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IONIC CHANNELS

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ABSTRACT

The chapter provides overview of passive and active ionic channels (intrinsic transmembrane proteins that span the membrane) which regulate the movements of ions into and out of biological cells and in the membrane-bound organelles; their role is central to the life of the cells, as the single genetic abnormalities that cause over 600 human diseases have now been identified, including variety of channelopathies**.** In introductory part, remarkable diversity of ionic channels is classified according to functional characteristics (electrophysiologic behavior, inhibition or stimulation by pharmacologic agents, activation by extracellular agonists, and modulation by intracellular messengers) or structural characteristics (amino acid sequence homology and the kinds of subunits of which they are composed). In the second part, the biophysical transport of ions through biological membranes is considered as an interplay between (gated) active membrane transport (which explains the origin of different ion concentrations from the inside and from the outside of the cell membrane) and (nongated) passive membrane transport (explaining the resting potential of the membrane, as a consequence of the concentration gradient established by active transport). In the last part, we summarize the major structural features of the known 18 distinct families of ionic channels that have been identified in mammals by cloning of their pore-forming subunits, where the hypothetical membrane topologies are based primarily on hydropathy analysis; when annotation of the human genome is completed, it will eventually be possible to provide a definitive survey of channel types and functions.

Keywords: Ionic channels; nongated and gated channels; transmembrane proteins; conformational transitions; functional and structural characteristics; passive and active membrane transport; channelopathies.

As the most of the molecules present in living systems are highly soluble in water *but* poorly soluble in nonpolar solvents (including the hydrophobic interior of the membrane lipid bilayer), biological membranes pose a formidable barrier to most water-soluble molecules, maintaining the large differences in concentration of many substances between the cytoplasm and the extracellular fluid. However, various proteins embedded within the membrane lipid bilayer, make also a mosaic of *highly selective permeability* functions, which regulate the movements of molecules (from smaller O_2, N_2, CO_2, and H_2O, to much larger nutrients, specific metabolites, secreted molecules and excrete waste products) and ions (Na^+, K^+, Cl^-, Ca^{2+}) into and out of the different types of cells and in the various membrane-bound organelles, which is central to the life of the cells [1].

The small, *nonpolar* (*hydrophobic*) *molecules*, including oxygen (O_2) and nitrogen (N_2), and small uncharged *polar* (*hydrophilic*) *molecules* such as carbon dioxide (CO_2), move through biological membranes simply by *diffusing* across the lipid membranes of cells [2]. Much larger, *polar organic molecules* not very soluble in the membrane lipids, such as glucose, amino acids and others, move trough membranes via *specific transport protein carriers* (*ionic channels*) [3]. On the other hand, hydrated *ions*, such as sodium (Na^+), potassium (K^+), chlorine (Cl^-), or calcium (Ca^{2+}), can move both through nonspecific (nongated or pores) and specific (gated or gated pores) ionic channels [4].

INTRODUCTION

As hydrophilic *ions* electrostatically attract water dipoles (*cations* attracted to oxygen atoms and *anions* attracted to hydrogen atoms of water molecules), thus being surrounded by electrostatically bound shells of water (waters of hydration), these hydrated ions are too bulky to dissolve effectively in the membrane. So, in order to traverse the membrane, ions can do so almost exclusively by going through the protein pores embedded in the membrane, called *ionic channels*. In contrast to the lipid bilayer, ionic channels provide an effective polar environment for ions (enabling their waters to pass to this polar medium, decreasing partly hydrated ionic overall effective diameter) and also the diameter of the channels is large enough (so that ions traversing the channel need not be stripped completely of their water shells). Although some transport proteins are *simple* aqueous ionic channels, many of these are *highly selective* for ions (Na^+, K^+, Ca^{2+}, and Cl^-) or, in the case of aquaporins, for water; others are enzymes or receptors for chemical messages from other cells [1].

Ionic channels are intrinsic transmembrane proteins which span the membrane (with inner diameters of about one-tenth of the membrane thickness (about 8 to 10 nm), and with distances between neighboring channels about ten times greater than the thickness of the membrane) [5]. Ionic channels themselves can be subdivided into two categories: *passive* or *active.*

Passive channels are *nongated*, being always open in the cell at rest (contributing significantly to the resting membrane potential, and influencing synaptic integration). *Active* channels are *gated*, having a gate either open or closed, controlled by membrane potential, by synaptic transmitters, or, in the case of receptor cells, by various physical stimuli (contributing significantly to the action, synaptic, and receptor potentials).

