A General Basis for Cognition in the Evolution of Synapse Signaling Complexes

S.G.N. GRANT

Genes to Cognition Programme, Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Correspondence: sg3@sanger.ac.uk

Beneath the complexity of the human brain are molecular principles shaped by evolution explaining the origins of the behavioral repertoire. The role of the nervous system is to provide a repertoire of behaviors allowing the animal to respond and adapt to changing environments during the course of its life. Multiprotein complexes in the postsynaptic terminal of synapses control adaptive and cognitive processes in metazoan nervous systems. These multiprotein complexes are organized into molecular networks that detect and respond to patterns of neural activity. Combinations of proteins are used to build different complexes and pathways producing great diversity. These complexes evolved from an ancestral core set of proteins controlling adaptive behaviors in unicellular organisms known as the protosynapse. Later expansion in numbers and interactions resulted in more complex synapses in invertebrates and vertebrates. The resultant combinatorial complexity has contributed to the neuroanatomical, neurophysiological, and behavioral diversity in these species. Mutations in genes encoding the complexes result in many human diseases of the nervous system. This general mechanism of cognition provides a useful template for studying evolution of behavior in all animals.

In his closing commentary on the Symposium, Dr. Brian Charlesworth (Edinburgh University) concluded that the two major evolutionary problems were the “origin of life” and the “evolution of human consciousness and cognition.” Toward the latter issue, this chapter traces a path linking the molecular origins of adaptive behaviors in unicellular organisms to the synaptic mechanisms of human cognition and its medical disorders. This framework has emerged from research using proteomic approaches to synapses of the mouse brain, which exposed a hitherto unappreciated molecular complexity (Husi et al. 2000). Experiments probing this complexity using both single-molecule and large-scale methods, with a particular emphasis on integration of neurophysiological, neuroanatomical, and behavioral approaches, lead to a general molecular theory for the origins of cognition and how animals interact and adapt to their environments.

This chapter is structured along the following lines. First, cognition is defined, and an overview of behavioral adaptation to the environment is presented. Next, a description of the molecular organization of the mammalian postsynaptic proteome and its complexity is introduced as background to molecular evolutionary studies. The remaining sections address electrophysiological, anatomical, and behavioral implications of synapse evolution.

COGNITION: FROM UNICELLULAR ORGANISMS TO HUMANS

Cognition is defined as the biology of information processing (Wilson and Keil 1999). This definition is generally applied in psychology where it embraces the acquisition and processing of information obtained by the sensory organs of animals. Specific cognitive mechanisms under-

lie perception, attention, learning, strategy choice, and other processes, which are not dealt with in detail here.

Processing of sensory information allows animals to detect and adapt to their immediate environment and changes in that environment or movement between environments. Considering the range of species that show cognitive processes, the 19th century biologist C.L. Morgan (1890) noted that unicellular organisms shared mechanisms with humans:

The primary end and object of the receptions of the influences (stimuli) of the external world or environment is to enable the organisms to answer or respond to these special modes of influence, or stimuli. In other words, their purpose is to set agoing certain activities. Now in the unicellular organisms, where both the reception and the response are effected by one and the same cell, the activities are for the most part simple, though even among these protozoa there are some which show no little complexity of response.

The importance of this observation arises when one considers the potential for conserved molecular mechanisms that underlie cognitive processes in animals, ranging from unicellular eukaryotic organisms to humans. Such core mechanisms, conserved across phyla, could form a basis around which species specificity and lineage-specific adaptations arose. In general, this aspect of neuroscience is neglected because most thinking is devoted to the function of neurons and their circuits, which are metazoan. Indeed most, if not all, models of cognition exclude unicellular organisms and are thus not general to all animals.

What form does cognitive processing take in unicellular organisms? To address this, we must consider how the information from the environment is converted into a meaningful adaptive response. Using yeast as an example

of a unicellular organism that detects changes in nutrients, pH, stressors, or other stimuli in its environment, its response through activation of signal transduction pathways regulating the expression of many genes is well known (Gasch et al. 2000). The output of this response is an orchestrated change in expression of large sets of genes, some common to many types of environmental changes and some gene sets specific to certain stimuli. This ability to detect different features of the environment and respond using signal transduction machinery to orchestrate transcription of overlapping combinations of genes is similar to signaling at mammalian synapses where neurotransmitter receptors can be activated with different stimuli to drive the expression of various sets of genes (Coba et al. 2008; Alberini 2009). More generally, receptor-mediated signal transduction systems are found in all cells and represent elementary cognitive mechanisms.

