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SYNAPSES – WHAT ARE THEY AND HOW DO THEY WORK?

Introduction: Neurons Are the Elementary Units of Our Nervous System

Every feature of ourselves that allows us to live as human beings is governed by our nervous system. It is not only responsible for our consciousness, but we also rely on it when we see, feel, listen, or move. Moreover, our nervous system controls most other functions of our body, such as our digestive system or our heart and blood circulation, which we become aware of only when something goes wrong.

Our brain is the master of our nervous system. It receives all signals from the outside or from other parts of our bodies, processes them, and governs the «output» which summarizes everything that is initiated by the brain, whether it be a movement, a change in our mood, or a new idea. How this happens is still fascinating for a large community of biologists, neuroscientists, physicists, and medical doctors. Despite more than one hundred years of research and enormous progress, particularly in the past few decades, we have only just begun to scratch the surface in our understanding of this miracle of evolution.

There are some similarities between our brain and a high-performance computer. Both systems are composed of elementary units – here the neurons and there the transistors. Both systems are specialized in the processing of information. However, that is where the similarities end. On one hand, modern computers are much more powerful and a lot faster than any nervous system. On the other hand, the complexity of a human brain and its performance still dwarfs that of high-performance computers in many aspects.

Let us zoom in on the neurons that make up our nervous system (Fig. I). A closer look reveals that relays are anything but simple. Neurons are struc-

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turally highly diverse, and each neuron is connected to many – sometimes more than a thousand – other neurons, resulting in complex and interconnected networks. Moreover, there are many different classes of neurons that differ in their functional properties and in the nature of their signaling molecules. Despite their diversity, however, all neurons share a set of common features. Like every other cell of our body, neurons are surrounded by a membrane, termed plasma membrane, that controls the exchange of molecules with the outside and that forms an electric insulator separating the cells' interior from the outside, a prerequisite for the generation and propagation of electric signals. Moreover, neurons have unique shapes, setting them apart from all other cells of our body. They consist of a cell body, termed soma, which contains the nucleus, and two types of thin and mostly branched processes that range from a few μm to many mm in length, and that are called dendrites and axons, respectively. For the most part, dendrites are highly branched, and they form the antenna region of the nerve cell at which signals are received. Frequently, individual neurons form many dendrites. In contrast, neurons mostly have only a single axon emanating from their somata that, however, may form highly branched networks at their distal ends. Axons serve as cables in which the signal output of the neuron is propagated in the form of stereotype electrical signals, the action potentials. The sheer number of neurons in our nervous system is staggering – close to 100 billion. Indeed, we are still barely able to understand the wiring diagram of a cubic millimeter of our brain, and even if we have this in hand it constitutes only a first step towards understanding, considering the biochemical and functional diversity of the neurons.

Signaling Between Neurons: An Overview of Synaptic Transmission

How are signals transmitted between neurons? Neurons are connected with each other at specialized contact sites. Information transfer is unidirectional: axons of the «sender» neuron connect with dendrites or cell bodies of the receiving neuron by forming specialized contact zones termed synapses (Fig. II). Here, the electrical signals reaching the axon terminals (also referred to as presynaptic terminals) are re-coded in chemical signals. The neurotransmitters that are released from the axon terminals into the extracellular space diffuse across a narrow cleft and bind to specific receptors in the membrane of the receiving, or postsynaptic, neuron. Binding to the receptors changes the electrical properties of the postsynaptic membrane, resulting in the generation of an electrical signal that is then integrated with signals received from other synapses, propagated passively as

far as the axon initial segment (Fig. I), where it is converted into action potentials as output signal. Action potentials are stereotype electrical signals, and the conversion of the graded electrical potentials into action potentials resembles an analog-digital conversion. The type of neurotransmitter determines whether synapses exert an excitatory or an inhibitory influence, i.e., whether the postsynaptic neuron increases or reduces the frequency of its action potentials. Moreover, neurons may be exposed to a tug-of-war: if both excitatory and inhibitory synapses are activated at the same time, the effect on the receiving neuron will depend on which influence is stronger. Thus, individual neurons are far more sophisticated than a transistor in that they are capable of complex signal integration and computation in time and space.

