

# Synapse signalling complexes and networks: machines underlying cognition

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## Summary

All thoughts and actions are encoded in patterns of neuronal electrical activity. Circuits of nerve cells connected by synapses are dedicated to processing information in these patterns. Information is not only transmitted across the synapse but also monitored by postsynaptic molecular machines. These machines are macromolecular complexes of ~100 proteins organised into a network of protein interactions. The network can be mathematically described as a scale-free network. Components of the complexes are necessary for decoding the neural code and converting electrical information into biochemical changes. The network properties of these complexes may explain many of the features of neuronal plasticity and cognitive function in rodents. Importantly, these multiprotein complexes and their network properties shed new light on the basis of human cognitive diseases including schizophrenia, autism, Huntington's disease and mental retardation. Supplementary material for this article can be found on the BioEssays website (<http://www.interscience.wiley.com/jpages/0265-9247/suppmat/index.html>). *BioEssays* 25:1229–1235, 2003.

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## Introduction

The machine analogy has been a longstanding tool of biologists.<sup>(1)</sup> Although many biological phenomena can be modelled with the machine analogy, perhaps the most important progress in the latter part of the 20th century has been

the development of tools for the identification of the molecular 'parts list' of biological machines. This has been facilitated by the genome projects in combination with classical reductionist molecular biological approaches. One of the most exciting areas of biology in this postgenomic era is the move from single gene/protein studies toward the study of whole sets of molecules that work together as machines. This should lead to an understanding of the emergent properties of the genome and converge with classical physiology.

Muscle contraction, the immune response or action potential generation are examples of physiological phenomena that are only partly explained by the discovery of a single 'key' molecule. For example, immunoglobulin gene rearrangement<sup>(2)</sup> provides an insight into clonal selection of B cells. It is clear, however, that this explains only a part (albeit a very important part) of the overall physiology of the immune response. It is now recognised that physiological phenomena will comprise multiple key processes, each one involving molecules organised into machines, and orchestrated by regulatory machinery. The success of single gene genetics and cell biology has been to link single molecules to physiological function, which in many cases has bypassed the machine. New methods, including those using proteomics and bioinformatics, will play an important role in filling this gap between single molecules and molecular machines and thus explain physiology.

Understanding the physiology of cognitive function has focussed attention on the identification of neural mechanisms involved with information processing.<sup>(3)</sup> All perceptions of the environment arise in the brain as a result of sensory end-organs encoding their signals as action potentials.<sup>(4)</sup> The pattern (rate, frequency etc.) of action potentials is passed from neuron to neuron across synapses and disseminated amongst circuits of billions of nerve cells each with thousands of connections.

A key feature of cognitive function is the storage and recall of experience—the phenomenon of learning. Historically, the notion that learning results from 'plastic' changes in the brain dates to antiquity.<sup>(5)</sup> Models where neuronal firing lead to changes in synapse 'resistance' emerged with the neuron doctrine of the late 19th century and shortly before Sherrington's discovery of synaptic transmission.<sup>(6)</sup> The single strongest model that was proposed by Hebb,<sup>(7)</sup> and was a

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Funding agencies: SGNG was supported by the Wellcome Trust and Genes to Cognition Programme ([www.genes2cognition.org](http://www.genes2cognition.org)).  
DOI 10.1002/bies.10381  
Published online in Wiley InterScience ([www.interscience.wiley.com](http://www.interscience.wiley.com)).

