Sticking and Signalling at the Synapse *

Two Sides of the Same Coin?

Kavita Babu

Our brain and nervous system coordinate all activities of our body and its interaction with the environment. Our brain is made up of a large number of cells called neurons that form specialised points of contacts with other neurons. These contacts are called synapses. The development and functioning of these synapses are clearly vital for nervous system function. A set of molecules call Cell Adhesion Molecules (CAMs) have been shown to be required for the development and maintenance of synapses. More recent work with CAMs indicates that these molecules are also required for signalling and hence normal functioning of synapses. Here, I discuss how CAMs function both in normal synapse development and in signalling at the synapse.

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Keywords
Synapse, cell adhesion molecules (CAMs), signalling, *C. elegans*.

Introduction

Our brain is one of the most important organs in our body. It allows us to move, think, talk, eat and perform all other daily functions. Problems in the functioning of our brain can affect multiple aspects of our day-to-day lives as is seen in patients with epilepsy, schizophrenia, Parkinson's disease, Alzheimer's disease or many other disorders involving our brain and/or the central nervous system. Our brain is made up of around a hundred billion neurons. These neurons form connections or synapses to multiple other neurons allowing for crosstalk between neurons. This crosstalk between neurons through synapses allows for normal functioning of our nervous system. Absence or alteration of the proteins required for normal cross-talk between neurons could lead to de-

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Box 1. Glossary of Terms

Cell Adhesion Molecule (CAM): Proteins located on the cell surface and required for interactions between cells. In many cases, the extracellular domain of a cell adhesion molecule binds to the extracellular domain of a cell adhesion molecule in the neighbouring cell, allowing for cell-cell contact.

Synapse: In the nervous system, a synapse is the region where a neuron passes information (chemical or electrical) to another neuron or muscle cell.

***C. elegans*:** *Caenorhabditis elegans* is a free-living non-hazardous nematode (worm) that lives in temperate soil and has been extensively studied to understand how proteins function. More then 35% of human genes have homologs or orthologs in *C. elegans*.

fects in the functioning of the nervous system manifesting as disease conditions or behavioural defects. We will discuss some of the proteins known to function at the synapse and their properties required for synaptic development as well as their ability to signal at the synapse. In order to understand the molecules that function at the synapse, we will start right at the beginning by understanding what a synapse is.

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1. What is a Synapse?

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In his book *The Integrative Action of the Nervous System* published in 1906, Sir Charles Scott Sherrington[1] coined the term 'synapse' referring to the nexus between cells that are separated from each other by surfaces. He used this term for the nexus between neurons or a neuron and muscle cell [1]. Decades of work has now allowed us to understand that a synapse in the nervous system is the interphase between the axon of one neuron and the dendrite of another neuron or the axon of one neuron and its target cell (e.g. body-wall muscle). At the synapse, one neuron (presynaptic neuron) passes on information that in many cases is a chemical signal called a neurotransmitter to the next neuron (postsynaptic neuron) or muscle (postsynaptic cell). The postsynaptic neuron or cell responds to the neurotransmitter released by

