

Getting to synaptic complexes through systems biology

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Abstract

Large numbers of synaptic components have been identified, but the effect so far on our understanding of synaptic function is limited. Now, network maps and annotated functions of individual components have been used in a systems biology approach to analyzing the function of NMDA receptor complexes at synapses, identifying biologically relevant modular networks within the complex.

Synapses are the intercellular contact sites where neurons communicate with each other. The classical theory of neuronal signaling states that presynaptically released chemical neurotransmitters bind postsynaptic receptors to depolarize neurons and initiate downstream signaling. At postsynaptic regions lies a cytoskeletal specialization known as the postsynaptic density (PSD) [1]. Clustered here are neurotransmitter receptors such as the NMDA receptor which responds to glutamate, associated regulatory proteins, and various proteins involved in downstream signaling and cytoskeletal organization [1,2]. Changes in the abundance of PSD-resident proteins are thought to mediate the strengthening or weakening of synaptic activity - long-term potentiation (LTP) or long-term depression (LTD), respectively - that are thought to underlie learning and memory. NMDA receptors in particular are critical for the induction of LTP [3]. Given the role of synapses in brain function, studying their molecular composition is a matter of considerable interest.

The number of identified synaptic components has recently received a boost by combining chromatography and tandem mass spectrometry with traditional subcellular fractionation and immunoaffinity complex purification [4]. More than 400 PSD components [5-10] and 186 NMDA receptor-associated proteins [11] have been identified in this way and several attempts have been made at analyzing these data [5,7]. But despite this increase, our understanding of synaptic

organization remains relatively unchanged. In fact, few proteomic studies contain an integrated functional analysis of the complexes they study. Pocklington *et al.* [12] have now elucidated the function of the NMDA receptor complex using a systems biology approach. They used literature searches to construct protein network maps and to assess the role of components of the NMDA receptor complex in various synaptic functions and brain pathologies. This effort has resulted in a prototype model of a postsynaptic network through which the authors attempt to explain several aspects of synaptic signaling.

Annotation of components of the NMDA receptor complex

Pocklington *et al.* [12] used a three-step process to annotate NMDA receptor complexes: first, they identified their components by proteomic-based methods; second, they performed bioinformatics and literature searches to identify domains, protein families and association to synaptic function and psychiatric disorders; and finally they constructed protein network maps using identified protein interactions and performed statistics and clustering. Work by Husi *et al.* [11] from the same laboratory had previously accomplished the first step. Using the components of the NMDA receptor complex identified by Husi *et al.* Pocklington and colleagues found that proteins with domains involved in intracellular

signaling (kinase, SH3, PDZ, GTP-binding domains and C2) were enriched 3-12-fold in NMDA receptor complexes compared with the mouse proteome. Proteins with IQ calmodulin-binding domains and PDZ domains were enriched 12- and 8-fold, respectively, over the mouse proteome, as expected given that calcium regulation and PDZ-dependent scaffolding abound at synapses. Overall, cell adhesion or cytoskeletal proteins and signaling molecules or enzymes represented the majority (39.8%) of NMDA receptor complex components. This reveals, as observed by others [5,7,8,10], that synapses have a relatively large capacity for downstream signaling.

Pocklington *et al.* [12] used literature searches to screen components of the complex for evidence of roles in long-term potentiation, long-term depression, spatial learning and cue or contextual conditioning. They found that 26% of the proteins had a link to behavioral paradigms, with 88% of these important for learning (17% linked to spatial learning and 13.5% to cue or contextual conditioning). NMDA receptor complex proteins could also be linked to psychiatric and neurological disorders: 18% to schizophrenia, 12% to mental retardation, 6.5% to bipolar disorder and 7.5% to depressive illness. These results are consistent with the established roles of NMDA receptors in synaptic and cognitive function. On the basis of these results, Pocklington *et al.* [12] speculate that the NMDA receptor complex may have an important role in neurological disorders that have cognitive dysfunction as a primary component (for example, mental retardation and schizophrenia).

The associations of protein families in the NMDA receptor complex with synaptic functions or neurological disorders were analyzed using statistical methods to exclude any association resulting by chance. Pocklington *et al.* [12] found a significant correlation between phosphatases and glutamate receptors and synaptic plasticity ($p < 10^{-2}$ and $p < 10^{-3}$, respectively), between G α -proteins and affective disorders ($p < 10^{-2}$) and between the C2 calcium-binding domain and behavioral plasticity ($p < 10^{-3}$). Overall, synaptic plasticity and behavioral plasticity were strongly connected with components of this complex ($p < 10^{-11}$). These studies reveal, at a systems level, the importance of NMDA receptors and associated proteins in synaptic and higher-order brain function.