Unicellular organisms exist in environmental niches where they are exposed to a range of stimuli external to the organism. In contrast, the environmental niche of the postsynaptic terminal, which receives the neurons input stimulus, is a highly specialized internal environment, built and maintained by the organism itself. We can envision the postsynaptic membrane as a specialized sensing point on the surface of neurons, receiving pulses of neurotransmitters released from the presynaptic terminal. Thus, it seems likely that synapses would share molecular features with unicellular organisms and have evolved molecular specializations for the purpose of responding to the information encoded within the patterns of neural activity.

That *patterns of neural activity* are a key aspect of the neurophysiological basis of cognition was discovered by E.D. Adrian (1928) in the 1920s. He found that nerve fibers from sensory end organs showed greater activity following greater stimulation, leading to the conclusion that information received by the brain from the environment is in the pattern or code of nerve action potentials. This concept of a “neural code” representing the environment has been extended from simple sensory inputs to abstract stimuli including perception of visual (Hubel and Wiesel 1959) and spatial information (O’Keefe and Nadel 1978), which is represented in the pattern of neural activity recorded in neurons in particular parts of the mammalian brain. It is therefore of paramount importance to understand how the postsynaptic terminal reads the neural code and ultimately to ask if these molecular mechanisms are relevant to the psychological representation of information in the brain.

Synapses have a major role in transmitting the neural code from one neuron to the next, as well as a role in “reading” or monitoring the information in the code. An action potential arriving at the presynaptic terminal elicits the release of neurotransmitter, which is then detected on the postsynaptic membrane by neurotransmitter receptors. The rapid (millisecond) role of these receptors is to change the electrical potential of the membrane through modulation of ion flux and thereby contribute to the generation of another action potential in the second neuron.

A slower (seconds to minutes) consequence of the activation of the neurotransmitter receptor is to activate enzymatic signal transduction pathways that can have prolonged

effects on the function of the synapse or indeed the whole neuron. As noted earlier, these changes include transcriptional responses as well as local biochemical and structural changes in the synapse itself. It is this property of long-term change that E.R. Kandel has shown to have a role in memory processes (Goelet et al. 1986). Importantly, there is a direct link between the neural code and the induction of changes in the neuron. For example, simple neural codes (e.g., pairs of action potentials) have transient effects on synapses, and complex neural codes (e.g., a 5-Hz train of 30 sec) result in long-term changes. An extensive literature shows that various experimentally induced patterns and timings of synaptic activity induce changes in neuronal function, indicating that synapses are capable of detecting and discriminating many different neural codes and differentially activating signaling mechanisms.

This summary presents the view that mammalian synapses and unicellular organisms share a fundamental capacity for detecting and discriminating signals from their environments and that shared mechanisms exist at the level of signal transduction. The postsynaptic terminal of synapses detects a diverse range of neural codes and the unicellular organism must detect “environmental codes.” This model by itself does not explain how complex nervous systems evolved or how the behavioral repertoire of animals arose. To address these issues, we need to consider the molecular composition of synapses, their signaling and information processing capacity, and their molecular evolution.

MOLECULAR COMPLEXITY AND ORGANIZATION OF THE POSTSYNAPTIC PROTEOME

The surprising complexity of the postsynaptic proteome was found using neuroproteomic methods (for review, see Bayes and Grant 2009). This complexity was first exposed when the excitatory neurotransmitter receptor known as the *N*-methyl-D-aspartate (NMDA) receptor was purified from mouse brain and found to have 77 proteins attached and named the NMDA receptor complex (NRC) (Husi et al. 2000). Since then, multiple affinity methods have been used to isolate NRCs, and the numbers of associated proteins have increased to ~100–200 (Collins et al. 2006; Fernandez et al. 2009). The NR2 subunit of the NMDA receptor binds to the Discs Large (Dlg) class of scaffold proteins (Kornau et al. 1995), also known as membrane-associated guanylate kinases (MAGUKs), of which mammalian PSD-95/Dlg4, SAP102/Dlg3, and PSD-93/Dlg2 are highly studied (Feng and Zhang 2009). Affinity isolation of PSD-95 allows MAGUK-associated signaling complexes (MASCs) to be isolated, which show considerable overlap in their composition with the NRC (Husi et al. 2000; Collins et al. 2006; Fernandez et al. 2009), and hereafter will be referred to as NRC/MASC. NRC/MASCs are core complexes within the postsynaptic terminal and contain the principle electrical machinery (e.g., glutamate receptors and voltage-gated potassium channels) necessary for the postsynaptic terminal to respond and transmit action potentials (Fernandez et al. 2009). The first direct evidence that NRC/MASC has a key role in cognition in

animals was obtained using mice carrying mutations in PSD-95 (Migaud et al. 1998) or in the intracellular interaction domains of the NR2 subunits (Sprengel et al. 1998).