Abbreviations: LTP, long-term potentiation; LTD, long-term depression; NMDA, N-methyl-D-aspartate; PSD-95, post-synaptic density 95; [www.PPID.org](http://www.PPID.org), protein protein interaction database; AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxasole-4-propionic acid; cDNA, complementary DNA; NRC, NMDA receptor complex; NF-1, Neurofibromatosis 1; Rsk-2, ribosomal protein S6 kinase; L1, nNeural cell adhesion molecule L1; mRNA, messenger RNA.

synthesis of the neurophysiological data of his day, is stated in his neurophysiological postulate:

*When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cell firing B, is increased.*

Hebb's neurophysiological postulate, which suggests a metabolic change is important, implies a requirement for a molecular machine(s) that detects and converts patterns of electrical activity into biochemical changes in the neuron. Several decades after Hebb's postulate, electrophysiologists found that artificially produced patterns of neural activity resulted in changes in the electrophysiological properties of nerve cells.<sup>(8,9)</sup> Using pharmacological and later genetic approaches, during the 1980s and 1990s many molecules were found that were involved with synaptic transmission and its plasticity. The most unexpected revelation was that synaptic plasticity was not accomplished by a small number of molecules, but was the manifestation of the function of dozens of proteins.<sup>(10)</sup> Thus the molecular basis of Hebb's postulate must involve a high degree of molecular complexity.

### **The NMDA receptor, plasticity and the molecular complexity of the postsynaptic terminal**

The central paradigm for the identification of single genes and proteins in synaptic plasticity has been the use of the rodent hippocampus slice preparation.<sup>(11)</sup> This preparation has a relatively simple neuroanatomy with readily identifiable populations of synaptic connected neurons (e.g. CA3 and CA1 pyramidal neurons). Moreover, the neuronal properties found in hippocampus neurons are similar to many other neurons in the central nervous system. Typically, 400  $\mu\text{m}$  thick sections of the hippocampus are bathed in an organ culture and impaled with electrodes for stimulation of presynaptic axons and the postsynaptic response is recorded, thereby allowing the quantification of efficacy of synaptic transmission. Patterns of neural activity can be elicited and the changes in synaptic strength measured in the presence or absence of a drug or mutation. A commonly used protocol is to stimulate with a train of 100 Hz for 1 second and measure the long-term potentiation (LTP) of synaptic transmission 60 minutes later. Other trains also produce LTP, whereas some protocols, such as 1 Hz trains, can produce long-term depression (LTD) of synaptic transmission.<sup>(12)</sup> In the synapses formed between the axon projections from CA3 to CA1 neurons, both LTP and LTD are dependent on the function of the NMDA subtype of glutamate receptor.<sup>(12,13)</sup> These NMDA receptor-dependent forms of synaptic plasticity are widely studied and also known to occur at most excitatory synapses in the mammalian central nervous system.

Using the experimental paradigm described above, dozens of synaptic localised proteins were found to be involved in