Mapping protein interactions

Pocklington *et al.* [12] identified 248 binary interactions between 105 proteins using publicly available studies and protein-interaction databases such as BIND [13], GRID [14] and NetPro [15]. A protein network map constructed by clustering the complex components and their interactions using an algorithm by Newman and Girvan [16] revealed a highly modular structure. They observed five highly connected nodes, containing around 75% of NMDA receptor complex proteins, and eight nodes with the remaining proteins.

Overall they observed that neighbors of highly connected nodes have low connectivity, a hallmark of stable protein network topology, and they speculated that these highly-connected nodes represented functional modular clusters. Cluster 1 contained all NMDA receptor subtypes and 50% of its components were essential in synaptic plasticity ($p < 10^{-2}$) and 40% were linked to schizophrenia ($p < 10^{-2}$). This represents a strong bias of cluster 1 towards cognitive function. Cluster 2 was enriched in metabotropic glutamate receptors and G-protein signaling proteins with 50% of its components associated with behavioral phenotypes ($p < 10^{-2}$). Moreover, a third of all the components of the NMDA receptor complex linked to depressive illness ($p < 10^{-2}$) were enriched in this group. The third major node, cluster 3, was enriched in signaling components such as tyrosine protein kinases and SH2-containing proteins and is centrally located - having connections with all other nodes. These results corroborate the hypothesis of Pocklington *et al.* [12] that the NMDA receptor complex is subdivided into biologically relevant modules.

Protein networks can shed light on the adaptability of biological mechanisms. Pocklington *et al.* [12] point to the surprising resilience of synaptic plasticity to perturbation and suggest that the less-than-expected effects of mutating important proteins, as found in previous studies [17-19], may be due to the pattern of connectivity in the network. They put forward a reasonable model stating that the more highly connected a protein is (which they call the protein's 'degree'), the larger its effect on synaptic function. Thus, in terms of long-term potentiation or depression, the mutation of highly connected proteins should have more severe effects on synaptic plasticity. To support their prediction, Pocklington and colleagues searched the literature for data on the quantitative changes in synaptic transmission to 100 Hz stimuli in mice expressing normal or mutant components of the NMDA receptor complex. This information was then used to plot each protein degree versus the absolute mean change in long-term potentiation resulting from its mutation. A plot using 11 available long-term potentiation studies on components of the NMDA receptor complex had a good linear fit ($p < 10^{-3}$, $R^2 = 0.85$), which corroborates their hypothesis. Indeed, the largest effects on long-term potentiation induced by a 100 Hz stimulus were observed in mice with defects in highly connected proteins such as PSD-95 (for example, PSD-95 knockout enhances long-term potentiation by around 120% over baseline). Thus their model can be used to predict the effects that the mutation of a component of the NMDA receptor complex would have on synaptic plasticity.

Are we there yet?

Studies that integrate large quantities of data into sensible models are essential first steps towards understanding macromolecular complexes. Pocklington *et al.* [12] have used a systematic approach to integrating the vast amounts

of data generated by proteomic-based methods and create a model for synaptic function. Their effort to gather existing literature on components of the NMDA receptor complex and assemble a rudimentary map of the synaptic network is highly laudable. Nevertheless, all will acknowledge that this must be considered a first step in a Herculean task, for the reasons we address below.

A crucial question to ask is exactly what is the NMDA receptor complex? Pocklington *et al.* [12] rightfully acknowledge that an immunopurified complex may represent a collection of different complexes. Husi *et al.* [11] identified the complex as proteins from crude forebrain extracts that co-precipitated using NMDA receptor immunopurification or NMDA receptor carboxy-terminal tail affinity purification [11]. This material therefore represents NMDA receptor complexes from extrasynaptic [20,21] and presynaptic [22] sites, those found in astrocytes [23], microglia and oligodendrocytes [24], as well as complexes located throughout the individual cell at various stages of maturation, trafficking or activation. Given the strong biological correlation between location and function, it is likely that each of these complexes will be significantly different. This study thus presents a map of superimposed NMDA receptor complex functions and locations, for example, complexes in pyramidal cells and interneurons, or at young and old synapses. It is also possible that the individual clusters identified by Pocklington *et al.* [12] represent the NMDA receptor complex at different intracellular locations (that is, presynaptic, Golgi, endoplasmic reticulum and endosomal). A number of factors may thus influence the relationship of the complex as defined here to individual complexes *in vivo*.

