

## Biotechnology of Human Nervous System

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### Abstract:

Ion Channels a cell membrane channel that is selectively permeable to certain ions (as of calcium or sodium) Synapses are region where nerve impulses are transmitted and received, encompassing the axon terminal of a neuron that releases neurotransmitters in response to an impulse, an extremely small gap across which the neurotransmitters travel, and the adjacent membrane of an axon, dendrite, or muscle or gland cell with the appropriate receptor molecules for picking up the neurotransmitters. Biological Neural networks draw much of their inspiration from the biological nervous system. It is therefore very useful to have some knowledge of the way this system is organized

### Introduction:

#### Ion Channels

The three main groups of ion channels are 1) the voltage-gated channels such as the sodium and potassium channels of the nerve axons and nerve terminals, 2) the extracellular ligand-activated channels which includes channels such as GABA and glycine receptor [1] channels, most of which are regulated by ligands that are “neurotransmitters”. These channels are often named according to the ligand they bind to. 3) Intracellular ligand-gated ion channels [2]. These include CFTR and some other ABC family members as well as ion channels involved in sense perception. These are often activated indirectly by GPCRs. Other common intracellular ligands which activate these kinds of channels include calcium ions, ATP, cyclic AMP and GMP as well as phosphatidylinositol [3] (PI). There are additional systems of nomenclature which have joined the second and third groups into the “chemically activated” or just simply “ligand gated” ion channels. It has been shown by sequence comparison that ion channels within the above groups will also show the greatest sequence similarity and are therefore most likely all be descended from a common ancestor. The mechanosensory and volume-regulated channels [4] have their own grouping, but they are still in the process of being classified. We have made a fifth “catch-all” group, miscellaneous 2, which includes any ion channels not included in the above there. This group includes the GAP junctions, peptide ion channels like Gramicidin, and various venomous insect toxins like the conus toxins from cone shells. At the end is a section on Recent Discoveries.

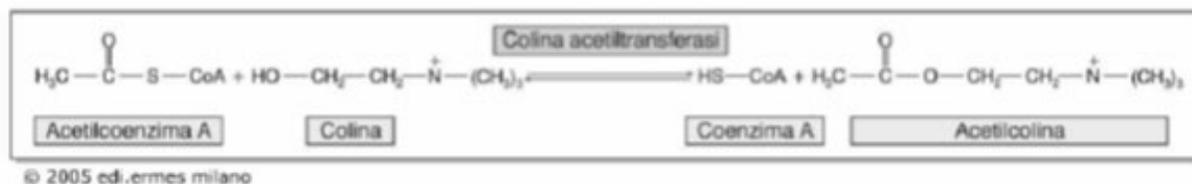
A few words regarding the classification and naming of ion channels: Of the several possible ways of arranging ion channels

into groups, so far the most successful seems to be a system based on how ion channels are regulated. It should be remembered that no method known is without drawbacks. For example, it is possible to arrange ion channels according to the ion that they conduct thru their pores rather than regulation mechanisms, however this method has the problem that very few other characteristics will be shared in common among the channels. For instance, the nAChR [5] channel conducts sodium ions, as does the voltage-gated sodium channels of the neurons. But other important features are very obviously not be shared between these two very different ion channels, including gating mechanisms, as well as the sequence of amino acids making up the channels themselves (i.e. they are not related evolutionarily). Many researchers believe that until there is a classification system based solely on sequences and molecular structures, as well as evolutionary history of all ion channels at the level of the genome, the method of classification (even the one used here based on activation mechanisms) will have to suffice. A good example which exemplifies a need for this new kind of system is given by the finding that certain cyclic nucleotide gated channels share enough sequence similarity with voltage-gated ion channels that they may in fact belong to the voltage-gated channel superfamily rather than the ligand-gated superfamily [6] they are presently placed in. Another problem with the present system is that while the glutamate ion channel, which is activated by the ligand glutamate [7], is often relegated to the family of ligand-gated ion channels, it shares no sequence similarity and therefore is probably unrelated evolutionarily to any other ligand-gated [8] ion channels in this group.

