The relative influence of different plasticity mechanisms could also influence variable effects. Adaptive changes are evidenced by differences in transmitter systems and functional properties below lesion sites (see above). These changes will alter the functional properties of a network, meaning that mechanisms that may be appropriate in the pre-lesion state may be inappropriate after injury. For example, networks below spinal lesion sites can become hyperexcitable, resulting in spasticity (Li *et al.* 2004); the addition of excitatory inputs to these networks, either through the regrowth of axons across lesion sites or electrically using neuroprostheses may exacerbate rather than overcome the pathological effects. While significant improvements in function may be possible without detailed insight into the network organization and mechanisms, information on these aspects must increase the probability of making functionally beneficial interventions. In the worse case scenario, the application of strategies based on false assumptions may actually have detrimental, rather than beneficial effects.

The presence of adaptive changes following injury could be of significant benefit to functional recovery. If effects at one site were to spread throughout the network through dynamic interactions between different plasticity mechanisms ('transsynaptic plasticity'; Fitzsimonds *et al.* 1997), the network could self-organize into an optimal functional state, without requiring that every step of the re-configuration was specified. This would remove the necessity of having detailed information on the network organization and functional properties, and could either directly optimize function, or could facilitate the effects of other strategies (e.g. regenerated inputs, training routines, or pharmacological interventions). However, insight would still be needed into the mechanisms of neuromodulation, activity-dependent plasticity, adaptive plasticity and meta interactions, which all rely on insight into the organization and properties of locomotor networks.

10. CONCLUSIONS

Is full information on a network possible? Probably not with current techniques. Is full information needed? Possibly not, but we cannot *a priori* know what information can or cannot be left out. Does it matter? The facilitation of technological applications (e.g. robotic systems with the speed, flexibility, and reliability of movement) is certainly possible without understanding all of the details of a network (we can mimic rather than match the nervous system). In quantum physics theories are still debated, but this has not prevented insight from being provided that has allowed the development of nuclear reactors, transistors, and lasers. We can hope that interventions to restore function to spinal cord networks after paralysis may be possible without full information on the organization and properties of spinal cord networks (Edgerton & Roy 2002), but this must be facilitated by optimizing any therapeutic interventions through genuine understanding of network function and plasticity.

Clearly, technological advances are needed to analyse the interactions between the large numbers of individual cellular and subcellular network components under conditions that are as physiologically relevant as possible. In addition, conceptual advances are needed that allow the multiple parallel and distributed network effects to be placed in context. The field is characterized by the production of an enormous amount of data that relate to molecular, chemical, cellular, synaptic, and developmental mechanisms. What is lacking is a coherent framework that

allows a unified view or general laws of how effects at these different levels interact, as well as the recognition of the level of analysis and explanation that should be attempted. Maybe we cannot hope for a unified theory of network or brain function, but a range of theories that help us to explain specific functions (e.g. anxiety or depression, or at least the biological component of these effects).

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