Synapse molecular complexity and the plasticity behaviour problem

Seth G. N. Grant

Abstract

Synapses are the hallmark of brain complexity and have long been thought of as simple connectors between neurons. We are now in an era in which we know the full complement of synapse proteins and this has created an existential crisis because the molecular complexity far exceeds the requirements of most simple models of synaptic function. Studies of the organisation of proteome complexity and its evolution provide surprising new insights that challenge existing dogma and promote the development of new theories about the origins and role of synapses in behaviour. The postsynaptic proteome of excitatory synapses is a structure with high molecular complexity and sophisticated computational properties that is disrupted in over 130 brain diseases. A key goal of 21st-century neuroscience is to develop comprehensive molecular datasets on the brain and develop theories that explain the molecular basis of behaviour.

Introduction

The most important technical advance in neuroscience in the latter decades of the 20th century was the introduction of molecular biology. In the late 1980s, gene cloning was first applied to molecules of the nervous system and since then there have been remarkable advances with innovative new methods and datasets that have transformed our thinking about how the brain is built and how it works. Thanks to the 'omic' approaches – genomics, transcriptomics, proteomics – we now know all the genes, RNAs and proteins that make up the brain. Genomic methods led to the discovery of thousands of gene mutations and variants causing hundreds of developmental, neurological and psychiatric disorders. In this chapter, I will present a perspective on the 'past, present and future' with a focus on the synapse. I contend that much of our thinking about the function of synapses comes from an era that predates the molecular revolution and we are only beginning to embrace molecular complexity and the challenges it presents to the standard dogma.

Synapses and the Neuron Doctrine

The basic model of how behaviour works remains rooted in the Neuron Doctrine, which arose in the late 19th century from the microscopy and neuroanatomical findings of Cajal ([1909] 1911) and his contemporaries. This model posits that neurons are the basic unit of the nervous system and that ensembles or circuits of connected neurons are the anatomical and physiological unit of each behaviour. At the same time, it was also proposed that the modification of behaviour – learning – leads to changes in the 'resistance' or strength of synaptic transmission (Berlucchi and Buchtel, 2009). This synaptic strength model (hereafter referred to as the Long-Term Synaptic Strength (LTSS) model) posits that the increased efficiency of the learned behaviour is to be found in the increased efficiency of synaptic transmission.

This is an example of 'top-down' and anthropomorphic logic as it postulates that the psychological phenomenon would be recapitulated in a homologous cellular neuroanatomical phenomenon. While anthropomorphic theories were commonplace in the 19th century, they are widely regarded today as cardinal scientific mistakes. It is also important to note that when the LTSS model was proposed, there were no cellular electrophysiological data to support it. Its enduring attraction may in part be because of the 'it-makes-sense' perspective. This is a potentially risky logical position because it encourages inductive reasoning and a search for supporting evidence, which by itself cannot be considered as proof of the model. Moreover, the quest to find data to support a model can bias thinking away from the development of

Genes to Cognition Programme, Centre for Clinical Brain Sciences, The University of Edinburgh, Edinburgh, UK

Corresponding author:
Seth G. N. Grant, Genes to Cognition Programme, Centre for Clinical Brain Sciences, The University of Edinburgh, Edinburgh EH16 4SB, UK.
Email: seth.grant@ed.ac.uk

alternative hypotheses. Is there any evidence that these are legitimate concerns and what kinds of data might lead us to consider alternative models?

In the classical circuit models of the Neuron Doctrine, the role of the synapse is very simplistic. Its main role is to transmit information so that a connected set of neurons (the circuit) fire action potentials and produce a behavioural output (e.g. muscle contraction or release of a hormone). Implicit in this connectionist model of behaviour is the view that each behaviour has its own circuit (Kandel et al., 1991). With learning, the circuit can be reinforced to fire because the strength of synaptic transmission is increased. It is important to keep these circuits separate so that learning in one circuit does not inadvertently interfere with another behaviour. And, if two behaviours are to be linked, then the model postulates that there is another set of neurons that connects the two circuits together through strengthened synapses. This theory for the organisation of behaviour was robustly articulated by Hebb (1949).