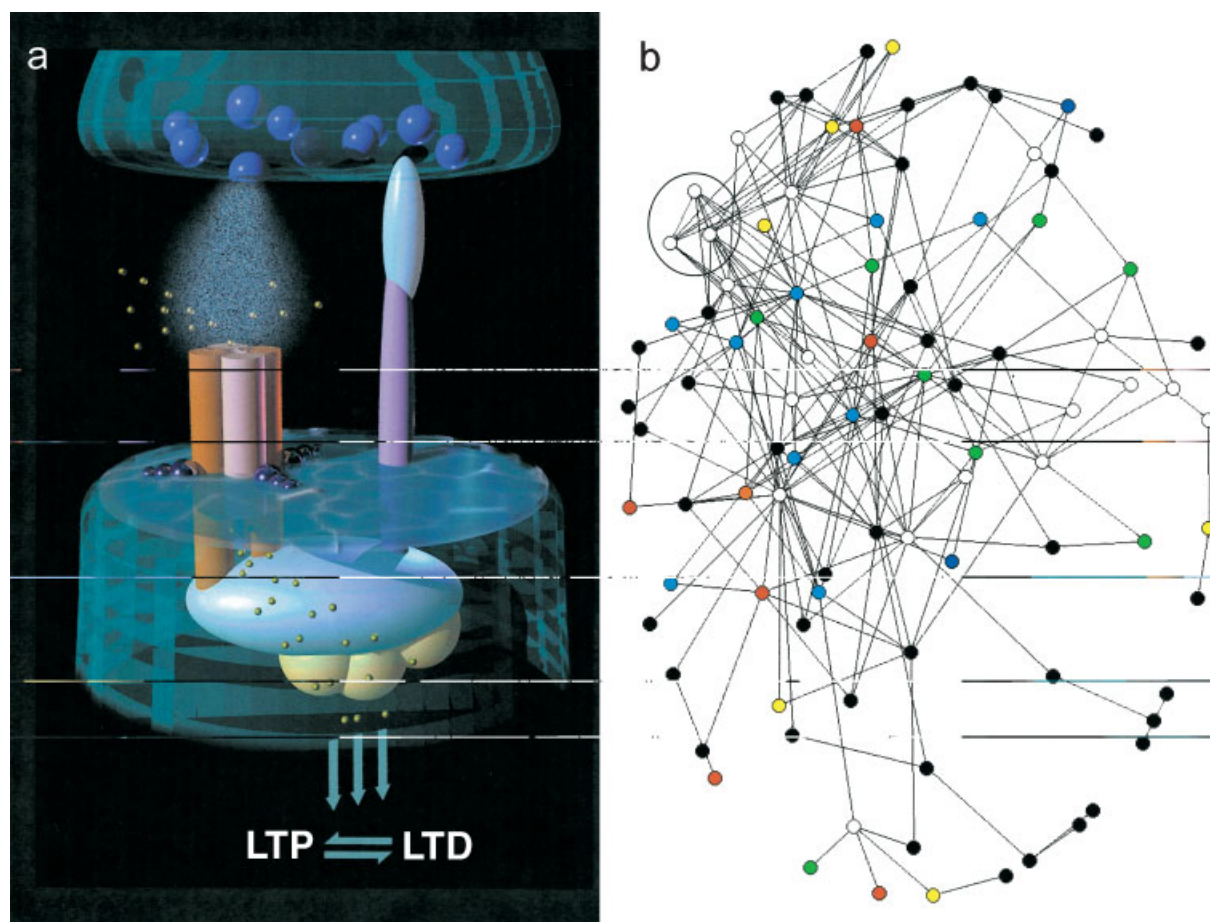
NMDA receptor dependent plasticity (reviewed in Ref. 10). In parallel with these physiological studies, considerable progress in recent years has been made in identifying proteins that are localised to the synapse (see reviews in Refs. 14,15). Since NMDA receptors are found on the postsynaptic membrane at the synapse, I will focus my discussion toward those proteins involved with the NMDA receptor and those forming a multiprotein complex with the receptor. For a general review of postsynaptic proteins and glutamate receptor regulation see Refs. 14,15.

### **Postsynaptic NMDA receptor multiprotein complexes for plasticity and cognition**

The first direct evidence that a postsynaptic multiprotein complex could be involved in plasticity and learning emerged from the study of synaptic plasticity in mice carrying a mutation in the Post Synaptic Density 95 gene.<sup>(16)</sup> PSD-95 is a postsynaptic protein capable of binding many proteins including the intracellular tail of NMDA receptor subunits,<sup>(17)</sup> thereby acting as a scaffold for assembly of signalling complexes. The PSD-95 mutant mice showed impaired spatial learning and altered synaptic plasticity. The mutation did not affect the NMDA receptor channel function but changed the intracellular signalling. This suggested that a complex, tethered to the channel, regulates the conversion of electrical signals to cellular changes. Figure 1a shows a schematic representation of an NMDA receptor complex.

The complexes of PSD-95 and NMDA receptor have been characterised using biochemical purification and subsequent analysis with proteomic methods<sup>(18,19)</sup> and also by cDNA cloning using the yeast 2-hybrid approach (see references within<sup>(15,19)</sup> and [www.PPID.org](http://www.PPID.org) for information on specific protein-protein interactions). NMDA receptor complexes (NRC) have been isolated using several affinity methods including antibodies to NMDA receptor subunits, PSD-95 and other complex components and also using peptides that bind the PDZ domains of PSD-95.<sup>(20)</sup> NMDA receptor complexes range in size between 2000 and 3000 kDa, which is a similar size to a ribosome.<sup>(18)</sup> Proteomic studies reveal a high degree of complexity with initial studies revealing 77 proteins and more recent studies, using modified protocols expanding this list to 185 proteins.<sup>(21)</sup> Although this appears a daunting number it is not unlike the increase in the number of spliceosome components from 46<sup>(22)</sup> to 145<sup>(23)</sup> seen with similar methods. Importantly, the AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid) class of glutamate receptor, found at synapses with the NMDA receptor, contain 10-fold less complexity.