The opening and closing of a channel involve conformational changes. In all ionic channels so far studied the channel protein has two or more conformational states that are relatively stable. Each of these stable conformations represents a different functional state. For example, each ionic channel has at least one open state and one or two closed states. The transition of a channel between these different states is called *channel gating.* The molecular rearrangements that occur during the transition from closed to open states appear to enhance ionic conduction through the channel not only by creating a wider lumen, but also by shifting relatively more polar amino acid constituents into the surface that lines the aqueous pore.

The direction and magnitude of ionic fluxes across membranes depend on both the *concentration difference* and the *electrical difference* (the membrane potential), and these two driving forces are collectively known as the *electrochemical gradient* (*electrochemical difference*) across a membrane [4]. The greater the number of open channels, the greater the ionic flux across the membrane for any given ion concentration difference. A single ionic channel may open and close many times in a single second, suggesting that the channel protein

fluctuates between two (or more) conformations. The results of various investigations show that ionic channels in nerve and muscle cell membranes can assume *multiple conformations,* one of which is more permeable to the ion in question than the others [4,7].

There are three general *physical models* for channel gating: (1) a localized conformational change that occurs in one region of the channel; (2) a generalized structural change that occurs along the length of the channel; and (3) a blocking particle that swings into and out of the channel mouth [8]. The two unresolved issues of the (semi)classically addressed problems in molecular biophysics should be especially pointed out: unreasonably long time necessary for *change* of biopolymer conformations *and* long-range *directivness* of selective molecular recognition processes – suggesting their *quantum-resonant* nature [9-11].

General Characteristics of Ionic Channels

All ionic channels have a basic glycoprotein component consisting of a large integral-membrane protein with carbohydrate groups attached to its surface. A central aqueous pore through the middle of the protein spans the entire width of the membrane, but in many channels the pore-forming region is made up of two or more subunits (dimers, trimers, tetramers, pentamers, hexamers), which may be identical or different. The ionic channels can be constructed as heterooligomers from distinct subunits, as homooligomers from a single type of subunit, or from a single polypeptide chain organized into repeating motifs, where each motif functions as the equivalent of one subunit. In addition to one or more pore-forming α-subunits, which comprise a central core, some channels contain auxiliary subunits (β, γ, or δ), which modulate the inherent gating characteristics of the central core. These subunits may be cytoplasmic or embedded in the membrane [8].

One way of making sense of remarkable diversity of ionic channels is to classify them according to their *functional characteristics* (*electrophysiologic* behavior, inhibition or stimulation by *pharmacologic* agents, activation by *extracellular agonists*, and modulation by *intracellular messengers*) or *structural characteristics* (amino acid *sequence homology* and the kinds of subunits of which they are composed) [4].

Electrophysiology. This approach consists of analyzing ionic currents by voltage-clamp techniques and then characterizing the ionic channels on the basis of their *selectivity* (Na^+, K^+, Ca^{2+}, and Cl^- channels), *voltage dependence* (in electrically excitable cells e.g., nerve, skeletal muscle, heart - these channels being generally also highly selective for Na^+, Ca^{2+} or K^+ ions), *gating* (regularly every millisecond or every second, or irregularly by bursts of activity followed by periods of relative inactivity).

Pharmacology. Currents that are virtually indistinguishable by electrophysiological criteria can sometimes be distinguished pharmacologically. For example, subtypes of voltage-gated Na^+ channels can be distinguished by their sensitivity to the peptide toxin μ-conotoxin, which is produced by *Conus geographus*, a venomous marine mollusk. This toxin strongly inhibits the Na^+ channels of adult skeletal muscle but has little effect on the Na^+ channels of neurons and cardiac myocytes.

Extracellular agonists. Some channels are characterized by their unique ability to be activated by the binding of particular molecule termed an *agonist.* These agonist-gated channels respond to different chemical activators such as acethylcholine (ACh), glutamate, serotonin (5-hydroxytryptamine [5-HT[), gamma-aminobutyric acid (GABA), and glycine. The types of agonist-gated channels are numerous and diverse, and they differ on the basis of electrophysiological and pharmacological criteria.

Intracellular messengers. Channels can be categorized by their physiological regulation via intracellular messengers. For example, channels underlying $[Ca^{2+}]_i$ stimulated K^+ and Cl^- currents are known as Ca^{2+}-gated K^+ channels and Ca^{2+}-gated Cl^- channels, respectively, while in light-sensitive rod cells of the retina a certain type of channel is directly activated by intracellular cyclic 3',5' -guanosine monophosphate (cGMP).