Although synapses are tiny (on average 1 μm in diameter), they are complex structures specialized for the rapid release of neurotransmitters. The most striking feature of synapses is the high concentration of small vesicular structures – the synaptic vesicles – that store neurotransmitters before release. While most synapses in our brain contain a few hundred of such vesicles, there are «industry-style» variants that are much larger and may contain several hundred thousand vesicles, such as the synapses regulating the contraction of our muscles. When an action potential arrives in the nerve terminal, the voltage across the membrane changes (it is depolarized), which triggers the influx of calcium ions into the synapse. Normally, the calcium concentration inside the synapse is very low, whereas on the outside it is high, with an almost ten thousandfold concentration difference. Thus, the opening of calcium channels results in an inwardly directed and jetlike spurt of calcium ions that is sensed by nearby synaptic vesicles and triggers their fusion with the plasma membrane. This process is termed exocytosis (see Fig. II), and results in the release of their neurotransmitter content.

As stated above, synapses are anything but simple relays. The amount of neurotransmitter that is released per arriving impulse depends on many variables, such as the past stimulation history or signals received from their surroundings. For instance, some synapses become stronger over time when excited by repetitive pulses (facilitation or potentiation), whereas others reduce their output under such conditions (desensitization or depression).

Although synaptic transmission is among the fastest known biological reactions, it is agonizingly slow when compared to a computer. At each synapse, there is a delay of about 1 millisecond before an incoming electrical signal results in an electrical response at the postsynaptic neuron, a far cry from the near light speed that governs electronic devices. Indeed,

synaptic delay poses an upper limit on our performance in time, which cannot be overcome even if we try very hard. For instance, due to synaptic delay it is impossible to beat a slot machine in a gambling casino – the number of synapses that need to be passed in the circuit from our eye to the control of our muscles is too high to react in time. Moreover, considering that neurons can fire many hundreds of times per second, synapses, particularly their synaptic vesicles, need to be continuously recycled, which puts additional constraints on the timing of information processing. The first step of recycling is termed endocytosis, where pieces of membranes corresponding to the membrane pieces incorporated during exocytosis are retrieved back into the interior of the presynaptic nerve terminal. Such coordinated retrieval also serves to maintain the shape of the synapse which enlarges if more and more of the vesicle membrane is incorporated into the plasma membrane. At least two different retrieval pathways are known to exist – one very fast and probably less accurate (ultra-rapid endocytosis, discovered only a few years ago, Watanabe *et al.* 2013), and a second, slower one that is associated with the regulated assembly of a protein coating surrounding the vesicle during its formation (see Fig. II). After retrieval, synaptic vesicles are regenerated within the nerve terminal, which sometimes involves intermediate steps. Individual vesicles can locally recycle many times, but at some point, they age and accumulate molecular defects. Such vesicles are eventually dismantled and shipped back towards the cell body for degradation. Conversely, vesicles freshly made by the protein and membrane factories close to the nucleus arrive by axonal transport for replenishment, thus ensuring that synapses continue to function for many years.

In our work, my collaborators and myself attempt to better understand the molecular basis of exocytosis and synaptic vesicle recycling, with a focus on two steps: (i) the regulated fusion of synaptic vesicles with the presynaptic plasma membrane, and (ii) the re-filling of synaptic vesicles with neurotransmitter after endocytosis and regeneration.