By the 1960s and 1970s, electrophysiologists studying synaptic transmission searching for activity-induced LTSS changes in central synapses had found many forms of short-term plasticity (e.g. facilitation) before eventually finding long-term potentiation (LTP; Bliss and Lomo, 1973). Irrespective of the psychological significance of these findings, they reveal that synapses are highly sensitive to information encoded in sequences of neural activity and that they can control synaptic strength on time scales from milliseconds to hours or more. Thus, synapses are not simple connectors, but capable of computing highly specific responses that draw upon their capacity to read the information in patterns of nerve cell activity (also known as the neural code).

Prior to molecular neurobiology and genetics, there was very little known about the molecular mechanisms employed by synapses to compute the information in patterns of activity or whether they were important in behaviour. Pharmacological approaches were the main avenue until 1992, when mouse gene targeting was introduced (Grant et al., 1992; Silva et al., 1992). The ability to interfere with synaptic molecular mechanisms using drugs or mutations allowed scientists to go beyond correlative studies and to test hypotheses about the role of specific molecules and the physiological correlates of behaviour.

Challenging the dogma with molecular approaches

Glutamate is the main neurotransmitter in vertebrate excitatory synapses and drugs were developed that blocked ionotropic glutamate receptors. During the 1980s, attention focussed on the role of the N-methyl-D-aspartate (NMDA) receptor in learning because NMDA receptor antagonists interfered with the induction of LTP and, if infused systemically or into the brain, interfered with the ability of rats to learn in a water maze (Bliss and Collingridge, 1993; Morris et al., 1986). This is a correlation and does not prove that LTP is the causal mechanism of learning – it merely shows that the NMDA receptor is involved in both LTP and the behaviour in this maze.

These experiments were conducted before the NMDA receptor was cloned, or for that matter any other synaptic molecule – it was an era in which our knowledge of the molecular biology of the synapses was virtually non-existent. It was also an era when very little was known about the electrophysiology and cell biology of the NMDA receptor, and we now know that the receptor controls many synaptic and cellular properties (in neurons and non-neuronal cells). For example, the NMDA receptor is required for forms of rapid short-term plasticity (Chamberlain et al., 2008; Larsen and Sjöström, 2015; Urban-Ciecko et al., 2014), transcription (Coba et al., 2008) and protein turnover (Kandel et al., 2014; Scheetz et al., 2000). Thus, the blockade of NMDA receptors and interference with LTP and behaviour cannot be simply assigned to any one of the many cell biological processes (including LTP) that the NMDA receptor regulates.

It is also important to recognise that *in vivo* recordings show that learning induces changes in multiple electrophysiological parameters including short-term and long-term plasticity (Gruart et al., 2015). The interpretation of the behavioural experiments that showed NMDA receptor antagonists specifically interfere with spatial learning is further complicated by the effects of the drugs on sensorimotor and other systems in which the NMDA receptor plays a role: the impairments in maze performance could arise from interference with these other behaviours (Cain, 1998). Relevant to this, NMDA receptor antagonists and mutations interfere with many innate instinctive behaviours (Ryan et al., 2013), further emphasising the fact that the NMDA receptor is not specific to learning (for example, it regulates behaviour in open fields, motoric performance, anxiety, among others) and that interference with these innate behaviours can interfere with performance in learning tasks (Cain, 1998). In fact, if rats are pretrained in the apparatus, then blocking the NMDA receptor (and LTP) does not interfere with spatial learning (Bannerman et al., 1995, 2014). This further supports the view that NMDA receptors independently regulate LTP and learning and that LTP is not causally linked to learning. A further challenge to the LTSS model was provided by the discovery that mutations in PSD95 (a postsynaptic protein that binds to the NMDA receptor) cause a learning deficit and an increase in LTP (Migaud et al., 1998; Nithianantharajah et al., 2013). This and many other genetic dissociations directly challenge the traditional model.

It is often overlooked when considering scientific proof, but dissociations are far more important than correlations, as they falsify the causal link between LTP and learning. These brief notes are by no means a comprehensive analysis or review of this literature but are presented to make the reader aware that the LTP model of learning remains highly controversial. These molecular perturbation experiments are one avenue of molecular research that has forced a reconsideration of our assumptions regarding the role of synapses in behaviour.