A larger set of proteins within the mammalian postsynaptic terminal of excitatory synapses can be isolated en masse using fractionation of a preparation known as the postsynaptic density (PSD). Approximately 1500 proteins were found in the rodent PSD, and the NRC/MASC represents ~10% of the PSD (Collins et al. 2006; Trinidad et al. 2008). Both the ionotropic (ion channel) and metabotropic (G-protein-coupled) glutamate receptors bind to scaffold proteins, which bind directly to signal transduction enzymes and structural proteins, forming multiprotein signaling complexes.

The concept of molecular networks complements the concept of multiprotein complexes and has proven to be useful in understanding the complexity imposed by the discovery of hundreds of postsynaptic proteins. For example, as shown in Figure 1, the proteins in NRC/MASC can be graphed as a molecular network of binary protein interactions (Pocklington et al. 2006). This reduces the complexity by grouping proteins into modules, and their relationship to one another can be examined. Specifically, the neurotransmitter receptors and their proximal interacting proteins form “upstream” modules that bind to “intermediate modules” of signaling proteins that in turn bind to “downstream modules” (Fig. 2). As discussed below, the

molecular evolution of this hierarchy of modules shows preferential expansion of upstream components in organisms with complex nervous systems.

Beyond a static network, we must ask how dynamic pathways linking receptors to downstream proteins function. A pathway may be made from a subset of the proteins, comprising components from each level of the hierarchy (Fig. 3). In other words, the complexity provides a combinatorial system for building many different pathways. It is now known that different combinations of PSD proteins are activated by different neurotransmitter receptors, and the PSD networks integrate this signaling information in a combinatorial manner (Coba et al. 2009).

Direct evidence showing that neuronal activity regulates a subnetwork of hundreds of PSD proteins was found using large-scale phosphoproteomics (Coba et al. 2009). Stimulation of the NMDA receptor led to rapid changes in the phosphorylation of more than 100 proteins on more than 200 phosphorylation sites. The proteins that were modulated contained a wide range of functional classes including other neurotransmitter receptors and ion channels, structural and cytoskeletal proteins, and enzymes regulating protein translation and transcription. Stimulation of other neurotransmitter receptors led to changes in distinct and overlapping sets of phosphorylation sites on these substrates (Fig. 3). At the level of information processing, this shows that the information associated with activation of

Figure 1. Protein network within the postsynaptic NRC/MASC complexes. Graph of 101 NRC/MASC proteins connected by 246 binary interactions and clustered into modules. The two uppermost modules contain the ionotropic (including NR2 and Dlg proteins) (pink) and metabotropic (brown) neurotransmitter receptors and thus “upstream modules.” The large “intermediate module” is connected to “downstream modules” at the bottom of the figure. A detailed description can be found in Pocklington et al. (2006), from which this was adapted, and a further simplified view is shown in Figure 2.

Figure 2. Modular structure and functional organization in the postsynaptic terminal. The synaptic cleft is at the top and neurotransmitters activate proteins in the input layer (purple top). The flow of information traverses the hierarchy of NRC/MASC modules through upstream (blue), intermediate (red), and downstream (yellow) modules connected by solid lines. These downstream modules regulate output effector mechanisms (green) that together produce the overall synaptic and neuronal changes. (Adapted from Pockington et al. 2006.)

different neurotransmitter receptors can recruit combinations of phosphorylation events in subsets of the many PSD proteins. Thus, the organization of the network and its differential activation provide a way to orchestrate the biological activity of hundreds of proteins.

The number of combinations of phosphorylation patterns induced by neurotransmitter receptor activation in the PSD is virtually limitless. Simple estimates indicate that 1000 proteins with an average of 10 phosphorylation sites can produce many more states ($2^{10,000}$) than there are synapses in the human brain. The full physiological range and diversity of PSD phosphorylation combinations have

yet to be measured, but it is clear that differential phosphorylation patterns are functionally significant. For example, the phosphorylation of specific combinations of sites on the GluR1 subunit of the AMPA (α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) receptor correlates with differential effects on synaptic plasticity (Delgado et al. 2007). In addition, the phosphorylation events regulate gene expression and differential activation of combinations of kinases, resulting in different patterns of gene expression (Coba et al. 2008). Thus, the picture is emerging that the molecular complexity of mammalian synapses provides a set of proteins from which combinations or subsets are used for processing of information.

Figure 3. Combinations of postsynaptic proteins activated by different stimuli. Three stimuli activate three receptors that drive subsets of the cytoplasmic networks of proteins organized into a three-layer hierarchy (circles). Each stimulus modulates or uses a distinct subset of proteins shown as the postsynaptic code (bottom).