Exocytosis of Synaptic Vesicles – Supramolecular Machines with Fascinating Properties

Synaptic vesicles are nanostructures of high complexity. They are smaller than viruses such as influenza or Sars-CoV2, but are structurally more diverse and contain many dozens of different proteins. Considering the abundance of neurons and considering further that each neuron on average forms 1,000 synapses, it is no surprise that synaptic vesicles are the most abundant organelles in our body. Each of us carries an estimated number of

more than 10^{16} synaptic vesicles, an almost unimaginably high number: over seven orders of magnitude more than our genome have letters (base pairs). If you put all your synaptic vesicles on top of each other, you would obtain a pile that would cover the distance from the earth to the moon several times. For a biochemist like myself, such abundance is an advantage because it is possible to isolate synaptic vesicles from animal brains in sufficient quantities for a detailed molecular analysis. Figure III shows a molecular model of an average synaptic vesicle with its main protein components. These proteins are instrumental for carrying out the two key jobs that synaptic vesicles must perform: (i) the attachment of the vesicle to the presynaptic membrane and its rapid fusion in response to an influx of calcium ions, and (ii) the re-loading of synaptic vesicles with neurotransmitter molecules.

Exocytosis is mediated by a set of three conserved proteins. They have funny names that were given to them as «brand names» before it was known what they did: Synaptobrevin (or VAMP) is anchored to the membrane of synaptic vesicles and the proteins syntaxin and SNAP-25 in the plasma membrane, and they are commonly referred to as SNAREs or SNARE proteins. These proteins work like a zipper at the nanoscale: when a vesicle close to the plasma membrane is getting ready for exocytosis, these proteins get in touch with each other at their distal ends. Then they spontaneously zipper up, thus pulling the membranes forcefully together and initiating the fusion reaction. Indeed, high-resolution structures obtained by X-ray crystallography have revealed that after zipping up, these proteins form extended bundles with a repetitive helical structure (Fig. IV). Moreover, biochemical experiments have confirmed that assembly is initiated at the membrane-distal ends and progresses towards the membrane anchors, supporting the zipper model of SNARE function. It is generally accepted that these three molecules are indeed the «working engine» of membrane fusion. For instance, numerous studies using genetic techniques have shown that deletion or modification of any of the proteins affects (or largely prevents) exocytosis. Moreover, all three proteins are selectively targeted by tetanus and botulinum neurotoxins, which exert their toxic effects by blocking signaling between neurons. When reaching the bloodstream, these bacterial toxins bind to the nerve terminals where they enter the cells and selectively cut either one of the three proteins. When this happens, synaptic vesicles cannot undergo exocytosis any longer – when action potentials arrive and calcium entry is initiated.

Due to intense research efforts by many laboratories, we now have a detailed molecular understanding of how exactly SNAREs mediate membrane fusion (Fig. V). SNARE proteins do not operate alone, but are regulated by a whole battery of additional proteins. In particular, initiation of

SNARE assembly – the «ignition» of the SNARE engine – is tightly regulated by a set of two proteins, Munc18 and Munc13, which by themselves are connected to a network of additional proteins at the site where fusion occurs. Although the details are still debated, it is becoming clear that these two proteins bind to the SNAREs before they assemble and guide them to the initiation of the zippering – the SNAREs need to be correctly «threaded», otherwise they will not work at the required speed. Another protein, complexin, facilitates subsequent fusion by binding to partially or fully assembled SNAREs.

How is calcium influx linked to SNARE-mediated exocytosis? This link is executed by another crucial regulatory protein, synaptotagmin. It is an abundant membrane protein of synaptic vesicles containing two calcium-binding domains. These domains operate like switches: they are «on» when calcium is bound, and as soon as calcium is removed from the synapse, they switch back into the «off» position. Synaptotagmin forms a small family of closely related proteins with slightly different functions, which allows for fine-tuning of the responsiveness of a given synapse to an arriving action potential. Although there is compelling evidence showing that synaptotagmin is indeed responsible for initiating exocytosis after calcium influx, exactly how this happens is still debated. Apparently, SNARE zippering is halted after initiation of their assembly at the membrane-distal ends, and synaptotagmin releases the brake somehow, thus allowing the SNAREs to assemble completely and mediate the fusion of the membranes. According to one view, synaptotagmin, when activated by calcium, directly binds to the partly zippered SNAREs, giving them a «push» to overcome the block and complete the zippering. According to another view, synaptotagmin binds to the membrane directly adjacent to the SNAREs in the plasma membrane, destabilizing it and thus enabling the SNAREs to progress in zippering towards fusion. In any case, the speed of synaptic transmission can only be achieved when the SNAREs are already correctly ignited and only need to flash through the superfast zippering process when triggered by synaptotagmin. Indeed, this step requires less than 200 μs, i.e., only one fifth of the one millisecond required for synaptic transmission.