How can the function of these complexes be characterised? There has been a limited amount of biochemical reconstitution approaches (e.g. expressing individual recombinant proteins and mixing subunits *in vitro*). The majority of experiments have involved the expression of cDNAs in



**Figure 1. a:** Connectivity of multiprotein complexes. Schematic of multiprotein complex at the synapse with presynaptic terminal with synaptic vesicles (top) and dendritic spine (bottom). NMDA receptor subunits (pink and orange cylinders) associate with PSD-95 (internal blue shape) and other proteins (yellow) in 2Mda complexes. The protein extending from PSD-95 across the synapse is an adhesions protein (neuroigin). Glutamate release from the presynaptic terminal leads to  $\text{Ca}^{2+}$  influx (gold spheres), which together with activates the complex and leads to synaptic plasticity (LTP and LTD, as indicated by arrows). Adapted from Ref. 16. **b:** Network connectivity of multiprotein complex proteins. Proteins identified in the complexes are drawn according to their binary protein interactions. The circle indicates the position of NMDA receptor subunits (NR1, NR2A, NR2B). The dense interconnectivity follows a scale-free network topology. Individual proteins are represented once.

transfected cells (neurons or heterologous cells), which have been useful for identifying interactions. The studies of synaptic plasticity, particularly those that require testing the response to different patterns or trains of activity, are best suited to brain slices and examination of the role of these proteins in cognition involves testing the animal in behavioural tests. In the whole animal setting, the dominant approaches have been transgenic methods using knockout or overexpression in genetically manipulated mice or drugs that have selective effects toward specific proteins. Table 1 (and supplementary material on the BioEssays website (<http://www.interscience.wiley.com/jpages/0265-9247/suppmat/index.html>)) shows a list of NRC proteins that have been disrupted

using genetic or pharmacological approaches in mice and rats and those that are necessary for normal synaptic plasticity (LTP or LTD) are specified. In summary, 37 NRC proteins were reported to be essential. The number of molecules can be increased (51) if the criteria are relaxed to include molecules that are reported to change in response to the induction of plasticity.

This set of NRC proteins was also annotated for those that are essential for forms of learning (spatial learning and cue/contextual conditioning). 33 proteins are required and 26 of these are required for synaptic plasticity. This annotation of 'single molecule' perturbation experiments suggests that these proteins have a common function in synapses, namely

**Table 1.** Summary of biological roles of NMDA receptor complex proteins

Category	Total	Plasticity	Behavior	Psychiatry
Channels and receptors	12	6	5	7
MAGUKs/adaptors/scaffolders	21	4	3	5
Ser/Thr kinases	8	4	4	2
Tyr kinase	2	2	1	0
Protein phosphatases	7	5	3	2
G-proteins and modulators	19	7	4	6
Signalling molecules and enzymes	51	11	11	15
Transcription and translation	5	0	0	0
Cytoskeletal and cell adhesion molecules	35	7	5	5
Synaptic vesicles and protein transport	22	5	3	4
Novel	3	0	0	0
Total	185	51	39	46

185 proteins in NRCs were annotated according to their molecular function and their reported roles in synaptic plasticity, rodent behaviour and human psychiatric diseases. A detailed list with individual molecules, accession numbers and specific references to annotated information is provided in the supplementary material on the BioEssays website (<http://www.interscience.wiley.com/jpages/0265-9247/suppmat/index.html>).