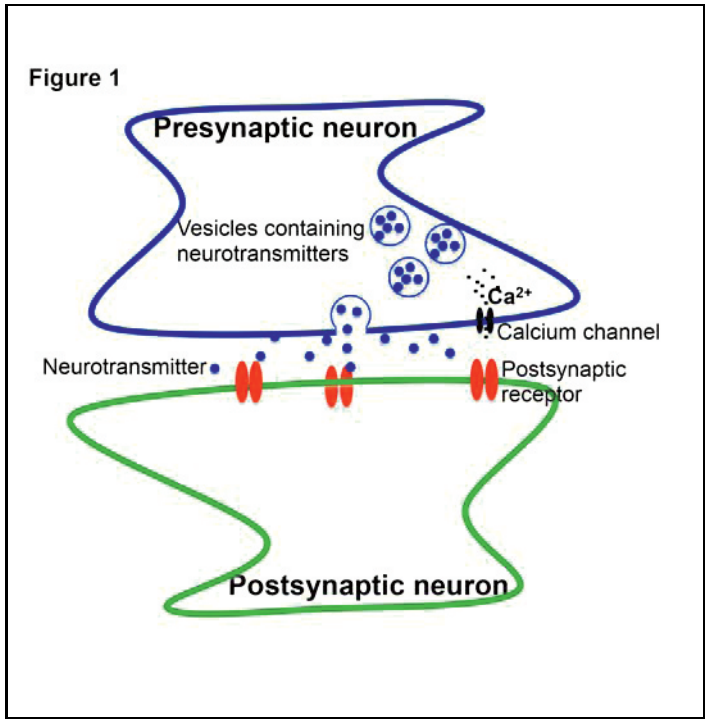

Figure 1. A cartoon of a synapse indicating the presynaptic and postsynaptic neurons, calcium channels that allow for neurotransmitter release, neurotransmitter vesicles, released neurotransmitter and postsynaptic receptors. A subset of postsynaptic receptors are activated upon neurotransmitter binding to the receptor and this, in turn, activates the postsynaptic neuron.

the presynaptic neuron through postsynaptic receptors to which the neurotransmitter binds. This, in turn, elicits a response in the postsynaptic cell. This process of synaptic signalling is illustrated in *Figure* 1.

In this short review, I will discuss some of the proteins that enable the development of the synapses, and how these same proteins could also be involved in signalling at the synapse.

2. What is a Sticky Synapse?

The synaptic apparatus is made up of a large number of proteins that have been revealed through genetic, biochemical, genomic and molecular biology experiments over the last half a century. Among the proteins present at the synapse are a class of proteins called the 'Cell Adhesion Molecules' or CAMs. CAMs are present both in the presynaptic neurons and in the postsynaptic target neurons or cells. These proteins as their name suggests,

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aid adhesive interactions across the synapse. These interactions occur when a CAM present on the presynaptic membrane bind to its partner on the postsynaptic membrane [2]. What is the function of CAMs at the synapse? Evidence shows that CAMs are required for target recognition and initiation of synaptic development, where the axon of the presynaptic neuron can recognise its postsynaptic target through CAMs expressed on pre- and postsynaptic sites that can interact with each other. This process is remarkable as it exemplifies the precision that is involved in synaptic development where the axon of one neuron is able to find its synaptic partner through proteins that are expressed on the surface of both these cells. This recognition is very precise as the neuron must make contacts with the correct target, and several CAMs aid this process. This initial foundation involving the axon of a neuron and its target cell allows for synapse development. CAMs are also involved in the maintenance of the synapse once it is formed. The strong interactions between presynaptic proteins and their postsynaptic partners allows for synapses to remain intact (*Figure* 2). This adhesion between the pre- and postsynaptic sites causes the synapse to be sticky and this stickiness of the synapse is essential for synaptic maintenance and hence normal functioning of the synapse [3, 2].

Evidence shows that CAMs are required for target recognition and initiation of synaptic development, where the axon of the presynaptic neuron can recognise its postsynaptic target through CAMs expressed on pre- and postsynaptic sites that can interact with each other.

3. Other Functions of CAMs

Although the adhesion function of CAMs has been fairly well-characterized with interaction partners for multiple adhesion molecules having been deciphered, other functions of these molecules at the synapses remain fairly unknown. It is conceivable that apart from their role in maintaining adhesion across the synapses, these molecules may also be involved in multiple aspects of signalling at the synapses to enable normal presynaptic neurotransmitter release and postsynaptic receptor maintenance. In the rest of this review, we will do a case study showing the cell adhesion and synaptic signalling role of three sets of well-studied synaptic CAMs. A number of studies involving understanding the signalling function of some of these CAMs have been addressed in

invertebrate organisms like the free-living nematode *Caenorhabditis elegans* or the fruit fly *Drosophila melanogaster*. These two model organisms are genetically tractable, and contain homologs or orthologs of many CAMs present in vertebrates including humans, making them attractive systems to further understand the functions of CAMs at the synapses.