As discussed above, SNARE zippering is spontaneous, and although regulated by other proteins, it is «exergonic»: the energy released during zippering is used to fuse the membranes. After fusion, however, SNAREs need to be regenerated to become active again, which involves the dissociation of the SNAREs from the complex (i.e., the re-opening of the zipper). How is this achieved? The protein responsible for this step is termed NSF, which belongs to a class of specialist proteins whose job it is to disentangle other protein assemblies by grabbing them and pulling on their parts. They

all are ring-shaped nanomachines, whose small arms extending from the ring grab, pull, and probably stuff the parts they pry loose from their targets through the hole in the middle of the ring. Obviously, such action requires energy that is supplied in the form of the molecular energy currency termed ATP. ATP-turnover allows the subunits of the ring to induce movement (conformational change in scientific terms) in their attached arm domains, thus transferring mechanical force to the bound substrate. Intriguingly, NSF does not directly connect to the SNAREs but rather uses an intermediate protein termed SNAP, i.e., it does not use its own «hands» to pull the SNAREs apart but uses a «crowbar» as a tool. SNAP is a stiff and twisted molecule, so well-suited for such action.

Disassembly by the NSF-SNAP system «closes» the SNARE cycle that re-generates the SNARE proteins for another round of exo-endocytosis. Obviously additional steps are required, not all of which are understood. Most importantly, after disassembly the liberated SNARE synaptobrevin needs to be separated from its partners in the plasma membrane and to join the recycling of synaptic vesicles. An overview over the SNARE cycle is shown in Figure V.

Even small problems in any of the steps governing synaptic vesicle exocytosis and recycling may quickly accumulate to have catastrophic consequences for the organism, as impressively shown by the effects of poisoning by tetanus or botulinum neurotoxins. We are only beginning to understand how it is ensured that these incredibly complex molecular machines can operate for many years in a «fail-safe» mode. As mentioned above, there are mechanisms that replenish damaged vesicles or damaged proteins with new ones while dismantling the dysfunctional ones into their molecular components. These replacements happen while the synapse continues functioning. Moreover, there are so-called «chaperones», i.e., proteins that help the key players to get back into shape if they end up with problems in their continuous conformational gymnastics. Intriguingly, mutations in one of these helping chaperones, termed synuclein, are in themselves causative for a major disease (Parkinson's disease), although Parkinson's is solely caused by irreversible aggregation of the protein itself and has nothing to do with its chaperone function in the SNARE cycle. Another mechanism for avoiding short-term problems is redundancy: the proteins crucial for exocytosis and membrane recycling are among the most abundant proteins of our brain – there is a huge reservoir of many of the critical components in the synapse and in nearby membranes, and this allows them to jump in and take over if one of the proteins has a problem in doing the job. Nevertheless, it is becoming evident that mutations in many of the critical proteins (most prominently the SNARE SNAP-25), while having only

very subtle and barely measurable effects on synaptic function, may result in altered performance of our nervous system, thus giving rise to neurological disease. Frequently, such mutations only become disease-inducing if combined with other similarly subtle defects in other proteins or pathways. Only when a «threshold» of such accumulating defects is crossed does the disease become apparent. At present, there has been rapid progress in identifying such defects, raising hopes that one day this knowledge may be instrumental for developing novel therapeutic approaches.