### Synapses; neurotransmitters, neuromodulators, ionotropic and metabotropic receptors.

**nAChR:** The “nicotine acetylcholine receptor” has the distinction of being the first ion channel sequenced: in 1983. It functions as a multimer of 5 subunits which form the channel (2 alpha, 1 beta, 1 gamma, 1 delta). It is found in nerve and muscle cells and is not to be confused with other nAChR receptors, some of which are not ion channels at all. The ion channel nAChRs[5] include those found in nerves (nerves release acetylcholine which binds to nAChR in muscle cells). When the channels open, they let pass just about all cations, including sodium, potassium, and calcium; and this depolarizes the cell membrane which can in turn trigger voltage-gated channels which in turn causes muscle contraction. nAChR is considered to be a model ion channel mainly because of its historical importance due to its high abundance from natural sources which made it easier to study especially before molecular biology techniques became more refined. nAChR is used in many detailed studies on ion channel kinetics and allosterism and was first isolated and characterized from the fish Torpedo[9] (tissue source was its electric organ). Torpedo is a marine ray). There are many different “variations” of the nAChR channel. For example, mammals have several just in the nervous system. The poison curare is known to shut down (and therefore act as an “antagonist” of) nAChR. Nicotine is an alkaloid drug from tobacco which exerts its physical effects on the body in part because it also binds to nAChR. However instead of simply shutting it down like curare did, nicotine activates it instead. Chlorpromazine, a tranquillizer, is able to block the channel pore. It is quite possible that general anesthetics exert their effects via direct binding to the transmembrane helices[10] of ion channels such as nAChRs. The nAChR family, together with the GABA and Glycine chloride channel families forms a superfamily of ligand-gated ion channels which is based on strong sequence similarity. These neurotransmitter ligand-gated channels are not related to voltage-gated channels or to the glutamate ligand-gated ion channel. They all have 4 distinct transmembrane segments.

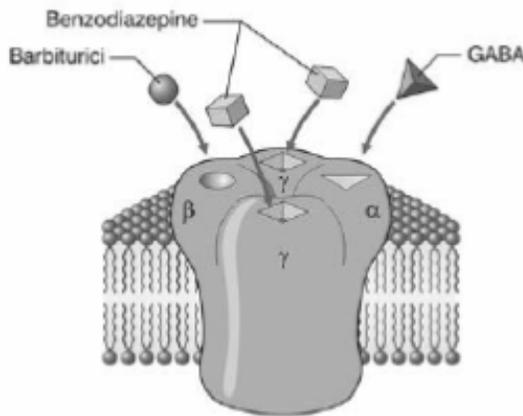
## Acetilcolina (ACh)



**GABA** and Glycine Receptors[11] (these are both families of chloride channels). GABA(A) and glycine chloride channels have relatively complex gating characteristics and are made up of 5 subunits, with each containing 4 transmembrane helices (TMs), and are characterized as having multiple states of conductance apparently due to them having more than one possible open state. TM2 has been found to contribute to pore (i.e. the five TM2 segments from the 5 subunits come together to line pore). Chloride channels in excitable tissues function in the same way as the potassium channel in that they serve to “dampen” the electrical excitability of the neuron. GABA(A) and glycine receptor chloride channels function in the postsynaptic neuron as well as skeletal tissue. GABA(A) is so named because it binds to gamma-aminobutyric acid. GABA and Glycine neurotransmitters act mostly on inhibitory neurons. Like glutamate, they are small molecules and therefore act as fast messengers in neurons. GABA inhibits ability of neurons to fire action potentials, and should not be confused with the unrelated GABA(B) receptor, which couples to the intracellular second messenger systems. As many as 1/3 of brain neurons use GABA.