A second avenue has been the characterisation of the synapse proteome – the protein constituents of synapses. In 2000, my research group performed the first proteomic studies on the NMDA receptor and postsynaptic protein complexes and found 77 proteins. Using more sensitive methods, this number increased to ~300 (Fernandez et al., 2009; Husi et al., 2000; Husi and Grant, 2001) and proteomics of the whole postsynaptic terminal of vertebrates recovers even more proteins: the postsynaptic proteome contains >1000 proteins and the overall synapse proteome comprises 2000–3000 proteins (Bayés et al., 2011, 2012, 2017b; Collins et al., 2006; Distler et al., 2014). This is a truly remarkable degree of molecular complexity because it had been thought that neurotransmission and LTP could be achieved with only a handful of proteins (Nicoll, 2017).

The reality is that the postsynaptic molecular machinery is vastly more complicated than had been anticipated. Moreover, phosphoproteomic studies showed that the activation of the

NMDA receptor modified hundreds of phosphorylation sites in over 120 postsynaptic proteins (Coba et al., 2009; Collins et al., 2005; Li et al., 2016). These proteins control short- and long-term plasticity, many intracellular signalling pathways, protein structure, interactions and synthesis, which further reaffirms the fact that the NMDA receptor cannot be thought of as specialised for LTP. It has a broad range of cell biological functions. More generally, the molecular complexity of the postsynaptic proteome poses an existential crisis to the traditional view that the synapse is a simple connector that is strengthened with learning. It is, in fact, a highly sophisticated molecular computer.

A 'bottom-up' molecular perspective on the synapse in behaviour

I have emphasised how the 'top-down' approach, which starts from behaviour, led to the discovery of synaptic physiological phenomena that appeared to fulfil the LTSS model of learning, but along the way there were unexpected findings that complicated and challenged the initial hypothesis. A completely different approach to thinking about the role of synapses in behaviour is to consider it from the 'bottom-up' – from the parts lists of proteins that comprise the highly complex proteome. As a starting point, we can ask a range of basic questions: What types of proteins are found in synapses and how are they physically organised? How do they detect and discriminate the patterns of neural activity? What are the roles of these proteins in short- and long-term plasticity and different behaviours? In the following sections, I will outline, using two different approaches, how this bottom-up molecular approach can be used to understand the role of synapses in behaviour. The first of these is an evolutionary approach, whereas the second will be one in which we examine how the individual proteins that comprise the complexity of the postsynaptic proteome are physically organised into higher-order assemblies. In contrast to the top-down approach, this bottom-up approach does not set out to understand learning as its primary objective; instead, its primary objective is to understand the physiological and behavioural characteristics endowed by synapse proteome complexity.

How evolution built synapses

One of the most insightful and instructive maxims is that 'Nothing in biology makes sense except in the light of evolution' (Dobzhansky, 1973). Indeed, the exploration of synapse molecular evolution has radically changed many aspects of our understanding of the brain and behaviour. Quantitative and systematic studies of synapse evolution were made possible by synapse proteomics together with genome data (Emes et al., 2008). A major surprise was that virtually every class of synapse protein found in mammals had first evolved in unicellular organisms, before metazoans, and therefore before the first neurons and nervous systems (Emes et al., 2008; Emes and Grant, 2011, 2012; Ryan and Grant, 2009). The synapse molecular machinery is therefore more ancient than the neuron. Furthermore, when considering the molecular machinery of the presynaptic terminal versus the postsynaptic terminal, it was discovered that the postsynaptic protein machinery was the most ancient, having first arisen in prokaryotes. Indeed, these fundamental protein components are present in the Last Universal Common Ancestor (LUCA) and therefore highly conserved since the earliest life forms ~4 billion years ago. The presynaptic vesicular trafficking machinery evolved much later, at the transition to eukaryotes ~1.5 billion years ago.

What does this ancient postsynaptic machinery do in prokaryotes, and what does it tell us about the function of synapses? A striking fact is that these prokaryotic proteins include receptor signalling complexes, which are basic multiprotein machines used by cells to detect the external environment and trigger intracellular adaptive responses (Emes and Grant, 2011, 2012; Ryan and Grant, 2009). Their counterparts in mammalian synapses are large (~1.5 MDa) supercomplexes built by PSD95 interacting with neurotransmitter receptors, ion channels and signalling proteins (Emes et al., 2008; Fernandez et al., 2009, 2017; Frank et al., 2016; Ryan and Grant, 2009). In mice, these supercomplexes detect and discriminate patterns of action potentials and control short- and long-term changes in synaptic strength, as well as regulate transcription, protein homeostasis and other intracellular mechanisms. Clearly, prokaryotes are not controlling 'synaptic strength', so what does this ancient machinery do?