Filling of Synaptic Vesicles with Neurotransmitters

Synaptic vesicles are containers for the signaling molecules, or neurotransmitters, that are released upon excitation and then «read» by the postsynaptic neuron. After exocytosis, the transmitter molecules quickly diffuse and are mopped up by powerful «vacuum cleaners» – transport proteins that very rapidly and with high efficiency pump them back into the neurons and surrounding glial cells. Moreover, each nerve terminal has the capacity to synthesize neurotransmitters from general metabolic precursors. However, in order to serve in signaling, they need to be transported from the synaptic cytoplasm into the lumen of synaptic vesicles to reach very high concentrations. Without such transport, synapses could not release their transmitters and signaling between neurons would not work.

Our nervous system uses only a handful of small molecules as neurotransmitters. In addition to specialists that are only made for the purpose of functioning as transmitters, such as acetylcholine, serotonin, dopamine, or noradrenaline, a few common metabolites also serve as neurotransmitters, most prominently the amino acids glutamate, glycine, and GABA. In the latter cases, the vesicular loading mechanism is responsible for deciding which transmitter is being released as they all are present as metabolites in the cytoplasm of all cells.

Unlike calcium influx discussed above, neurotransmitters need to be transported against their concentration gradient. The intravesicular concentrations that need to be achieved are a lot higher (in some cases several orders of magnitude) than those in the cytoplasm. This is achieved by transport proteins termed «secondary active» since they link the transport of their target to the transport of another molecule or ion that exhibits a concentration gradient in the reverse direction. This may be likened to a device that pumps fluids uphill while being powered by water driving a water wheel while flowing downhill. Using appropriate reduction gears, fluids can be pumped up much higher than the height difference of the water

powering the wheel. This is exactly how some of these transport proteins operate: they can achieve concentration gradients far higher than those of the «driving» ion, simply by linking the inward transport of one molecule to the outward transport of two or more of the driving ions.

In the same way that the water flowing downhill needs to be replenished by rain, the gradients of the driving ions must be maintained. This is particularly important for the synaptic vesicles which, due to their small size, can only accommodate a few hundreds of ions at any given time. Using such ions for coupled transport would very quickly drain the gradient, and could result in stopping or even reversing transport unless the gradient is continuously replenished. Synaptic vesicles use protons as the driving ion, with the gradient being maintained by the continuous activity of a specialized proton pump termed V-ATPase, which pumps protons into the synaptic vesicle at the expense of metabolic energy. Indeed, the continuous operation of this pump is critical for vesicle loading, particularly since each synaptic vesicle contains only one (rarely two) copies of these pumps. Moreover, it turns out that this pump is by far the biggest energy consumer of our brain: it burns even more ATP than the ion pumps that operate at the neuronal plasma membrane and maintain the membrane potential, i.e., all the electrical activities of the brain.

A handful of vesicular transporters specialized for their respective neurotransmitters are known. In some cases, there are several transporters for a single transmitter, such as glutamate, or transporters for several different transmitters, such as serotonin and the monoamine transmitters. Among the most abundant ones are the vesicular transporters for the amino acid glutamate (termed VGLUTs, see Fig. III) that serves as the main excitatory neurotransmitter in our central nervous system.

In our group, we are primarily interested in understanding how synaptic vesicles can be filled with high amounts of transmitter in seconds. When a vesicle is regenerated by endocytosis, it does not contain any transmitter, but rather extracellular fluid with a defined ionic composition. Pumping in huge amounts of transmitter, particularly those that are ions such as glutamate, acetylcholine, or the amines, requires that not only charge neutrality is maintained during transport, but also that the intravesicular increase in osmotic pressure is compensated in one way or another. We also want to understand how vesicle filling is regulated. Are all vesicles stereotypically filled with the same amount (like when the gas tank of a car is completely filled), or are there factors controlling the degree of filling, and thus the amount of transmitter that is released from a vesicle? To achieve this, we are adapting and developing methods that make it possible to monitor vesicle filling and associated ion fluxes with high precision, high time resolu-

tion, and high sensitivity. For instance, a few years ago we used appropriate fluorescent sensors to show that the proton gradients can be monitored at the level of single synaptic vesicles, despite the fact that they are too small to be resolved in the light microscope unless the most advanced super-resolution methods are applied. Another approach is to construct our own vesicles with predefined properties, either by using purified proteins or by inserting the membrane of native synaptic vesicle into large artificial vesicles, which can be achieved by SNARE-mediated fusion. We hope that in the next few years we will have made progress along these lines and successfully addressed at least some of these open issues.