GABA is manufactured by the body from glutamate, however vitamin B6 is a necessary component. Lack of it can cause seizures. Alcohol and barbituates like Valium on the other hand act on GABA(A) receptors as agonists (activators), by increasing burst time of the channels. They bind at sites where GABA doesn't normally bind to, and potentiates the action of GABA when it binds at it's own site. This often makes these types of drugs beneficial for epilepsy. Glycine Receptors tend to be more localized in brain, but can be found in spinal cord and other places. Some molecules bind GABA(A) Receptors but do not open them, thereby blocking them from activation by the endogenous neurotransmitter[12] itself. Diversity of GABA(A) receptors can be increased due to the fact that each channel is made up of distinct amounts of various individual protein subunits (each subunit is a distinct protein). There are 6 alpha subunit isoforms for GABA(A) receptors (3 beta and 3 gamma and one delta). All subunits have a GABA ligand-binding site, unlike nAChR in which only a single subunit is able to bind its ligand, ACh. Their mRNA transcripts can also be alternatively spliced to provide further diversity. Note: the majority of all known ion channels consist of more than one protein subunit, as with GABA(A) and Glycine Receptors. Glycine Receptor is also chloride channel, but binds glycine instead of GABA. The glycine and GABA(A) Receptors are both found in the post-synaptic membranes of neurons and are both composed of oligomers consisting of several homologous subunits (each subunit is about 50 KDa) with the amino terminus facing out of the cell and the second transmembrane helix lining the pore (like with nAChR). Each subunit most likely consists of 4 membrane-spanning helices, not unlike the nAChR subunits. The glycine receptor has 4 different alpha subunits and one beta subunit. Both GABA(A) and Glycine Receptors are involved in producing inhibitory responses and serve

to dampen the action potential of neurons and show amino acid sequence similarities. All of the subunits are of similar size. Note: the various glutamate receptors are in a different family completely due to differences in sequences. Blocking inhibitory receptors causes convulsions. The GABA(A) Receptor is widely found in brain. The disease called “Startles Disease” [13] results from a mutant form of the glycine receptor alpha-1 subunit [14]. Patients with this disorder are subject to muscle rigidity in response to external stimuli believed to be due to a lowered affinity of the ion channel to its ligand, glycine, which results ultimately in a lowered chloride conductance into neurons. This reduces the inhibitory effects of chloride on certain neurons. Strychnine acts as a competitive antagonist. It binds the alpha-subunits of the Glycine Receptor. These channels, like the GABA(A) ion channel, form mostly heteromeric pentamers. Both GABA(A) and glycine receptors conduct chloride in the range of 10 to 90 pS. Both conduct bicarbonate anions as well. Glycine receptors can be found throughout the CNS, not unlike GABA(A). Note: GABA and Glycine neurotransmitters act mostly on the inhibitory type of neurons. As many as 1/3 of the neurons in the brain have them.



**Sodium Voltage-Gated Ion Channels:** In 1978, these channels were first purified (Agnew et al.) from electric eel electric organs. Found to be a single peptide of almost 2000 amino acids in length (but with internal repeats corresponding to the equivalent of subunits). However, in other tissues such as mammalian skeletal muscle or brain, it can be found as subunits (4 subunits in the case of voltage-gated channels, but 5 in the fast ligand-gated receptors. GAP junctions have 6. It seems that the more subunits an ion channel is composed of, the less selective it is for its respective ions. This may be because the pore is correspondingly larger the more subunits it is composed of). The channel from electric eel was also found to have 30% of its weight in carbohydrates (500 sugars of mostly sialic acid and N-acetylglucosamine) and 6% as attached fatty acids. Some Sodium Voltage-gated channels may have as many as 6 different kinds of neurotoxins which bind and inhibit them to various degrees and each toxin appears to bind at a different site, which is unusual. Some of these toxins are classified as peptides, while others are alkaloids, cyclic polyethers, esters, and heterocycles. Most peptide neurotoxins are 60-100 amino acids in length, which allows them to assume a defined shape, but curiously, the peptide toxins made from cone shells are often only between 10 and 30 amino acids long. They accomplish their inhib-