Signalling complexes measure time

The signalling complexes in the membrane of a bacterium enable it to sense the chemical signals in its environment and then swim towards or away from the source (Wadhams and Armitage, 2004). It is crucial to appreciate that a bacterium is so small that the receptors on either end of the cell body would not be able to discriminate a chemical gradient. Therefore, the bacterium senses, then swims and senses again, and the differential signal between these sensing events allows it to decide if it is moving in the appropriate direction. This is fundamentally important as it is shows that the most ancient mechanism of adaptive behaviour is in the ability to detect the timing of signals, and the detection of time is a property of the multiprotein signalling complexes. Remarkably, this is precisely what the postsynaptic supercomplexes do – they measure the interval of time between pulses of neurotransmitter. These evolutionary observations indicate that the most ancient and fundamental property of the postsynaptic machinery is the integration of temporal information. It also indicates that temporal integration by signalling complexes is a basic and ancient memory mechanism.

This raises a fascinating and simple alternative to the classical model of synaptic resistance and the LTSS model, namely, that it is temporal detection that is the fundamental property and that the adjustment of strength is a secondary and much later evolved function. It is also worth noting that the capacity to detect patterns of activity is significantly altered in synapses carrying mutations in the scaffold proteins that organise the vertebrate signalling complexes (Carlisle et al., 2008; Cuthbert et al., 2007; Migaud et al., 1998). This indicates that interfering with the measurement of time by synapses results in behavioural disorders.

Synapse diversity as a way of encoding information

Because this temporal decoding capacity is a property of the postsynaptic proteins, it follows that differences in protein expression between synapses will result in differences in the capacity of those synapses to detect and respond to patterns of activity. But how much difference is there in the proteomes of

synapses? We have developed genetic labelling methods that fuse fluorescent proteins to postsynaptic scaffold proteins, making the synapses that express these proteins visible with light microscopy (Broadhead et al., 2016; Fernandez et al., 2017; Zhu et al, 2018). We find different amounts of these proteins in different synapses and that different combinations of two or more proteins generate a variety of different synapse types. We have examined millions of individual synapses in the mouse brain and found that there is a spatial patterning to the different synapse types (Zhu et al., 2018). Mapping synapses across the whole brain generates a 'synaptome map'. The synaptome is the full complement of brain synapses and represents a new frontier in brain 'omic' approaches. The synaptome patterning means that the functional properties of synapses will be determined by the synaptome map. Because the synaptome map is genetically programmed, it follows that this could be a way of encoding instincts. Learning could also be encoded in synaptome maps by changing the proteome composition of the relevant synapses. We refer to this emerging model as the 'synaptomic' model of behaviour.

In the synaptomic model, there is no need to change the long-term strength of synapses to encode information. Information can be encoded by molecular changes that alter short-term plasticity (Zhu et al., 2018). Since short-term plasticity generates an instantaneous postsynaptic response to incoming patterns of activity, then changing synapse proteins would change this response profile.

Another important distinction to the Hebbian connectionist model (Hebb, 1949) is that the synaptomic theory does not even require a behaviour to be encoded by a connected set of neurons: synapses distributed across the brain can participate in a behavioural representation. To illustrate this in the simplest way, imagine that there are two types of synapses (A and B), where each has a particular subset of postsynaptic proteins that determine the pattern of activity that these synapses preferentially respond to (e.g. Type A responds with large amplitudes to 1-Hz and Type B to 10-Hz frequency trains). These synapse types can be distributed anywhere in the brain and can respond to the same pattern of activity. In essence, the molecular composition is a zip code (or postcode as it is known in the United Kingdom) that determines where in the brain any given pattern of activity will produce its response. If, as a result of experience, synapses modify their postsynaptic proteome composition, then there will be new spatial distribution of these zip codes and this can encode a representation of the learned experience.