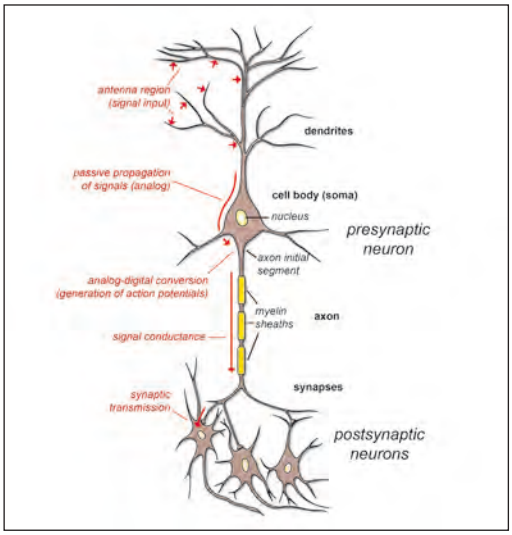
Cited references

Sutton, B. – Fassahuer, D. – Jahn, R. – Brünger, A.T. 1998. "Crystal Structure of a SNARE Complex Involved in Synaptic Exocytosis at 2.4 Å Resolution." *Nature* 395, pp. 347-53.

Stein, A. – Weber, G. – Wahl, M.C. – Jahn, R. 2009. "Helical Extension of the Neuronal SNARE Complex into the Membrane." *Nature* 460, pp. 525-28.

Takamori, S. – Holt, M. – Stenius, K. – Lemke, E.A. – Grønberg, M. – Riedel, D. – Urlaub, H. – Schenck, S. – Brügger, B. – Ringler, P. – Müller, S.A. – Rammner, B. – Gräter, F. – Hub, J.S. – De Groot, B.L. – Mieskes, G. – Moriyama, Y. – Klingauf, J. – Grubmüller, H. – Heuser, J. – Wieland, F. – Jahn, R. 2006. "Molecular Anatomy of a Trafficking Organelle." *Cell* 127, pp. 831-46.

Watanabe, S. – Rost, B.R. – Camacho-Pérez, M. – Davis, M.W. – Söhl-Kielczynsk, B. – Rosemund, C. – Jorgensen, E.M. 2013. "Ultrafast Endocytosis at Mouse Hippocampal Synapses." *Nature* 504, pp. 242-47.



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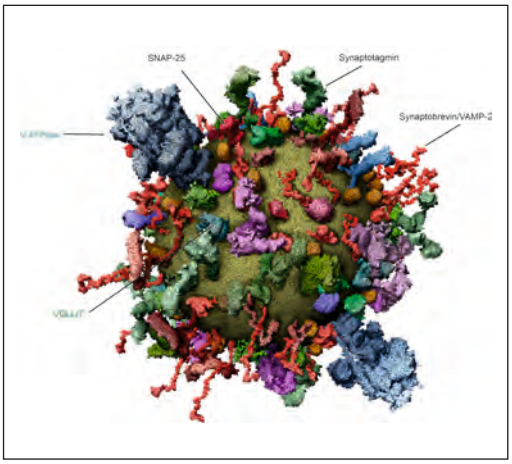