4. Neurexins and Neuroligin are Required for Both Sticking and Signalling

A rigorously studied example of proteins required for synapse development is neurexins and its partner neuroligins. These two proteins form interactions with each other across the synapse with neurexin expressed on the presynaptic neuron and neuroligin on its postsynaptic partner. The discovery of the interaction between these two molecules has allowed us to get some of the first indications of molecular specificity governing CAMs at the synapse and how this specificity may allow for normal synapse formation and maintenance. Both neurexin and neuroligins are transmembrane proteins that possess large extracellular regions that interact with one another across the synapse (*Figure* 2). Initial insights into the function of these proteins in synapse formation were concluded from a set of experiments where these two proteins were expressed in non-neuronal cells co-cultured with neurons; This gave rise to synapse like contacts between the non-neuronal cells and neurons. Further, increased expression of neurexin and neuroligin in neurons showed an increase in the numbers of synapses formed between the neurons. Understanding how neurexin/neuroligin interaction allows for precise and specific synapse development is more difficult to address. One possibility lies in the fact that both these proteins have multiple splice variants[2]. It has been hypothesised that these splice variants may be involved in allowing for specific interactions between variants of neurexin and neuroligin [4].

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[2] Splice variants are multiple gene products obtained from the same gene locus. These different gene products are called isoforms of the gene.

Having assessed the role for the neurexin/neuroligin pair in synapse development, multiple studies have tried to address how these

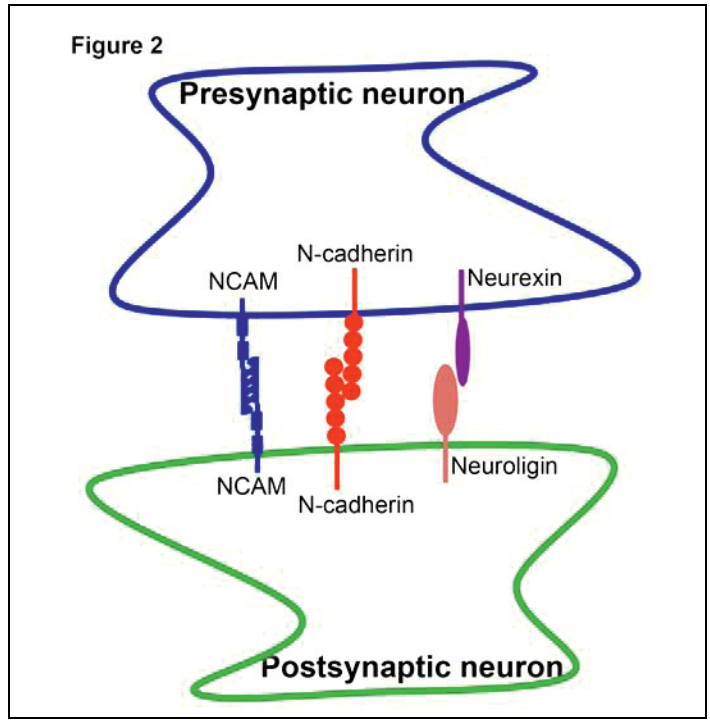

Figure 2. This illustration shows the CAMs at a sticky synapse. The presynaptic and postsynaptic regions are seen to be brought together by CAMs. These cell adhesion molecules include neurexin and its partner neuroligin, NCAMs and N-Cadherins (Source: Redrawn with modifications from [5].

proteins operate either separately or together in synaptic signalling. Work using the invertebrate model system *C. elegans*, has shown that apart from its function in synapse formation, neurexin also mediates neurotransmitter release from the presynaptic neurons onto their targets – the body-wall muscles. Experiments have shown that neurexin binds to and inhibits the function of a calcium channel that is present on the presynaptic neuron. Inhibition of the calcium channel, in turn, maintains the amount of neurotransmitter release from the neuron at the synapse between the neuron and muscle, called the 'neuromuscular junction' (NMJ). These experiments show a requirement of neurexin that is different from its requirement of functioning with neuroligin in synapse development, indicating a possible signalling function for this protein [5].