Evolution of synapse complexity

Evolutionary studies also exposed a surprising molecular explanation for the sophisticated behavioural repertoire of vertebrates. The Neuron Doctrine has underpinned the commonest explanation for the increase in complexity that distinguishes the vertebrate behavioural repertoire from that of invertebrates: more neurons give more behaviours. The study of the molecular evolution of synapses revealed that vertebrates have undergone a remarkable expansion in the molecular complexity of synapses and the genomic mechanisms responsible are now well understood. This was first realised when the postsynaptic proteomes of *Drosophila* and mice were compared and it was discovered that there are many more postsynaptic proteins in vertebrates than in invertebrates (Emes et al.,

2008). Since then, a range of vertebrate species have been examined, including humans, and it is now clear that there are ~1000 highly conserved proteins in the postsynaptic proteome of vertebrate excitatory synapses (Bayés et al., 2011, 2012, 2017b; Collins et al., 2006; Distler et al., 2014). Importantly, in all these species, the protein classes that first evolved in unicellular organisms are found in all metazoans. Thus, the vertebrate expansion in proteome complexity is essentially an increase in the size of each gene/protein family rather than an invention of new proteins.

This expansion in synapse proteome complexity is a hallmark of vertebrate synapse complexity and occurred following two whole-genome duplications early in the vertebrate lineage ~550 million years ago (Emes and Grant, 2012; Grant, 2016). These extra genes and the overall high complexity of vertebrate synapse proteomes must have been of great functional importance since the genes have been under strong purifying selection (Bayés et al., 2011, 2012, 2017a). Indeed, the functional significance has been tested using genetic engineering experiments in mice: it was found that the increased postsynaptic proteome complexity gave rise to a more complex behavioural repertoire and a more diverse capacity of synapses to detect and decode patterns of neural activity (and multiple forms of synaptic plasticity; Grant, 2016; Nithianantharajah et al., 2013; Ryan et al., 2013). These studies led to the Synapse Proteome Expansion theory of vertebrate behavioural complexity (Grant, 2016), whereby each behaviour is controlled by a combination of different postsynaptic proteins. This is an important departure from the classical model where neural circuits specify each behaviour, although these two models are not necessarily incompatible. The increase in neuron number could provide more synapses in which the greater number of protein combinations could be expressed.

The hierarchical organisation of the postsynaptic proteome complexity

A second and orthogonal 'bottom-up' approach to understanding synapses and behaviour is one based on the structural organisation of the proteome. Just as the evolutionary analyses revealed how the very simple molecular machines that first arose in the earliest life forms were the antecedents to the highly sophisticated molecular machinery of synapses in humans, the structural approach reveals that these multiprotein complexes are building blocks of synapses and behaviour. A recent biochemical study of the postsynaptic proteome showed that none of the ~60 proteins examined was found as a monomer; instead, all were assembled into higher-order complexes and supercomplexes (Frank et al., 2016). In total, ~220 of these supramolecular assemblies were identified and fewer than 20 of them were previously known.

How these molecular machines are built from individual proteins is an area of intense interest. One of the best-studied examples in the intact animal is the supercomplexes formed between postsynaptic scaffold proteins (PSD95 and PSD93), NMDA receptors and other molecules. It was discovered that there is a hierarchy of supramolecular complexity, as individual proteins assemble into complexes, and complexes assemble into supercomplexes (Frank et al., 2016, 2017; Frank and Grant, 2017). For example, combinations of NMDA receptor subunits assemble to form the tetrameric ionotropic receptor and combinations of these tetramers assemble with scaffold protein complexes, other ion channels and signalling complexes to form supercomplexes

(e.g. the NMDAR–PSD95 supercomplexes, also called membrane-associated guanylate kinase (MAGUK)-associated signalling complexes). This is a combinatorial assembly process and it is therefore important to know if there are mechanisms that restrict promiscuous combinatorial interactions from occurring. Indeed, it was shown that there are highly specific genetic rules that determine which complexes can assemble into supercomplexes (Frank et al., 2016, 2017; Frank and Grant, 2017). Moreover, these genetic rules control the production of families of complexes and supercomplexes (Frank et al., 2017). These principles are likely to apply to all postsynaptic proteins and will therefore define how the whole postsynaptic density is built.

These molecular studies of protein assemblies are converging with studies of the nanoscale architecture of synapses, a field that has rapidly advanced following the invention of super-resolution microscopy. Fluorescent labelling of postsynaptic proteins (so that they can be visualised with super-resolution microscopes) has shown that the protein complexes/supercomplexes are not randomly distributed within the postsynaptic terminal but are localised into domains called nanodomains or nanoclusters (Broadhead et al., 2016). Examination of synapses in different regions of the hippocampus has revealed that certain cell types and dendritic layers have characteristic numbers of nanoclusters.