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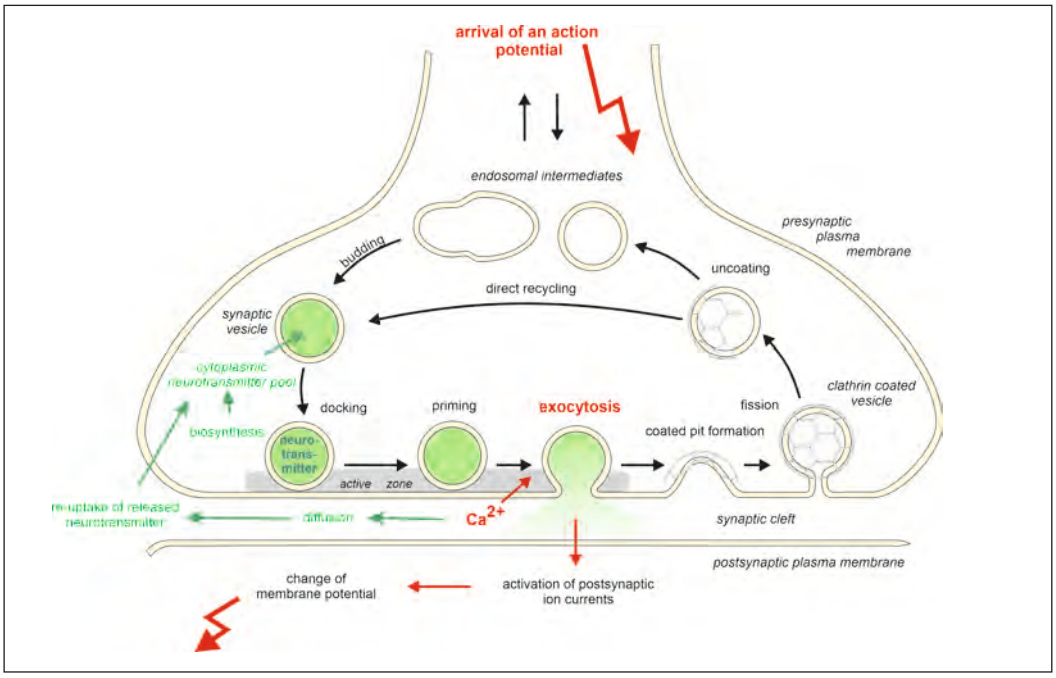
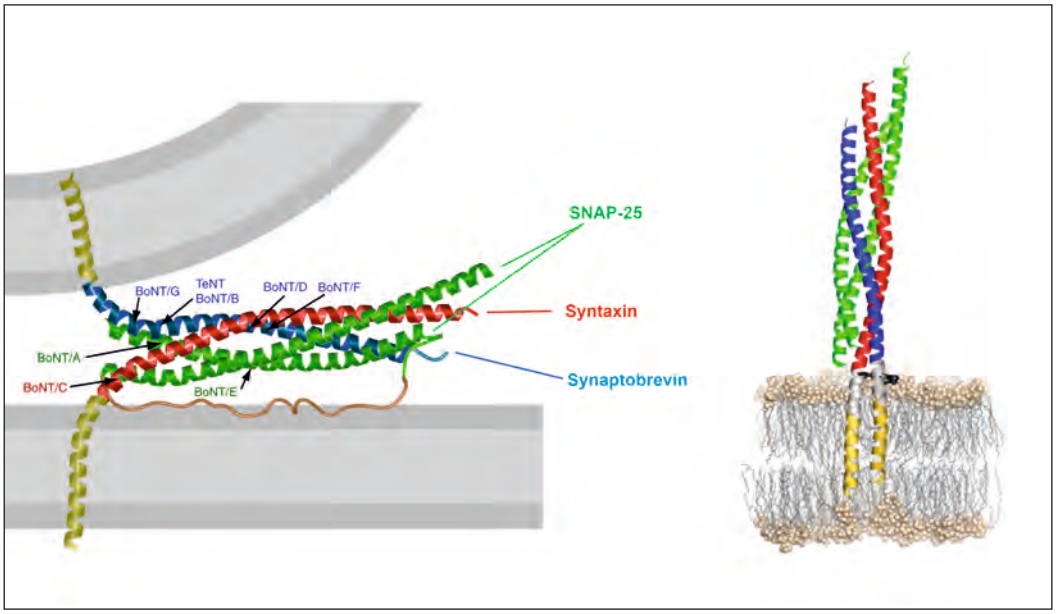