itory task by forming disulfide bonds with each other. Usually 2 or 3 come together and form these larger structures. Voltage-gated Sodium channels are responsible for the action potential of neurons while the voltage-gated potassium channels help to re-establish the membrane potential back to normal. Pore sizes are estimated to be  $\sim 3 \times 5 \text{ \AA}$  for the selectivity filter region. Potassium channels are more diverse, and yet it is also true that sufficient diversity exists among sodium channels for different monoclonal antibodies to distinguish sodium channels from different tissues (axons from muscle, etc). Sodium channels deactivate quickly compared to calcium channels. This is the reason calcium ions are used by the cell for more of a sustained response to external stimuli. Some other members of this family: mH1, mH2, SCN4A (skeletal muscle), PN1, PN3, SkM1, RSMK, Kat1, EAG, ELK, Drk1 [15].

### Simple circuits nervous: the control of body movement

Since the species Homo sapiens appeared on Earth, man has begun to observe and to try to predict the behavior of animals. Currently, the study of the way in which animal behavior is produced and controlled is surely motivated by the search for patterns of human behavior. L' "hardware" at the base of the behavior, however, is formed by neuronal networks, or interconnected neuronal circuits. Unlike electrical circuits, connected in a predetermined way, the neural networks are not "installed in a rigid way". In fact, one of the properties of neural networks is the plasticity, that is, the ability to change from a functional point of view, and in part also anatomic, in response to the experience. The simplest nervous circuit is represented by the arc reflection, in which the sensory input is transmitted through a number of synapses to a motor neuron which produces the motor output that determines the muscle contraction. It is supposed that the primordial reflex arc was made from a sensory receptor that, rooted directly an effector cell. In the pharynx of the nematode *Caenorhabditis elegans* have in fact been identified cells that are likely to serve both the function of perceiving the stimulus that that of producing the locomotor signal. In the course of evolution, the neurons have become more and more numerous, the progressively more complex neural circuits and the nervous system has been compacted and central forming the central nervous system. The spatial contiguity [16] between neurons and the central nervous system increases the possibility that you establish connections between individual nerve cells. Moreover, since many of the neurons are localized in the central nervous system, peripheral receptors and effectors are connected to the central nervous system through long axons sensory and motor. A simple circuit, very common in modern animals is the monosynaptic reflex arc. In this type of reflex arc a sensory neuron (receptor) synapses with a motor neuron located in the central nervous system, which innervates a muscle (effector); are thus three elements of this simple reflex: the sensory neuron, the motor neuron and the muscle fiber. Each time that the sensory receptor is activated by a stimulus in a sufficient way, that excite the motor neuron, in turn, controls a reflex muscle contraction. Reflex pathways in postsynaptic, much more common, however, one or more interneurons localized in the central nervous system bring into connection the sensory receptors and motor neurons. In the course of evolution, animals organisms have become increasingly complex and parallel interneurons have become progressively more numerous; is increased accordingly

also the complexity of the behavior, which is extremely elaborate in higher animals.

### **Neural networks and their meaning morpho-functional**

The connections between neurons are much more complex than those implemented in neural computing architectures. The basic kinds of connections between neurons are chemical synapses and electrical gap junctions. One principle by which neurons work is neural summation, i.e. potentials at the post synaptic membrane will sum up in the cell body. If the depolarization of the neuron at the axon goes above threshold an action potential will occur that travels down the axon to the terminal endings to transmit a signal to other neurons. Excitatory and inhibitory synaptic transmission is realized mostly by inhibitory postsynaptic potentials and excitatory postsynaptic potentials.

On the electrophysiological level, there are various phenomena which alter the response characteristics of individual synapses (called synaptic plasticity) and individual neurons (intrinsic plasticity). These are often divided into short-term plasticity and long-term plasticity. Long-term synaptic plasticity is often contended to be the most likely memory substrate. Usually the term “neuroplasticity” refers to changes in the brain that are caused by activity or experience.