How might this hierarchy of supramolecular organisation be relevant to behaviour? In short, the complexes/supercomplexes are performing the task of temporal discrimination and decoding of the patterns of activity. Behavioural genetic experiments show that each component of the behavioural repertoire is controlled by particular combinations of these proteins (Nithianantharajah et al., 2013). Thus, the postsynaptic multiprotein assemblies are specifying the components of the behavioural repertoire and these assemblies are in turn defined by a genetic programme.

Synapse proteome complexity and disease

Prior to the molecular biology revolution, the only known synaptic disease was myasthenia gravis, which is an autoimmune disorder that impacts on neurotransmitters in the peripheral nervous system. With the discovery of synaptic proteins, it begun to emerge that genetic disorders disrupt synaptic proteins. This was established from the proteomic studies of NMDAR–PSD95 complexes, which identified several proteins that cause human intellectual disability (Bayés et al., 2011; Fernandez et al., 2009; Husi et al., 2000; Pocklington et al., 2006). A recent analysis showed that over 145 genes encoding components of PSD95 supercomplexes are now linked to genetic disorders (Bayés and Grant, 2016). The proteomics of the human postsynaptic proteome identified hundreds of mutations in 199 genes that cause over 130 brain diseases (Bayés et al., 2011; Bayés and Grant, 2016b). Thus, the fundamental biology of a huge number of brain diseases is caused by dysfunction in postsynaptic proteins.

It is also very interesting to ask in what way do these disease mutations impact on the hierarchical assembly of proteins into supercomplexes and whether there is any link with the molecular evolution of vertebrate synapses. Mutations can have highly specific effects on the hierarchical assembly of proteins. For example, mutations in PSD93 (as occur in schizophrenia and autism) interfere with the assembly of NMDAR–PSD95 complexes into supercomplexes (Frank et al., 2016). As was found in the mouse, combinations of human mutations in postsynaptic proteins impact upon specific components of the behavioural repertoire (Nithianantharajah et al., 2013). It is very clear that the proteins that evolved from the genome duplications and vertebrate synapse proteome expansion are involved in specific brain disorders. For example, PSD95, SAP102 and PSD93, which are paralogues in the gene family of MAGUK scaffold proteins, are each involved in different mental disorders (Cuthbert et al., 2007; Nithianantharajah et al., 2013; Tarpey et al., 2004). Thus, there is a simple nexus between evolution of the synapse and brain disease: the increase in molecular complexity contributed to more sophisticated and specific regulation of behaviour that expanded the behavioural complexity of vertebrates, and these same genes are rendering the organism susceptible to deleterious mutations that manifest as a spectrum of brain diseases.

Future perspectives

For a century, neuroscience has been dominated by neuroanatomy and electrophysiology, but in the past 25 years the molecular biological revolution has had a profound impact on our understanding of the biology of the brain and disease. We are still in the midst of this revolution and new theories are required that comprehensively describe and explain the many new and surprising observations that continue to emerge. A perspective that is underappreciated, even by many molecular biologists, is that genome evolution is central to understanding the brain and behaviour. It is a truism that the genome built the brain for the genome. It might also be time to review the dominance of the Neuron Doctrine. Perhaps, there should be a Synapse Doctrine based on the organisation of proteomes and molecular machines.

Finally, we are now in an era when large-scale studies are producing comprehensive datasets, such as genomics, transcriptomics, proteomics, synapse maps of the brain, electrophysiology and behaviour. In the next few years, we will see comprehensive maps of molecules and cells across the brain and across the lifespan. These represent a major conceptual change compared with more typical anecdotal approaches. They allow one to look beyond 'the keys under the lamppost' and survey the entire landscape. This comprehensive approach will be increasingly useful for rigorous testing of old models and developing new theories.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

References

Bannerman DM, Good MA, Butcher SP, et al. (1995) Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. *Nature* 378(6553): 182–186.

Bannerman DM, Sprengel R, Sanderson DJ, et al. (2014) Hippocampal synaptic plasticity, spatial memory and anxiety. *Nature Reviews Neuroscience* 15(3): 181–192.

Bayés À and Grant SG (2016) Synapse proteomes and disease: The MASC paradigm. In: Sala C and Verpelli C (eds) *Neuronal and Synaptic Dysfunction in Autism Spectrum Disorder and Intellectual Disability*. London: Elsevier, pp. 85–100.

Bayés À, Collins MO, Croning MD, et al. (2012) Comparative study of human and mouse postsynaptic proteomes finds high compositional conservation and abundance differences for key synaptic proteins. *PLoS ONE* 7(10): e46683.