MOLECULAR EVOLUTION AND DEVELOPMENT OF THE POSTSYNAPTIC PROTEOME

With the insight that synapses in the mammalian brain have evolved a signaling system capable of sensing many different types of stimuli and transducing these stimuli into a combinatorial multistate signaling output of modulated postsynaptic proteins, we must ask how this system originated and evolved. Through the analysis of species differences, this question can be answered in an outline form and summarized in the following six points.

Point 1. Examining the genomes of representative eukaryotes, metazoans, and chordates for orthologs of the mouse NRC/MASC and PSD genes showed that ~25% of the proteins were found in *Saccharomyces cerevisiae* and ~50% in invertebrate species (Fig. 4) (Emes et al. 2008;

Figure 4. Evolution of postsynaptic complexes. Animals representing vertebrates (mouse, monkey, bird, and human), invertebrates (fly, worm), and unicellular eukaryotes (yeast) are shown adjacent to their complement of synaptic proteins represented using the modular representation of postsynaptic networks into upstream (blue circle), intermediate (red box), and downstream (yellow triangle). The protosynapse first found in unicellular organisms is expanded in invertebrates and vertebrates with preferential expansion of upstream (blue circles) proteins. A neuron is shown to the left of the invertebrate synapse, emphasizing its origins after the protosynapse. Bracketed above the vertebrate synapse is a set of four variants of NRC/MASCs, illustrating that combinations of proteins generate diversity.

Ryan et al. 2008; Ryan and Grant 2009). The core set of synapse proteins, conserved in all animals, is referred to as the *protosynapse*. In unicellular eukaryotes, protosynapse proteins are necessary for responding to changing environments and include genes (e.g., *NF1*) that in humans result in heritable forms of learning impairment, attesting to the conservation in cognitive processes. Molecular dating of eukaryotes places the origins of this protosynapse to ~1.4 billion years ago (Hedges and Kumar 2009; Ryan and Grant 2009), indicating that the origins of the brain are in deeply conserved adaptive machinery predating neurons.

Point 2. The eukaryote protosynapse set was expanded through addition of new proteins and functional domains. By 1 billion years ago, the protosynapse may have contained many of the essential signaling components of mammalian synapses as evidenced by the presence of homologous genes in the genomes of unicellular choanoflagellates (considered to be the last unicellular ancestor of animals) and the multicellular porifera (sponges), which lack neurons (King 2004; Emes et al. 2008; Kosik 2009; Ryan and Grant 2009). These include ion channels, neurotransmitter receptors, adhesion proteins, and their associated scaffold and signaling enzymes. Thus, the key functionality of the postsynaptic machinery arose in simpler animals before the origins of electrically active neurons, a model referred to as the “synapse first model” (Ryan and Grant 2009). With evolution of primitive metazoans, such as cnidarians, ionotropic glutamate receptors and other proteins were added, giving the invertebrate NRC/MASC the classes of receptors, scaffolds, and signaling enzymes that are commonly studied in mammalian synapses. Moreover, mutations in some of these genes in *Drosophila* impair learning, showing the functional conservation between invertebrates and vertebrates in this cognitive mechanism (Wu et al. 2007).

Point 3. The evolution of vertebrate synapses was associated with a major expansion of synaptic genes and proteins, shown by both proteomic and genomic methods (Emes et

al. 2008). Vertebrates show a preferential expansion in the upstream proteins, rather than the downstream proteins and modules in the signaling networks (Fig. 4). The genomic mechanism for this expansion in gene families was gene and genome duplication. Two key examples that have been extensively investigated are the NMDA receptor *NR2* and *Dlg* genes that expanded from a single gene in invertebrates to four in vertebrates (Ryan et al. 2008).

Together, points 2 and 3 illustrate that major expansions in complexity occurred around the ancestral protosynapse proteins to give rise to an invertebrate synapse. The vertebrate postsynaptic proteome is largely characterized by a further major expansion in complexity in gene families, which means that more receptors and the upstream module components of the pathways that give specificity to signaling responses are available to synapses in these species (Fig. 4). Linking this increase in complexity to the earlier discussion of combinatorial signaling pathways found in phosphoproteomic studies, it is reasonable to expect that the vertebrate complexity provides a greater number of combinations of postsynaptic signaling subnetworks (Fig. 3) than those found in invertebrates.

Point 4. In addition to complexity in numbers, vertebrate molecular evolution in protein–protein interaction domains reveals that the *organization* of NRC/MASC complexes and networks has added to the complexity. Using the NMDA receptor *NR2* example and considering its four vertebrate paralogs and comparing their structure with their single invertebrate homolog, it was found that the cytoplasmic carboxy-terminal domain (C-tail) in invertebrates lacks most of the enzyme interaction sites possessed by vertebrate *NR2* subunits (Fig. 5) (Ryan et al. 2008).