5. Role of NCAMs at the Synapse

'Neural Cell Adhesion Molecules' (NCAMs) are members of the immunoglobulin superfamily of proteins. These are among the most abundant proteins in the vertebrate and invertebrate nervous systems. Work from a large number of groups has shown that NCAMs are required for the development, maturation and maintenance of synapses. Studies have shown that NCAM is the first protein to accumulate at the nascent synapse and allows for synaptic development. NCAMs are thought to be expressed on both the pre- and postsynaptic membranes promoting interaction between two NCAM proteins across the synapse. Further, NCAM overexpression in cultured neurons from the rodent hippocampal region of the brain stimulates synapse formation, while the loss of NCAM proteins from synapses causes thinner or smaller synapses indicating the important role played by NCAMs in synapse development. Again NCAMs like neurexins and neuroligins have multiple isoforms that may be involved in rendering specificity to NCAM–NCAM interactions. For example, NCAM140 interacts specifically with NCAM180 across the synapse allowing for normal synaptic development and maintenance (illustrated in *Figure* 2 and reviewed in [6]).

We have worked on an NCAM-like protein in *C. elegans* and have found that this protein, RIG-3, does not function in synapse development. Instead, it is involved in synaptic signalling, hence allowing for normal synaptic functioning in the nervous system. Our work has shown that RIG-3 is localised to the synapse in presynaptic neurons that form synapses with the body-wall muscles. We have further shown that RIG-3 functions to maintain neurotransmitter receptor levels on the body-wall muscles. How a CAM, expressed and localised in the presynaptic neuron, affects the postsynaptic receptor levels is interesting. RIG-3 directly interacts with a Wnt receptor on the muscle, this, in turn, modulates postsynaptic receptor levels (*Figure* 3). Wnts are secreted proteins that upon secretion from a cell, bind to a surface protein on a neighbouring cell (Wnt receptor) to enable changes in gene expression in the cell that expresses the Wnt receptor.

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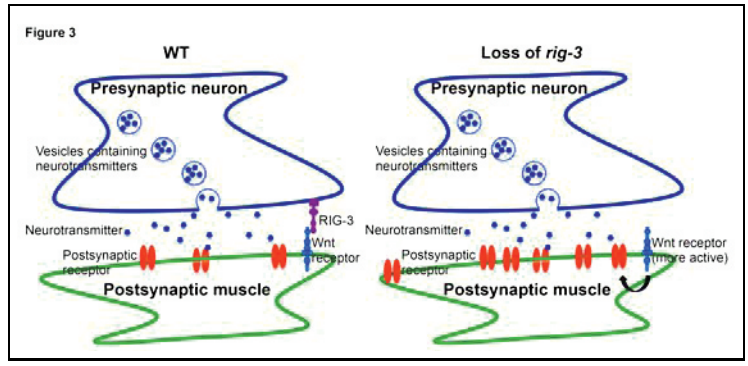

Figure 3. Illustration of RIG-3 signalling at the neuromuscular synapse RIG-3 functions presynaptically in neurons that synapse onto the body-wall muscles. RIG-3 binds to a Wnt receptor present on the muscle, which, in turn, is required for maintaining normal levels of postsynaptic receptor on the muscle. In the absence of *rig-3* the Wnt receptor is free for Wnt binding. This, in turn, causes more postsynaptic receptor at the neuromuscular junction (Source: Redrawn with modifications from [8].

The Wnt signalling pathway has been shown to be required in many developmental and functional processes and is considered essential for normal formation and functioning of an organism. Hence, when *rig-3* is absent, the Wnt receptor is free to bind Wnt and leads to excess postsynaptic receptors on the muscle [7, 8]. These experiments indicate that similar to the non-adhesion function of neurexin, NCAMs may also have signalling functions that are independent of their functions in synapse development and maintenance.

6. Multiple Functions of Cadherins

Cadherins (named for 'Calcium-Dependent Adhesion') are cell adhesion molecules that are required for adhesion between two cells. The adhesion is brought about by the cadherin domains located outside the cell and that bind to its partner cadherin located outside the neighbouring cell. Hence, cadherins are required for cell-cell interactions. The human genome encodes for as many as 115 cadherins that are grouped under multiple subgroups based on their sequence similarities. The family of cadherins that is best studied at the synapse is a class of cadherins called the N-cadherins. Analysis of the localization pattern of N-cadherins has revealed that they are localised to the synapse. Further, these cadherins are known to interact with either the same cadherin or a different cadherin, giving rise to the hypothesis that cadherins interact across the synapses allowing for both the development and