Bayés À, Collins MO, Reig-Viader R, et al. (2017a) Evolution of complexity in the zebrafish synapse proteome. *Nature Communications* 8: 14613.

Bayés À, Collins MO, Reig-Viader R, et al. (2017b) Zebrafish synapse proteome complexity, evolution and ultrastructure. *Nature Communications* 8: 14613.

Bayés À, van de Lagemaat LN, Collins MO, et al. (2011) Characterization of the proteome, diseases and evolution of the human postsynaptic density. *Nature Neuroscience* 14(1): 19–21.

Berlucchi G and Buchtel HA (2009) Neuronal plasticity: Historical roots and evolution of meaning. *Experimental Brain Research* 192(3): 307–319.

Bliss TV and Collingridge GL (1993) A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature* 361(6407): 31–39.

Bliss TV and Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology* 232(2): 331–356.

Broadhead MJ, Horrocks MH, Zhu F, et al. (2016) PSD95 nanoclusters are postsynaptic building blocks in hippocampus circuits. *Scientific Reports* 6: 24626.

Cain DP (1998) Testing the NMDA, long-term potentiation, and cholinergic hypotheses of spatial learning. *Neuroscience & Biobehavioral Reviews* 22(2): 181–193.

Cajal SR ([1909] 1911) *Histologie du système nerveux de l'homme et des vertébrés*. Paris: Maloine.

Carlisle HJ, Fink AE, Grant SG, et al. (2008) Opposing effects of PSD-93 and PSD-95 on long-term potentiation and spike timing-dependent plasticity. *Journal of Physiology* 586(Pt 24): 5885–5900.

Chamberlain SE, Yang J and Jones RS (2008) The role of NMDA receptor subtypes in short-term plasticity in the rat entorhinal cortex. *Neural Plasticity* 2008: 872456.

Coba MP, Pocklington AJ, Collins MO, et al. (2009) Neurotransmitters drive combinatorial multistate postsynaptic density networks. *Science Signaling* 2(68): ra19.

Coba MP, Valor LM, Kopanitsa MV, et al. (2008) Kinase networks integrate profiles of N-methyl-D-aspartate receptor-mediated gene expression in hippocampus. *Journal of Biological Chemistry* 283(49): 34101–34107.

Collins MO, Husi H, Yu L, et al. (2006) Molecular characterization and comparison of the components and multiprotein complexes in the postsynaptic proteome. *Journal of Neurochemistry* 97(Suppl. 1): 16–23.

Collins MO, Yu L, Coba MP, et al. (2005) Proteomic analysis of in vivo phosphorylated synaptic proteins. *Journal of Biological Chemistry* 280(7): 5972–5982.

Cuthbert PC, Stanford LE, Coba MP, et al. (2007) Synapse-associated protein 102/dlgh3 couples the NMDA receptor to specific plasticity pathways and learning strategies. *Journal of Neuroscience* 27(10): 2673–2682.

Distler U, Schmeisser MJ, Pelosi A, et al. (2014) In-depth protein profiling of the postsynaptic density from mouse hippocampus using data-independent acquisition proteomics. *Proteomics* 14(21–22): 2607–2613.

Dobzhansky T (1973) Nothing in biology makes sense except in the light of evolution. *American Biology Teacher* 35(3): 125–129.

Emes RD and Grant SG (2011) The human postsynaptic density shares conserved elements with proteomes of unicellular eukaryotes and prokaryotes. *Frontiers in Neuroscience* 5: 44.

Emes RD and Grant SG (2012) Evolution of synapse complexity and diversity. *Annual Review of Neuroscience* 35: 111–131.

Emes RD, Pocklington AJ, Anderson CN, et al. (2008) Evolutionary expansion and anatomical specialization of synapse proteome complexity. *Nature Neuroscience* 11(7): 799–806.

Fernandez E, Collins MO, Frank RAW, et al. (2017) Arc requires PSD95 for assembly into postsynaptic complexes involved with neural dysfunction and intelligence. *Cell Reports* 21(3): 679–691.

Fernandez E, Collins MO, Uren RT, et al. (2009) Targeted tandem affinity purification of PSD-95 recovers core postsynaptic complexes and schizophrenia susceptibility proteins. *Molecular Systems Biology* 5: 269.