Point 5. Expansion in complexity is amplified by the combinatorial nature of complexes. Again, *NR2* and *Dlg* families have expanded from a single homolog in invertebrates to four in vertebrates. Because each *NR2* binds a single *Dlg* to form a complex, invertebrates have only one complex, whereas vertebrates could generate tenfold

Figure 5. Evolution of NMDA interactions. Comparison of NMDA receptor complexes of *Drosophila melanogaster* containing a single NR2 subunit (*a*) with the mouse containing four NR2 subunits (*b*). PDZ-binding domains at the carboxyl termini of both *D. melanogaster* (NR2; SVL) and mouse NR2 (ESDV) are indicated. Note that the mouse NR2 intracellular domain is five times larger than that of *D. melanogaster*. The vertebrate NR2B carboxyl termini has numerous primary and secondary interacting proteins (colored shapes in *c*) and therefore a greater degree of NMDA-receptor-signaling complexity. Furthermore, the number of potential interactions of the NMDA receptor with Dlg proteins (yellow hexagons, Dlg, PSD95, SAP102, PSD93) is much greater for vertebrate synapses. (Reprinted, with permission, from Ryan and Grant 2009 [©Nature Publishing Group].)

more (Fig. 5). Considering the many other expanded families of proteins that also bind to NR2 and Dlg, one can readily appreciate the multiplicative increase in potential postsynaptic diversity.

Point 6. The organization of these complexes and networks of postsynaptic proteins requires them to be synthesized and transported to synapses during development of the nervous system. Genome-wide studies of messenger RNA (mRNA) (Valor et al. 2007) and microRNA (miRNA) (Manakov et al. 2009) show that the synthesis of PSD proteins proceeds in an orchestrated and orderly pattern of coregulation. As discussed in the next section, the coexpression of the sets of proteins is not identical in all neurons and all synapses. Variations in the relative levels of the proteins between neurons occurs, and this has a distinct evolutionary signature.

AN EVOLUTIONARY BASIS TO NEUROANATOMICAL MAPS OF SYNAPSE PROTEOME COMPOSITION

Vertebrates are characterized by having larger brains than invertebrates, and this difference is generally accepted as the explanation for their different cognitive abilities. This anatomical perspective also dominates the comparisons among vertebrates where brain size, encephalization quotients, and other measures of anatomical complexity are correlated with behavior. Not only is anatomical complexity a poor index of cognitive function and species-specific behaviors, but it lacks rigorous testing methods and is based almost exclusively on correlative approaches.

A large-scale study of the expression of NRC/MASC and PSD proteins in forebrain regions of the mouse found that more than 90% of the genes were coexpressed in neurons and different brain regions (Emes et al. 2008). Unsurprisingly, expression patterns of individual proteins were different, with some showing similar levels of expression in all regions and some showing very different levels in one or other region. At first glance, there was no prevailing logic to these patterns until the molecular phylogeny of the genes was considered. A clear picture emerged, in which the ancestral protosynapse set of proteins was expressed in all neurons and regions at equivalent levels, which was in contrast to more recently evolved genes that showed the most variety in expression patterns (Fig. 6). This pattern was functionally informative because the *upstream module* proteins, which contain neurotransmitter receptors, contributed more to anatomical diversification and regional specialization. Thus, the evolutionary expansions in postsynaptic complexity were used to generate diverse neurons and synaptic types (Fig. 4). These anatomical data again reflect on the combinatorial usage of postsynaptic proteins to generate diversity.

It is interesting to note that the ancestral chordate (~600 mya), which had the complex complement of synaptic proteins, evolved before species with large brains such as vertebrates. In other words, the complex synapse evolved before neuroanatomically complex brains.

NEUROPHYSIOLOGICAL CONSEQUENCES OF EXPANSIONS IN POSTSYNAPTIC COMPLEXITY

Returning to the fundamental neurophysiological basis of cognition—the encoding of information in patterns of action potentials—we can examine evidence supporting the following conclusion: Expanded molecular complexity conferred upon vertebrate synapses the ability to respond to a greater range of patterned stimuli. To specifically address this, different patterns of neural activity (e.g., trains of 1-Hz, 5-Hz or 100-Hz stimuli) are experimentally presented to the CA1 synapse in the hippocampus slice and their effects measured by examining the change in efficiency of synaptic transmission. These cellular phenomena (e.g., long-term potentiation [LTP], long-term depression, and spike-timing-dependent plasticity) share molecular mechanisms with behavioral cognitive processes. From