The validity of the conclusions from bioinformatics analysis will also depend strongly on the quality of the complex, whose composition and purity will reflect its means of preparation. Single affinity-based purification methods are commonly contaminated with nonspecific interactions. A computational analysis of large protein-interaction databases suggested that 30-50% of these were biologically relevant [25]. Husi *et al.* [11] identified the NMDA receptor complex from the SDS-based elution of the affinity matrix, which may include a significant number of contaminants, and thus the components should be independently verified. Several methodologies have been developed to reduce the introduction of nonspecific interactions, such as tandem affinity purification (TAP) [26]. Moreover, immunoprecipitations eluted with the antigenic peptides are significantly 'cleaner' than whole-matrix elution. Future refinements of protein complex preparation should reduce these concerns.

Another problem could be literature bias. The years of research on synaptic function and dysfunction require some means of systematic correlation and interpretation, and the effort made by Pocklington *et al.* [12] is highly commendable. Nonetheless, concerns should be recognized about both

a time bias introduced by literature searches, and about combining the results of experiments performed with a wide-range of protocols. Thus, it is possible that NMDA receptor complex proteins are more likely to be linked to older topics with more literature. For example, more components were associated with schizophrenia (18%) and mental retardation (12%) than with bipolar disorder (6.5%) or depressive illness (7.5%). But a PubMed search of those terms reveals 70,080 articles on schizophrenia, 68,892 on mental retardation and significantly fewer on bipolar disorder and depressive illness (34,487 and 50,007, respectively) - a significant correlation with the functional distribution of NMDA receptor complexes. Pocklington *et al.* [12] find that of proteins involved in learning, 17% were associated with spatial learning and 13.5% with cue or contextual learning. Again, this is similar in distribution to the available literature (12,151 articles for spatial learning and 8,724 for cue or contextual learning). This bias will especially impact on the construction of protein network maps. It is not surprising that PSD-95, which attracts considerable interest among the scientific community, should have the greatest number of reported connections. At the time of writing this article, there were some 629 referenced works in PubMed for PSD-95 (16 interactions) compared with around 57 for Shank, another PSD scaffolding protein (four interactions). A prevalent trend was observed: some 15 citron publications and four interactions, around 38 stargazin publications and four interactions, and more than 800 calmodulin publications and 19 interactions. While it is possible that a protein with more interactors will be published more often, we cannot help but notice that the proteins with highest connectivity are those with the longest history, that is, tubulin, PSD-95, calmodulin, actin and NR-1. The extent to which the clustering of nodes and association of NMDA receptor complex components with brain pathologies depends on the clustering of the scientific literature rather than on biological function remains to be determined.

Beyond reductionism

Reductionist biology, while responsible for the vast majority of biological data, is insufficient to fully understand complex systems. The advent of proteomic-based identification of macromolecular structures has resulted in an avalanche of data, although the biological interpretation of these data lags woefully behind. The approach of Pocklington *et al.* [12] takes a big step towards overcoming this lag. Ultimately, a rigorous experimental biological interpretation will be required to separate the credible interactions from background noise.

Finally, the notion of the NMDA receptor complex itself and its physical and functional organization and apparent modularity may be subject to change. Indeed, the NMDA receptor not only connects to intracellular protein complexes, but it also connects through PSD-95 to cell adhesion molecules, specifically the neuroligins, which bind to presynaptic

neurexins and in turn to the presynaptic cytomatrix that includes the vesicle-release machinery [27]. Indeed, it is possible to 'walk' along molecules from the NMDA receptor to PSD-95 and on to the molecules of the presynaptic active zone. Similarly, extensive walks are possible postsynaptically, for example, through PSD-95 to the specialized AMPA glutamate receptor subunit, stargazin, and to the AMPA receptors themselves. Moreover, the NMDA receptor complex is certainly highly dynamic, and may vary in ways not yet fully appreciated. Thus, the definition of a mammalian NMDA receptor complex, although surely meaningful, is somewhat subjective. The method of systematic annotation for correlating and making sense of the large amounts of information now collecting on the structural, functional, pathologic and other levels is an excellent first effort, but the approach itself will most probably evolve and increase its power to make sense of this vast collection of information.

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