II

Reinhard Jahn, *Synapses – What Are They and How Do They Work?*

Fig. I. Architecture of a typical neuron. See text for details.
Fig. II. Schematic overview over the main steps in synaptic transmission. When an electrical signal (action potential) arrives, the presynaptic membrane changes its voltage, resulting in the opening of voltage-gated calcium channels. The resulting influx of calcium ions triggers the exocytosis of synaptic vesicles, resulting in the release of their neurotransmitter content. Synaptic vesicles are regenerated within the nerve terminal by endocytosis (which may involve the formation of clathrin-coated vesicles) and regenerated, involving endosomal intermediates. See text for details.
Fig. III. Molecular model of an average synaptic vesicle from mammalian brain. Proteins involved in the exocytosis of synaptic vesicles are labeled in black, those involved in the filling of the vesicle with neurotransmitters are labeled in green. Adapted from Takamori *et al.* (2006).

IV



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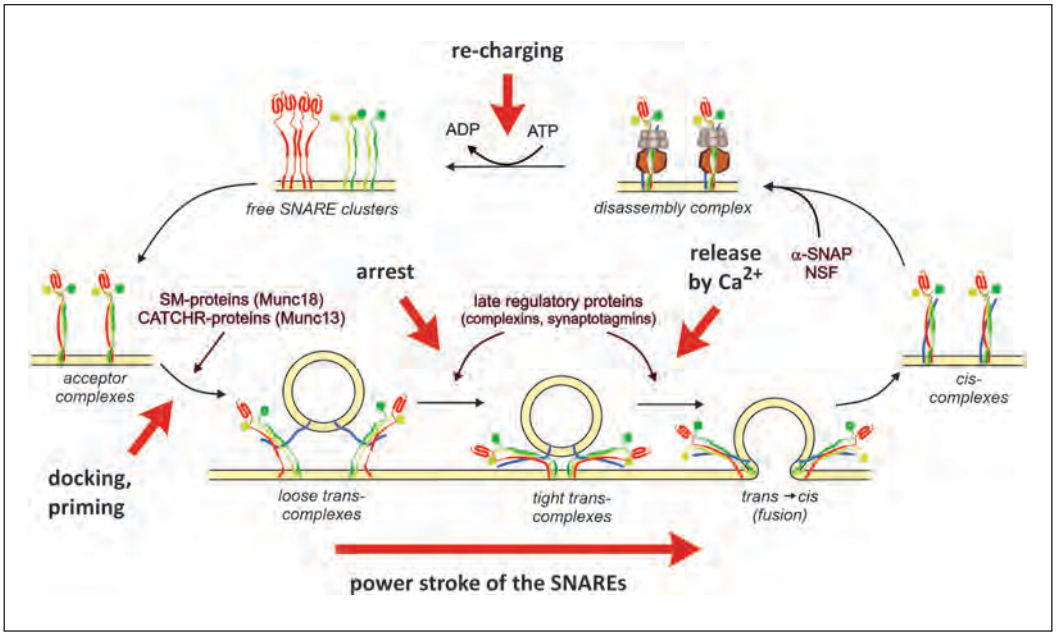

Fig. IV. Structures of the partially (left) and fully (right) assembled SNARE proteins as determined by X-ray crystallography. Note that only fragments of SNAP-25 and syntaxin were crystallized in these experiments. The sites where the various botulinum neurotoxins (BoNT) and tetanus toxin cleave the SNARE proteins (and thus inactivate them) are indicated. Adapted from Sutton *et al.* (1998) (left) and Stein *et al.* (2009) (right).

Fig. V. Schematic overview over the cyclic assembly (zippering) and disassembly of the synaptic SNARE proteins, which drives exocytotic fusion of synaptic vesicles with the plasma membrane. The «power stroke» is the step in which energy is liberated to overcome energetic barriers for the fusion of membranes. To re-charge the SNARE «machine», the proteins need to be disentangled and separated, which is mediated by an energy-consuming enzyme termed NSF (see text).