Figure 6. Combinatorial NRC/MASC diversity in brain regions. Modular representation of the NRC/MASC is shown in the synapse schematic (*bottom*). Bracketed above are four variants built from combinations of the evolutionarily expanded protein families, which are found in particular brain regions (arrows to brain).

mice with deletions of PSD-95/dlg4, SAP102/dlg3, or PSD-93/dlg2, a distinct set of changes was observed in each mutant. For example, both Dlg4 and Dlg3 mutants showed enhanced LTP, whereas Dlg2 showed reduced LTP compared to normal mice (Migaud et al. 1998; Cuthbert et al. 2007; Carlisle et al. 2008). Further separating Dlg3 and Dlg4, some patterns of stimuli only showed abnormal effects in Dlg4. Other examples of paralog-specific effects were found in knockout mice of other protein families including the Src family (Grant et al. 1992) and NR2 subunits (Sprengel et al. 1998). Together, these data show that vertebrate expansion in synapse complexity has endowed the CA1 synapse with greater signaling specificity and a greater range of responsiveness to different neural codes (Fig. 7).

Because the NR2, Dlg, and Src family proteins interact in NRC/MASC complexes, these results indicate that complexes of different compositions could account for different synaptic responsiveness. Thus, the combinations of interacting postsynaptic proteins could generate an enormous diversity of *functional synapse types* in the mammalian brain. Because combinations of these proteins are characteristic of particular neurons and brain regions, it can then be expected that the mapping of synapse diversity at the molecular level will align with maps of synaptic electrophysiological diversity.

BEHAVIORAL ROLES FOR SYNAPSE EVOLUTION

The simplest evidence that evolutionary expansion in postsynaptic proteome complexity was important for behavior comes from vertebrate-specific genes, which if knocked out in mice or humans results in cognitive impairments (e.g., Dlg3/SAP102). A particularly important paradigm of how evolution of *complexity* and *organization*

Figure 7. Different complexes respond to different neural codes. Patterns of action potentials shown as two distinct stimuli (*top*) and three types of NRC/MASC complexes composed of different proteins. The biochemical output is shown as three boxes and could represent transcription, receptor trafficking, and local protein synthesis or other downstream outputs of synaptic stimulation. The green shading indicates which of the three outputs is activated by the type of complex in response to the type of stimulus.

both influence behavior is provided by examples of the paralogs of the NR2 and Dlg genes and their interactions via the NR2 cytoplasmic C-tail. The four paralogs of NR2 found in vertebrates have diverged at the level of protein sequence, particularly in the C-tail, whereas the structure of channel-forming and ligand-binding domains are highly conserved. This divergence in the C-tail sequence has been responsible for its selectivity in binding particular postsynaptic signaling proteins (Ryan et al. 2008) including PSD-95/Dlg4, SAP102/Dlg3, CamKII, and SynGAP, all of which when knocked out in mice result in cognitive deficits (Silva et al. 1992; Migaud et al. 1998; Komiyama et al. 2002; Cuthbert et al. 2007). In addition, the specific deletion of C-tails of the NR2A and NR2B subunits in mice results in distinct behavioral consequences (Sprengel et al. 1998).

More than 50 diseases in humans are caused by NRC/MASC gene mutations (Grant et al. 2005; Fernandez et al. 2009), reinforcing the observations in mice that different NRC/MASC and PSD proteins have common and distinct behavioral roles. Dlg proteins are again useful examples because Dlg2 mutations result in schizophrenia (Walsh et al. 2008), Dlg3 mutations in X-linked mental retardation (Tarpey et al. 2004), and the Neuroligins, which bind Dlg, underlie forms of autism (Jamain et al. 2003). Thus, different Dlg proteins have roles in different human cognitive disorders. In general, it appears that the evolution of increasing synaptic complexity has provided a larger set of molecular building blocks for the cognitive repertoire of animals.

IMPLICATIONS OF POSTSYNAPTIC COMPLEXITY AS A SOURCE OF SYNAPSE DIVERSITY

With the capacity to generate postsynaptic diversity from the combinatorial evolutionary molecular mechanisms described above, there could exist a vast or even limitless

number of synapse types. If the number of synapse types is important for the overall function of the brain, it is then interesting to speculate that the traditional model of brain size as the index of cognitive capacity could be replaced by a model with a number of synapse types. Indeed, brain size may limit the number of synapse types. The full range of synapse diversity as measured by molecular markers or physiological properties is not known for any animal.