Columns: Category, protein type; Total, number of molecules found in NRC; Plasticity, number of molecules involved with synaptic plasticity (long-term potentiation or depression); Behaviour, rodent behaviour includes evidence of involvement in spatial learning, cued/contextual learning; Psychiatry, major psychiatric disorders (schizophrenia, mental retardation, bipolar disorder and depression).

the participation in synaptic plasticity. This function may itself underpin the role that these proteins have in learning.

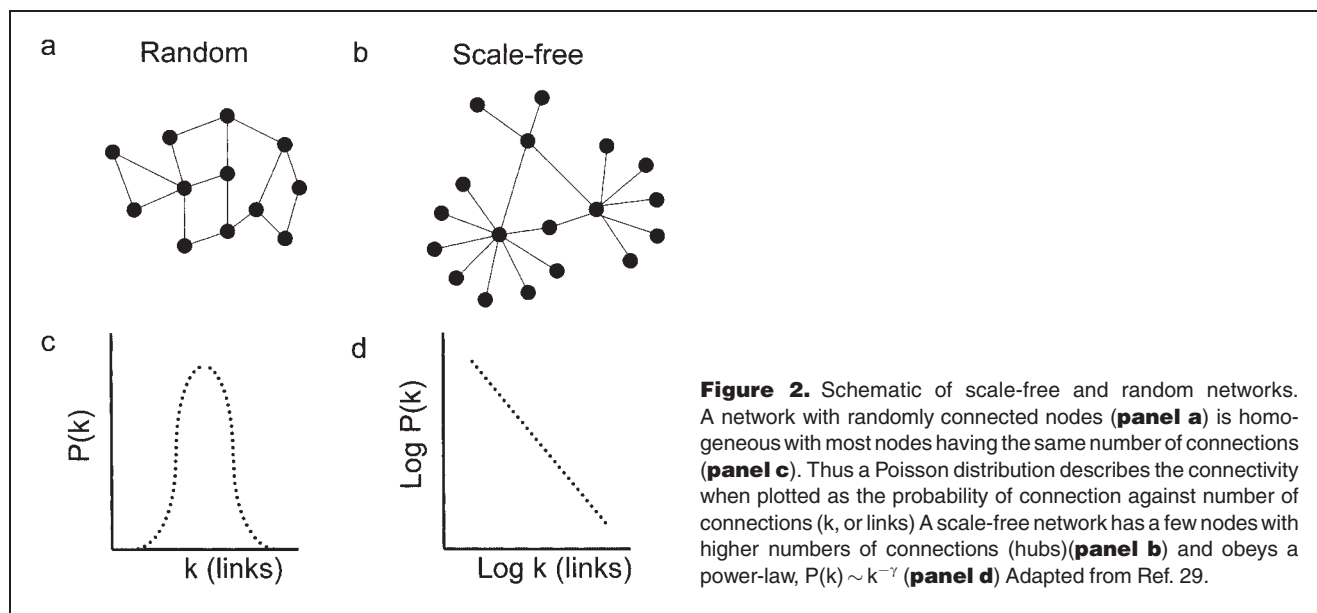
### Network approaches to the higher-order organisation of complexes

Motivated by the knowledge that many binary protein–protein interactions had been defined and that mutations in NMDA receptor interacting proteins altered the signalling of the receptor, a protein–protein interaction database ([www.PPID.org](http://www.PPID.org)) for analysing mammalian protein interactions was constructed.<sup>(24)</sup> It contains 4000 interactions for ~1700 proteins harvested from the reported literature. PPID also has a ‘route finder’ tool for identifying the known interaction routes between pairs of proteins. Using 100 protein in NRCs, we mapped their connectivity (Fig. 1b). Proteins are represented once in these graphs and thus illustrate the functional impact of their disruption and their role in multiple pathways. Hub proteins are those with high numbers of connections. At first glance these maps look confusing and do not appear to help. However, the mathematics of networks, which has been developed in non-biological settings has proved useful in simplifying this complexity.<sup>(25,26)</sup>