It should be noted that the four presented functional criteria (electrophysiology, pharmacology, extracellular agonists, and intracellular regulators) for characterizing channels are not mutually exclusive. For example, Ca^{2+}-gated K^+ channels and cGMP-activated channels are both structurally related to the family of voltage-gated channels.

Sequence homology. Because of the diversity of channels based on functional criteria, a molecular biologic approach is ultimately required to classify ionic channels *structurally*. Amino acid sequencing of purified channel proteins provided the information needed to prepare oligonucleotide probes that could be used to isolate the coding sequences of channels from coding deoxyribonucleic acid (cDNA) clones derived from messenger ribonucleic acid (mRNA). This approach has led to the cloning of many different types of ionic channels and has confirmed that the diversity of channels foreshadowed by physiological research corresponds to an enormous diversity at the molecular level.

Molecular information obtained from sequence analysis and structural information on channel proteins has revealed possibility to gain insight into the *evolutionary inter-relationships* of proteins by comparing their deduced primary amino acid sequences, as well as nucleotide sequences of genes that encode them. By aligning various protein sequences and computing the relative similarity of each pair protein sequences, it is possible to reconstruct a hypothetical family tree (*dendrogram*) of evolutionary relationships. The branch lengths of the tree correspond to relative evolutionary distances as measured by sequence similarity. In case where the functional properties of cloned channel genes are known from their behavior in expression systems, their functions are consistent with the classification of channel subtypes based on molecular evolution.

BIOPHYSICS OF IONIC PASSIVE AND ACTIVE MEMBRANE TRANSPORT

Biophysical transport of ions through biological membrane will be considered here as an interplay between (gated) *active membrane transport* (which explains the origin of different ion concentrations: Na^+, K^+, Cl^-, ... from the inside and from the outside of the cell membrane) and (nongated) *passive membrane transport* (explaining the resting potential of the membrane, as a consequence of the concentration gradient established by active transport) [12,13]. Although there is good evidence that the membrane has separate gated channels for Na^+, K^+, Cl^-, and Ca^{2+}, it is not clear whether different ions have separate nongated channels or whether they all share a common (leakage) pathway [6].

Passive Membrane Transport. Living cells are characterized by 10-20 times increased concentration of K^+ ions and decreased concentrations of Na^+ and Cl^- ions inside the cells compared to the cells environment ($C^i_{K^+} >> C^e_{K^+}$; $C^i_{Na^+} << C^e_{Na^+}$; $C^i_{Cl^-} << C^e_{Cl^-}$). As a consequence of these ionic gradients across the (~ 10 nm thick) membranes, there appears experimentally observed *potential difference* between the cell cytoplasm and its environment ($\Delta\varphi = \varphi_i - \varphi_e \sim -80$ mV).

This potential difference (*resting potential*) is a consequence of dynamic equilibrium of *nongated* ionic *diffusion* across the membrane (caused by ionic gradients across the membrane) and

nongated ionic drift under the embedded electric field within the membrane (which counterbalances the diffusion process), whence the *Hodgkin-Katz formula* for the membrane *resting potential* can be deduced:

$$\Delta\varphi = \frac{RT}{\mathrm{F}} \ln \frac{P_{K^+} C^i_{K^+} + P_{Na^+} C^i_{Na^+} + P_{Cl^-} C^e_{Cl^-}}{P_{K^+} C^e_{K^+} + P_{Na^+} C^e_{Na^+} + P_{Cl^-} C^i_{Cl^-}}, \quad (1)$$

(where F is Faradey's number, R is the molar gas constant, T is the absolute temperature, and P_K+, P_{Na}+, P_{Cl}- are permeability coefficients of the membrane for K^+, Na^+ and Cl^- ions, respectively ($P = \mu RT/\mathrm{F}\Delta x$, where μ is the ion mobility, and Δx is the membrane thickness)).

Active Membrane Transport. An increased concentration of K^+ ions and decreased concentration of Na^+ ions inside the cell are determined by the active membrane transport which proceeds against the electrochemical potential gradient. It should be pointed out that active transport is one of the most important features of life processes in general, as it resolves the contradiction between the preservation of spatial heterogeneity and metabolism – the exchange of matter and energy with the surrounding medium – in the framework of *nonequilibrium thermodynamics* of open biological systems.