Connections display temporal and spatial characteristics. Temporal characteristics refer to the continuously modified activity-dependent efficacy of synaptic transmission, called spike-dependent synaptic plasticity. It has been observed in several studies that the synaptic efficacy of this transmission can undergo short-term increase (called facilitation) or decrease (depression) according to the activity of the presynaptic neuron. The induction of long-term changes in synaptic efficacy, by long-term potentiation [17](LTP) or depression [18](LTD), depends strongly on the relative timing of the onset of the excitatory postsynaptic potential and the postsynaptic action potential. LTP is induced by a series of action potentials which cause a variety of biochemical responses. Eventually, the reactions cause the expression of new receptors on the cellular membranes of the postsynaptic neurons or increase the efficacy of the existing receptors through phosphorylation.

Back propagating action potentials cannot occur because after an action potential travels down a given segment of the axon, the voltage gated sodium channels' (Na<sup>+</sup> channels) m gate becomes closed, thus blocking any transient opening of the h gate from causing a change in the intracellular [Na<sup>+</sup>], and preventing the generation of an action potential back towards the cell body. In some cells, however, neural back propagation does occur through the dendritic arbor and may have important effects on synaptic plasticity and computation.

A neuron in the brain requires a single signal to a neuromuscular junction to stimulate contraction of the postsynaptic muscle cell. In the spinal cord, however, at least 75 afferent neurons are required to produce firing. This picture is further complicated by variation in time constant between neurons, as some cells can experience their EPSPs [19] over a wider period of time than others.

While in synapses in the developing brain synaptic depression has been particularly widely observed it has been speculated that it changes to facilitation in adult brains.

A receptive field is a small region within the entire visual field.

Any given neuron only responds to a subset of stimuli within its receptive field. This property is called tuning. As for vision, in the earlier visual areas, neurons have simpler tuning. For example, a neuron in V1 may fire to any vertical stimulus in its receptive field. In the higher visual areas, neurons have complex tuning. For example, in the fusiform gyrus, a neuron may only fire when a certain face appears in its receptive field. It is also known that many parts of the brain generate spatiotemporal patterns of electrical activity that spatially correspond closely to the layout of the retinal image (this is known as retinotopy). It seems further that imagery that originates from the senses and internally generated imagery may have a shared ontology at higher levels of cortical processing. About many parts of the brain some characterization has been made as to what tasks are correlated with its activity.

In the brain, memories are very likely represented by patterns of activation amongst networks of neurons. However, how these representations are formed, retrieved and reach conscious awareness is not completely understood. Cognitive processes that characterize human intelligence are mainly ascribed to the emergent properties of complex dynamic characteristics in the complex systems that constitute neural networks. Therefore, the study and modeling of these networks have attracted broad interest under different paradigms and many different theories have been formulated to explain various aspects of their behavior. One of these—and the subject of several theories—is considered a special property of a neural network: the ability to learn complex patterns.

### **Biotechnology of the nervous system.**

See that dust bunny hiding under your bed when you clean your room? Where did it come from? Did you know that dust bunnies are often made from our dead skin cells that fall off when we move around? Our hair grows, our nails get longer, and our red blood cells get replaced every four months. What is responsible for all these areas of growth in our bodies? Stem cells in each of these areas produce the cells needed to grow these structures. In some ways, stem cells are like factories that can mass produce different types of cells. Stem cells in your skin produce enough cells to replace your skin every four weeks. Stem cells in your blood produce 2.5 million red blood cells a second! In teenagers and adults, stem cells are found in practically every tissue in the body and are used to build tissues that are growing and maintain tissues that have cells that are continually replaced. Stem cells are even found in our brains, and they usually form cells called astrocytes and oligodendrocytes [20] (cells that support neurons and cells that wrap around neurons and insulate them so they can send information over long distances). Researchers are studying what neural stem cells (stem cells of the central nervous system) do in normal brains and after injury and disease. A recently published study has achieved, through the use of astrocytes derived from human embryonic precursors, to regenerate experimental spinal cord injury in rats. It is not the first study to use glia in an attempt to repair these injuries: it has been tried before (with spectacular results in experimental animals) the ability of olfactory ensheathing glia to regenerate spinal cord injuries. However, this study refines previous knowledge, since it makes a distinction: there is a specific subpopulation of astrocytes responsible for achieving a robust regeneration, and most importantly, the recovery of body movement lost after the injury.