Networks are represented in graph theory by nodes connected by edges (links), and the simplest networks comprise randomly linked nodes, where each node has a similar number of links and is known as a random network<sup>(27)</sup> (Fig. 2). A network that includes occasional long-range connections and a small number of highly connected nodes (hubs) produces a very different network, which is heterogeneous and known as a scale-free network.<sup>(28)</sup> These two classes of network can be readily identified by graphing the distribution of the probability,  $P(k)$ , that a node has  $k$  links; a random network follows a

Poisson distribution whereas a scale-free network has no peak, but follows a power-law ( $P(k) \sim k^{-\gamma}$ ) where it appears as a negatively sloped straight line on a log–log plot.<sup>(28,29)</sup> Large datasets of binary protein interactions have been obtained and represented in complex networks for *Saccharomyces cerevisiae* (1870 nodes, 2240 links).<sup>(30)</sup> We reported elsewhere that mammalian protein interaction networks and the proteins in the NRC are connected also obey a scale-free topology ( $P < 10^{-5}$ ).<sup>(21)</sup> Moreover, Barabasi and colleagues have documented that metabolic networks in all eukaryotes follow a scale-free architecture.<sup>(29)</sup> This suggests that the simple design principles underlying protein interaction networks and pathway organisation are evolutionary conserved from yeast to humans and contribute to the architecture of multiprotein complexes.

A striking feature of scale-free networks is their ‘small world’ property, which has been used to explain the short distances between individuals in social networks and pages on the world wide web amongst others.<sup>(25,26)</sup> This property can be quantified using the network diameter, which is the average of the shortest path (number of links) between all pairs of nodes in a network. The diameter of the NRC network was 3.26 and the overall postsynaptic proteome was 3.82. Thus, the dendritic spine, which is the subcellular compartment that harbors the postsynaptic proteome, is a small world where its proteins are on average less than 4 connections from any other and organized into a scale-free network. The small world nature of multiprotein complexes implies they are tightly organised, highly interconnected structures. It also suggests that changes in one protein could readily alter the action of many other proteins. This idea might help explain how knockout of any one protein often alters the overall function of the complex.