A general model of psychological function is that stimuli, ideation, action plans, and other constructs are *representations* of neural activity, as measured in functional brain imaging. Somehow, the neural activity is selectively activating particular brain regions. Considering that brain regions are characterized by combinations of PSD proteins and that these protein combinations lead to preferred responses to neural codes, synapse types in particular brain regions might “tune” that region to respond to the neural pattern of activity. Synapse diversity will also render different synapses susceptible to particular genetic disorders and influence the clinical manifestations including disease predilection to particular brain regions. At the cellular electrophysiological level, the importance of synapse diversity in connecting neural circuits is poorly understood.

CONCLUSIONS

The origins of human adaptive behavior and cognition from the ancestral protein machinery found in unicellular organisms was followed by the evolution of increasing complexity giving rise to synapses with extraordinary combinatorial computational signaling properties. The number and organization of these combinations were greatly enhanced during evolution, particularly with metazoans and chordates, and shown through genetic studies to directly contribute to fundamental cognitive properties at the synaptic level, including processing the patterns of neural activity and cognitive behaviors in the whole animal.

That the phylogeny of synapse complexity maps onto the anatomical organization of the brain, and that the global regulation of NRC/MASC and PSD development is coordinated during neural development, point to genomic mechanisms for generating and controlling the diversity in different neurons and synapses. This anatomical and functional diversity may be relevant to the formation of psychological representations or environment feature detection.

The differences and diversity of composition and organization in NRC/MASC and PSD proteins offer a molecular template for analyzing and understanding species diversity and behavioral specialization. Perhaps the increase in cognitive mechanisms that postsynaptic molecular evolutionary events provided contributed to the geographical spread and specialization of animals into novel environmental niches. This model helps to explain the evolutionary basis of the high degree of molecular complexity of mammalian synapses and its role in animal behavior. The data sets of proteins open many new approaches to the study of human brain disease, which must reflect the importance of these evolutionary mechanisms.

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REFERENCES

- Adrian ED. 1928. *The basis of sensation: The action of the sense organs*. Norton, New York.
- Alberini CM. 2009. Transcription factors in long-term memory and synaptic plasticity. *Physiol Rev* **89**: 121–145.
- Bayes A, Grant SG. 2009. Neuroproteomics: Understanding the molecular organization and complexity of the brain. *Nat Rev* **10**: 635–646.
- Carlisle HJ, Fink AE, Grant SG, O'Dell TJ. 2008. Opposing effects of PSD-93 and PSD-95 on long-term potentiation and spike timing-dependent plasticity. *J Physiol* **586**: 5885–5900.
- Coba MP, Valor LM, Kopanitsa MV, Afinowi NO, Grant SG. 2008. Kinase networks integrate profiles of *N*-methyl-D-aspartate receptor-mediated gene expression in hippocampus. *J Biol Chem* **283**: 34101–34107.
- Coba MP, Pocklington AJ, Collins MO, Kopanitsa MV, Uren RT, Swamy S, Croning MD, Choudhary JS, Grant SG. 2009. Neurotransmitters drive combinatorial multistate postsynaptic density networks. *Sci Signal* **2**: ra19.
- Collins MO, Husi H, Yu L, Brandon JM, Anderson CN, Blackstock WP, Choudhary JS, Grant SG. 2006. Molecular characterization and comparison of the components and multiprotein complexes in the postsynaptic proteome. *J Neurochem* (suppl. 1) **9**: 16–23.
- Cuthbert PC, Stanford LE, Coba MP, Ainge JA, Fink AE, Opazo P, Delgado JY, Komiyama NH, O'Dell TJ, Grant SG. 2007. Synapse-associated protein 102/dlg3 couples the NMDA receptor to specific plasticity pathways and learning strategies. *J Neurosci* **27**: 2673–2682.
- Delgado JY, Coba M, Anderson CN, Thompson KR, Gray EE, Heusner CL, Martin KC, Grant SG, O'Dell TJ. 2007. NMDA receptor activation dephosphorylates AMPA receptor glutamate receptor 1 subunits at threonine 840. *J Neurosci* **27**: 13210–13221.
- Emes RD, Pocklington AJ, Anderson CN, Bayes A, Collins MO, Vickers CA, Croning MD, Malik BR, Choudhary JS, Armstrong JD, Grant SGN. 2008. Evolutionary expansion and anatomical specialization of synapse proteome complexity. *Nat Neurosci* **11**: 799–806.
- Feng W, Zhang M. 2009. Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density. *Nat Rev* **10**: 87–99.
- Fernandez E, Collins MO, Uren RT, Kopanitsa MV, Komiyama NH, Croning MD, Zografos L, Armstrong JD, Choudhary JS, Grant SG. 2009. Targeted tandem affinity purification of PSD-95 recovers core postsynaptic complexes and schizophrenia susceptibility proteins. *Mol Syst Biol* **5**: 269.
- Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO. 2000. Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* **11**: 4241–4257.
- Goebel P, Castellucci VF, Schacher S, Kandel ER. 1986. The long and the short of long-term memory—A molecular framework. *Nature* **322**: 419–422.
- Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER. 1992. Impaired long-term potentiation, spatial learning, and hippocampal development in *fyn* mutant mice. *Science* **258**: 1903–1910.
- Grant SG, Marshall MC, Page KL, Cumiskey MA, Armstrong JD. 2005. Synapse proteomics of multiprotein complexes: En route from genes to nervous system diseases. *Hum Mol Genet* (spec no. 2) **14**: R225–R234.