Researchers have come to this conclusion when deriving different types of astrocytes from the same precursors. To this end, these precursors have been isolated from embryonic nervous tissue and divided into two different types of astrocytes according to different growth factors. This way, it has been identified one subpopulation of astrocytes able to regenerate the damage, while the other is not. As results have shown, not all human astrocytes are equal when it comes to repairing the nervous system. The clinical implications are clear: it will not be enough to obtain the necessary precursors, they certainly need to be treated and specifically differentiated before transplantation. In this issue, we have two examples of that. In “Stem Cells,” the iPSC process includes gene therapy—making cells express (or activate) four genes they don’t usually express. In the same article, it’s gene therapy when stem cells are made to produce more neurotrophins than they would if left on their own. Fortunately, a biotechnology breakthrough in 2006 has allowed the creation of cells that seem to retain many of the qualities of embryonic stem cells. A scientist from Kyoto, Japan, named Shinya Yamanaka, realized that by having a cell express just four different genes, he could reprogram a mature, specialized cell back into an immature, embryonic stem cell-like cell. This technique created induced pluripotent stem cells (iPSCs) that seem to act like embryonic stem cells but can be made from any mature cell type. The implications of this biotechnological advance are tremendous. Potentially any cell from a patient can be used as a source to make that person’s own pluripotent stem cells, without the need for embryonic stem cells. For any stem cell therapy to be successful, the donor stem cells need to match the patient. The biotechnology breakthrough of iPSCs circumvents this problem. In theory, a doctor could take skin cells from a patient, create iPSCs from the mature skin cells, and instruct these iPSCs to become any cell type the patient needed.

#### **Biotechnological Approaches for Neurodegenerative Diseases**

Dementia is increasingly being recognized as one of the most important medical problems in older people with a prevalence rising from 1% at the age of 60 to at least 35% at the age of 90 [Ferri et al. 2005]. [21] Within the spectrum of dementias, Alzheimer’s disease (AD) is the most prevalent subtype, accounting for about 60% of all dementias. It is characterized clinically by progressive memory and orientation loss and other cognitive deficits, including impaired judgment and decision making, apraxia and language disturbances resting tremor, rigidity and postural instability. The estimation is that about 25 million people live with Alzheimer’s disease and more than 6 million live with PD today. Every 7 seconds there is a new case of Alzheimer’s disease and every 36 seconds a new case of PD. “Cellular therapies” utilize cell or tissue grafts to treat diseases or injury. Treatment objectives of stem cell therapies typically center on cellular replacement or providing environmental enrichment. Cellular replacement for neurodegenerative diseases involves the derivation of specific neuronal subtypes lost in disease and subsequent grafting into affected areas of the nervous system. The newly transplanted neurons may then integrate, synapse and recapitulate a neural network similar to the one lost in disease. Alternatively, stem cells may provide environmental enrichment to support host neurons by producing neurotrophic factors, scavenging toxic factors or creating auxiliary neural net-

works around affected areas. Many strategies for environmental enrichment utilize stem cells to provide de novo synthesis and delivery of neuroprotective growth factors at the site of disease. Growth factors such as glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF) [48] are protective in neurodegenerative disease models and provide in situ support at the main foci of disease. [22-25] The appropriate objective of cellular therapy for each neurodegenerative disease must be based on the specific neuronal pathology of each disorder. While cellular replacement may be effective in diseases like PD where a specific neuronal subpopulation is lost, ALS is most likely to benefit from cellular therapies that enrich the local spinal cord environment to support the remaining MNs. Factors such as how well grafted neurons integrate and migrate within the host tissue, and the distances that axons must extend to reach their targets, must be considered when determining the potential efficacy of cellular therapies for neurodegenerative diseases.

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