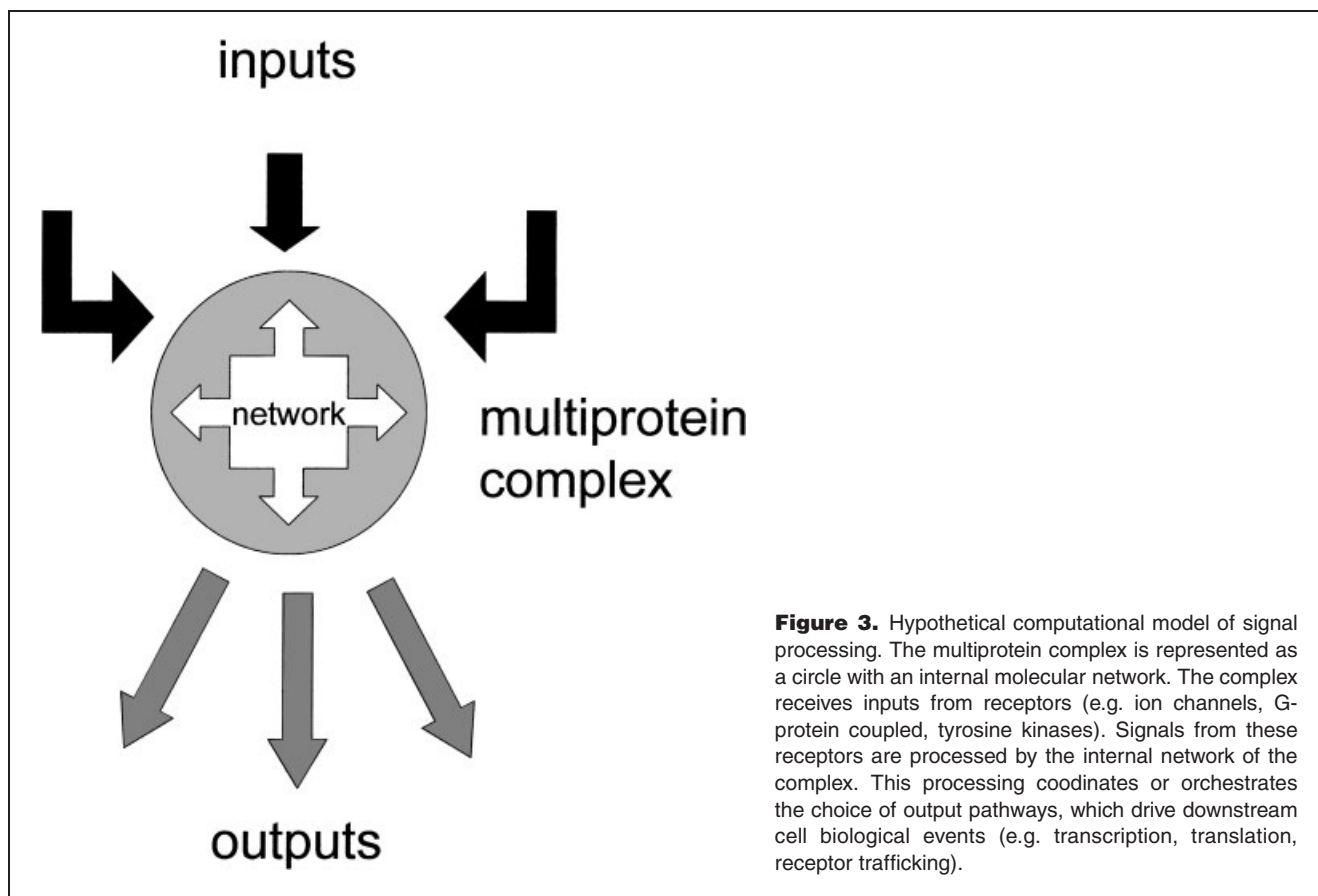
### The biological implications of network organisation of complexes

We have used the NRC model to ask if the network diagrams might be useful for understanding general biological properties of signalling complexes. Although the following sections are quite speculative, it is possible to ask two general questions: i) can a network be used to model the computational properties of a signalling complex?, and ii) how could a network-based signalling machine explain features of cognitive function and mental illness?

#### A network model of signalling within multiprotein complexes

The simplest model is that NRCs 'compute' information at three levels; the inputs, internal (intracomplex) signalling, and outputs (Fig. 3). The inputs to the complex are receptors. These include ion channels (e.g. NMDA receptors, voltage-dependent calcium channels), metabotropic (G-protein coupled) glutamate receptors and growth factor receptors (kinases).<sup>(31)</sup> These receptors feed into the common target of the NRC. Within the complex, there are many signalling proteins and components of known pathways (e.g., Ras-ERK pathway). Also, the major kinase/phosphatase components of synaptic plasticity signalling pathways are components of the complexes. Although the details of their interactions and cross-talk are poorly understood, the small world nature of this complex offers ample opportunity for cross-talk. The output from the complex is to downstream effector mechanisms. The receptors in the complex and many of the internal enzymes are known to regulate translation, transcription, dendrite structure and trafficking and dynamics of synapse components (notably AMPA subtypes of glutamate receptors).