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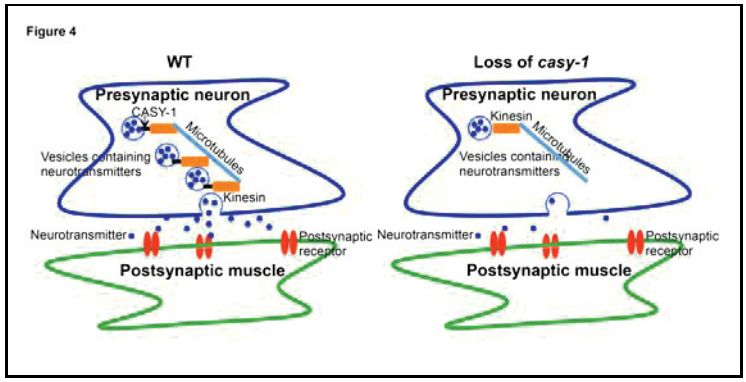

Figure 4. Illustration of CASY-1 function at the neuromuscular junction The shorter isoforms of CASY-1 are required for neurotransmitter release at the synapse. They are involved in this process by interacting with vesicles containing neurotransmitter through the motor protein kinesin that allows the vesicles to be delivered at the synapse along the microtubule network in the axon. In the absence of *casy-1*, this process is hampered and less neurotransmitter is released at the neuromuscular synapse (Source: Redrawn with modifications from [12]).

maintenance of synaptic structures (*Figure* 2). Loss of cadherin has shown defects in the morphogenesis and the maintenance of synapses, where mice in which N-cadherin was genetically ablated in late stages of brain development showed decreased synaptic density of a subset of synapses. Experiments have also shown that loss of another type of cadherin called E-cadherin shows defects in the development of another subset of synapses. Removal of E-cadherin from cultured neurons showed reduction in the density of synapses made by these neurons. A third type of cadherin—Cadherin-7—is required for synapse formation in the cerebellum. These and other studies show the importance of cadherins in synapse formation [9].

Apart from functioning to develop and maintain synapses, the cadherin superfamily proteins are also required for the functioning of synapses. We have recently shown that in *C. elegans*, a cadherin superfamily protein—CASY-1—works in the nervous system in an isoform dependent manner. Our work shows that loss of *casy-1* does not affect synapse development or maintenance, but affects synaptic function. Interestingly, the smaller isoforms of CASY-1 are required for neurotransmitter release from motor neurons at the NMJ (*Figure* 4). On the other hand, the large CASY-1 isoform is required to maintain neurotransmitter release in the neurons of the head of *C. elegans*. Together these studies give more insight into the signalling role of another CAM at the synapse [10–12]).

7. CAMs in Disorders of the Nervous System

Mutations in CAMs would likely affect synaptic formation and normal signalling at the synaptic site and hence brain development and function. A number of brain disorders are thought to be a result of mutations in CAMs.

As seen from the above examples, CAMs appear to be very important in the development, maintenance and functioning of synapses. Hence, mutations in CAMs would likely affect synaptic formation and normal signalling at the synaptic site and hence brain development and function. A number of brain disorders are thought to be a result of mutations in CAMs. For example, copy number variations, small deletions and single or small point mutations in the neuroligin or neurexin genes can give rise to autism (commonly thought of as autism spectrum disorders) [13]. Mutations in the NCAM gene have been associated with psychiatric and neurodegenerative disorders in the human brain (reviewed in [6]). Finally, a human counterpart of the *casy-1* cadherin gene has been implicated in memory performance. A genome-wide screen aimed to identify single neucleotide polymorphisms associated with human memory, identified a gene similar to *casy-1* called Calsyntenin 2 (*CLSTN2*). A single change of neucleotide in the first intron of this gene from T to C was shown to enhance the memory and cognitive performance of the subject [14]. Apart from these examples, changes in the expression other cell adhesion molecules are also thought to be linked to various aspects of brain function. Together, all of these evidence builds a strong case for the study of both the adhesion and the signalling properties of CAMs at the synapses.

Suggested Reading

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