- Hedges SB, Kumar S. 2009. *The timetree of life*. Oxford University Press, Oxford.
- Hubel DH, Wiesel TN. 1959. Receptive fields of single neurones in the cat's striate cortex. *J Physiol* **14**: 574–591.
- Husi H, Ward MA, Choudhary JS, Blackstock WP, Grant SG. 2000. Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nat Neurosci* **3**: 661–669.
- Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, et al. 2003. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat Genet* **34**: 27–29.
- King N. 2004. The unicellular ancestry of animal development. *Dev Cell* **7**: 313–325.
- Komiyama NH, Watabe AM, Carlisle HJ, Porter K, Charlesworth P, Monti J, Strathdee DJ, O'Carroll CM, Martin SJ, Morris RG, O'Dell TJ, Grant SG. 2002. SynGAP regulates ERK/MAPK signaling, synaptic plasticity, and learning in the complex with postsynaptic density 95 and NMDA receptor. *J Neurosci* **22**: 9721–9732.
- Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. 1995. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* **269**: 1737–1740.
- Kosik KS. 2009. Exploring the early origins of the synapse by comparative genomics. *Biol Lett* **5**: 108–111.
- Manakov SA, Grant SG, Enright AJ. 2009. Reciprocal regulation of microRNA and mRNA profiles in neuronal development and synapse formation. *BMC Genomics* **10**: 419.
- Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, et al. 1998. Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396**: 433–439.
- Morgan CL. 1890. *Animal life and intelligence*. Arnold, London.
- O'Keefe J, Nadel L. 1978. *The hippocampus as a cognitive map*. Clarendon/Oxford University Press, Oxford.
- Pocklington AJ, Cumiskey M, Armstrong JD, Grant SG. 2006. The proteomes of neurotransmitter receptor complexes form modular networks with distributed functionality underlying plasticity and behaviour. *Mol Syst Biol* **2**: 2006.0023.
- Ryan TJ, Grant SG. 2009. The origin and evolution of synapses. *Nat Rev Neurosci* **10**: 701–712.
- Ryan TJ, Emes RD, Grant SG, Komiyama NH. 2008. Evolution of NMDA receptor cytoplasmic interaction domains: Implications for organisation of synaptic signalling complexes. *BMC Neurosci* **9**: 6.
- Silva AJ, Paylor R, Wehner JM, Tonegawa S. 1992. Impaired spatial learning in α -calcium-calmodulin kinase II mutant mice. *Science* **257**: 206–211.
- Sprengel R, Suchanek B, Amico C, Brusa R, Burnashev N, Rozov A, Hvalby O, Jensen V, Paulsen O, Andersen P, et al. 1998. Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. *Cell* **92**: 279–289.
- Tarpey P, Parnau J, Blow M, Woffendin H, Bignell G, Cox C, Cox J, Davies H, Edkins S, Holden S, et al. 2004. Mutations in the *DLG3* gene cause nonsyndromic X-linked mental retardation. *Am J Hum Genet* **75**: 318–324.
- Trinidad JC, Thalhammer A, Specht CG, Lynn AJ, Baker PR, Schoepfer R, Burlingame AL. 2008. Quantitative analysis of synaptic phosphorylation and protein expression. *Mol Cell Proteomics* **7**: 684–696.
- Valor LM, Charlesworth P, Humphreys L, Anderson CN, Grant SG. 2007. Network activity-independent coordinated gene expression program for synapse assembly. *Proc Natl Acad Sci* **104**: 4658–4663.
- Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, Nord AS, Kusenda M, Malhotra D, Bhandari A, et al. 2008. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* **320**: 539–543.
- Wilson RA, Keil FC. 1999. *The MIT encyclopaedia of the cognitive sciences*. MIT Press, Cambridge, MA.
- Wu CL, Xia S, Fu TF, Wang H, Chen YH, Leong D, Chiang AS, Tully T. 2007. Specific requirement of NMDA receptors for long-term memory consolidation in *Drosophila* ellipsoid body. *Nat Neurosci* **10**: 1578–1586.

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S.G.N. Grant

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