The network model predicts that the complex is both robust to perturbation and also susceptible to interference by disruption of its many components. At first this may seem paradoxical, however, a simple analogy to the automobile may be useful: the loss of any component will interfere with its overall function but most bits are accessories with little major role and are only used for quite specific functions (e.g. the rear view mirror). Using network simulation tools designed to examine how airline networks, the world wide web and other networks are resistant to loss of nodes, we simulated the effect of knocking out each node from the NRC network and measured the change in network diameter. As is the case for scale-free networks, this showed that the most highly connected (hub) proteins were particularly important for network integrity. In addition, the severity of the biological phenotype was also predicted by the modelling of network disruption. Knockout of individual proteins in synaptic plasticity does not completely abolish plasticity, but only partially impairs it. We used quantitative data from synaptic plasticity studies and found a strong correlation between the severity of the phenotypes (to mutation or drug) and the predicted impact on the network diameter. Similar results have also been observed for growth and robustness to mutation in *S. cerevisiae* that shows a strong relationship between links and phenotype.<sup>(30)</sup> Thus the network model may be useful in explaining how many molecules can work together to produce a phenotype, and to predict the impact of disruption. A useful analogy for thinking about a network of signalling proteins is that of traffic through an airline network. The hub proteins are like hub airports, which provide links to most other proteins and thereby produce the small world property. If a node is lost, then this will alter the traffic in the network and signals will have to proceed in alternative



routes. These alternative routes may provide the redundancy and robustness seen in signal transduction pathways and present a picture of how signalling enzymes engage in cross-talk.

#### *Complexes in cognition and mental illness*

As shown in Table 1, many NRC proteins were important for cognitive function in rodents. Earlier proteomic studies of the NRC found three component proteins (NF-1, Rsk-2, L1) where human mutations in the genes encoding these proteins are responsible for the cognitive deficits (childhood learning disability).<sup>(19)</sup> As an extension of this analysis, Table 1 shows a summary of all NRC proteins that are reported to change in human psychiatric conditions (a complete list is shown in supplementary material on the BioEssays website (<http://www.interscience.wiley.com/jpages/0265-9247/suppmat/index.html>)). These reports include correlative evidence, such as changes in the level of mRNA or protein expression in tissue samples of affected individuals. Overall, 46 NRC proteins were implicated in mental illness. Surprisingly, there was an apparent bias toward human cognitive disorders: 26 in schizophrenia, 18 in mental retardation, 7 in Bipolar disorder and 6 in depressive illness. Several important

proteins are closely linked to PSD-95; PSD-95 directly binds neuroligins,<sup>(32)</sup> ERB4<sup>(33)</sup> (neuregulin receptor) and huntingtin<sup>(34)</sup> which are genes involved with autism,<sup>(35)</sup> schizophrenia<sup>(36)</sup> and Huntington's disease<sup>(37)</sup> respectively. These diseases have in common the feature that cognitive impairments are in their primary symptoms. These reports support the view that alterations in the expression of NRC proteins can be directly or indirectly involved with human cognitive diseases.

#### **Conclusions**

It is now emerging that NRCs are molecular machines found at synapses, where they process information encoded in patterns of neural activity. These multicomponent complexes can be activated via  $\text{Ca}^{2+}$  influx from channels and receptors allowing networks of interacting proteins and pathways to orchestrate the multiple signaling pathways and cellular mechanisms for the expression of plasticity. Different patterns or frequencies of synaptic activity use different proteins<sup>(38)</sup> and routes in the network. In addition to molecular biological and biochemical approaches, the future study of molecular machines will be dependent on novel bioinformatics tools. The protein interaction database ([www.PPID.org](http://www.PPID.org)) was essential

for our studies as well as mathematical model of networks. The network model opens an entirely new platform on which to design future experiments. The network has proven useful in predicting the relative importance of proteins found in our lists and they can be prioritised for investigation. Moreover, the network model can be refined by the inclusion of more detailed information other than protein interactions. For example, regulatory information on phosphorylation and other enzymatic activities can be modelled.

One overzealous prediction is that the world of single gene/protein biology is now giving way to the world of complexity, networks, emergent biology and the problems of many molecules working together. We may see a shift away from the 'single molecule switch' point of view, toward the regulatory machine models. Along with this shift will be a growth in the complexity of molecular interactions and perhaps the disappearance of the simple linear biochemical pathway.

### Acknowledgments

J.D. Armstrong, H. Husi, M. Cumiskey, N.H. Komiyama, P.M. Visscher, W. Blackstock, J. Choudhary and T.J. O'Dell for discussion, J.D. Armstrong for network modelling and A. Delaney for assistance with Supplementary Table 1.

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