Frank RA and Grant SG (2017) Supramolecular organization of NMDA receptors and the postsynaptic density. *Current Opinion in Neurology* 45: 139–147.

Frank RA, Komiyama NH, Ryan TJ, et al. (2016) NMDA receptors are selectively partitioned into complexes and supercomplexes during synapse maturation. *Nature Communications* 7: 11264.

Frank RAW, Zhu F, Komiyama NH, et al. (2017) Hierarchical organization and genetically separable subfamilies of PSD95 postsynaptic supercomplexes. *Journal of Neurochemistry* 142(4): 504–511.

Grant SG (2016) The molecular evolution of the vertebrate behavioural repertoire. *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences* 371(1685): 20150051.

Grant SG, O'Dell TJ, Karl KA, et al. (1992) Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science* 258(5090): 1903–1910.

Gruart A, Leal-Campanario R, Lopez-Ramos JC, et al. (2015) Functional basis of associative learning and its relationships with long-term potentiation evoked in the involved neural circuits: Lessons from studies in behaving mammals. *Neurobiology of Learning and Memory* 124: 3–18.

Hebb DO (1949) *The Organization of Behavior: A Neuropsychological Theory*. New York: Wiley.

Husi H and Grant SG (2001) Isolation of 2000-kDa complexes of N-methyl-D-aspartate receptor and postsynaptic density 95 from mouse brain. *Journal of Neurochemistry* 77(1): 281–291.

Husi H, Ward MA, Choudhary JS, et al. (2000) Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nature Neuroscience* 3(7): 661–669.

Kandel ER, Dudai Y and Mayford MR (2014) The molecular and systems biology of memory. *Cell* 157(1): 163–186.

Kandel ER, Schwartz JH and Jessell TM (1991) *Principles of Neural Science* (3rd edn). Norwalk, CT: Appleton & Lange.

Larsen RS and Sjöström PJ (2015) Synapse-type-specific plasticity in local circuits. *Current Opinion in Neurology* 35: 127–135.

Li J, Wilkinson B, Clementel VA, et al. (2016) Long-term potentiation modulates synaptic phosphorylation networks and reshapes the structure of the postsynaptic interactome. *Science Signaling* 9(440): rs8.

Migaud M, Charlesworth P, Dempster M, et al. (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* 396(6710): 433–439.

Morris RG, Anderson E, Lynch GS, et al. (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature* 319(6056): 774–776.

Nicoll RA (2017) A brief history of long-term potentiation. *Neuron* 93(2): 281–290.

Nithiananantharajah J, Komiyama NH, McKechanie A, et al. (2013) Synaptic scaffold evolution generated components of vertebrate cognitive complexity. *Nature Neuroscience* 16(1): 16–24.

Pocklington AJ, Cumiskey M, Armstrong JD, et al. (2006) The proteomes of neurotransmitter receptor complexes form modular networks with distributed functionality underlying plasticity and behaviour. *Molecular Systems Biology* 2: 2006.0023.

Ryan TJ and Grant SG (2009) The origin and evolution of synapses. *Nature Reviews Neuroscience* 10(10): 701–712.

Ryan TJ, Kopanitsa MV, Indersmitten T, et al. (2013) Evolution of GluN2A/B cytoplasmic domains diversified vertebrate synaptic plasticity and behavior. *Nature Neuroscience* 16(1): 25–32.

Scheetz AJ, Nairn AC and Constantine-Paton M (2000) NMDA receptor-mediated control of protein synthesis at developing synapses. *Nature Neuroscience* 3(3): 211–216.

Silva AJ, Paylor R, Wehner JM, et al. (1992) Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science* 257(5067): 206–211.

Tarpey P, Parnau J, Blow M, et al. (2004) Mutations in the DLG3 gene cause nonsyndromic X-linked mental retardation. *American Journal of Human Genetics* 75(2): 318–324.

Urban-Ciecko J, Wen JA, Parekh PK, et al. (2014) Experience-dependent regulation of presynaptic NMDARs enhances neurotransmitter release at neocortical synapses. *Learning & Memory* 22(1): 47–55.

Wadhams GH and Armitage JP (2004) Making sense of it all: Bacterial chemotaxis. *Nature Reviews Molecular Cell Biology* 5(12): 1024–1037.

Zhu F, Cizeron M, Qiu Z, et al. (2018). Architecture of the mouse brain synaptome. *Neuron* 99(4): 781–799.