Active transport is accomplished through the sophisticated *indirect coupling* (in *stationary nonequilibrium state*) of diffusion fluxes to the exergonic reactions that take place in the bulk of the membrane: the transfer of matter occurs at the expense of the free energy liberated in chemical reactions (as a rule, in hydrolysis of adenosine triphosphate (ATP). This is a transport facilitated by a chemical reaction (*facilitated*/*mediated transport*), which is accelerated due to the presence of *carriers*, the membrane proteins which interact with the transported ions or molecules, but do not leave the membrane, so that the circulation of carrier occurs.

In case of the *sodium pump*, the key role is given to the protein carrier K^+,Na^+-activated adenosine triphosphatase - ATPase (C), which is the enzyme with high affinity for K^+ (CK) in dephosphorylated state, and with high affinity for Na^+ (CFNa) in phosphorylated state (as a result of corresponding conformational changes of the ATPase, accompanied by hydrolysis of ATP). So, the overall membrane process, which makes use of the free energy of ATP for the active transport of Na^+ and K^+ ions in the direction of their increasing concentrations, might be described as:

$$\mathrm{CK} + \mathrm{ATP} + \mathrm{Na}^+ \rightleftharpoons \mathrm{CFNa} + \mathrm{ADP} + \mathrm{K}^+, \quad (2)$$

and can be understood as a sophisticated *gated ionic transport* facilitated by a chemical reaction, consisting of two cycles which drive each other [12,13]: (1) the exchange of K^+ and Na^+ ions (*ion-exchange cycle*) and (2) the phosphorylation and dephosphorylation of ATPase (*chemical cycle*).

Neuronal Ionic Currents

The previous discussion of the sodium pump is only a simplified model of active membrane transport: the search of the electrophysiologial basis of the varying intrinsic properties of different types of neurons of vertebrates and invertebrate revealed a wide variety of ionic currents. So, in vertebrate neurons, two distinct Na^+ currents have been identified and five distinct Ca^{2+} currents and a plethora of distinct K^+ currents are known (cf. Table 1) [14]. Each type of ionic current is characterized by several features: (1) the type of ions conducted by underlying ionic channels (e.g., Na^+, K^+, Ca^{2+}, Cl^-, or mixed cations), (2) their voltage and time dependence, and (3) their sensitivity to second messengers.

Table 1. Neuronal ionic currents [14], modified

Current	Description	Function
Na^+ currents		
$I_{Na,t}$	Transient; rapidly activating and inactivating	Action potentials
$I_{Na,p}$	Persistent; noninactivating	Enhances depolarization; contributes to steady-state firing
K^+ currents		
I_K	Activated by strong depolarization	Repolarization of action potential
I_C	Activated by increase in $(Ca^{2+})_i$	Action potential repolarization and interspike interval
I_{AHP}	Slow afterhyperpolatization; sensitive to increases in $(Ca^{2+})_i$	Slow adaptation of action potential discharge; its block by neuromodulators enhances neuronal excitability
I_A	Transient; inactivating	Delayed onset of firing; lengthens interspike interval; action potential repolarization
I_M	Muscarine sensitive; activated by depolarization; noninactivating	Contributes to spike frequency adaptation; its block by neuromodulators enhances neuronal excitability
I_h	Depolarizing (mixed cation) current; activated by hyperpolarization	Contributes to rhythmic burst firing and other rhythmic activities
$I_{K,leak}$	Contributes to neuronal resting membrane potential	Its block by neuromodulators results in a sustained change in membrane potential
Ca^{2+} currents		
$I_{T,}$ low threshold	Transient; rapidly inactivating; threshold negative to - 65 mV	Underlies rhythmic burst firing
$I_{L,}$ high threshold	Long-lasting; slowly inactivating; threshold around - 20 mV	Underlies Ca^{2+} spikes prominent in dendrites; involved in synaptic transmission
I_N	Neither; rapidly inactivating; threshold around - 20 mV	Underlies Ca^{2+} spikes prominent in dendrites; involved in synaptic transmission
I_p	Purkinje; threshold around - 50 mV	?

MAJOR STRUCTURAL FEATURES OF KNOWN FAMILIES OF IONIC CHANNELS

Here we summarize the major structural features of the known families of ionic channels that have been identified in mammals by cloning of their pore-forming subunits, where the hypothetical membrane topologies are based primarily on hydropathy analysis. Thus the folding diagrams should be considered a "best-guess" representation - and on the basis of the data bank of mammalian channel-protein sequences derived from cloning methods, at least 18 distinct families of channel proteins are recognized [4]. When annotation of the human genome is

completed, it will eventually be possible to provide a definitive survey of channel types and functions.

Connexins. The connexins have four transmembrane segments. Six connexins surround a central pore to form a connexon. Two connexons from neighboring cells stack end to end to form a *gap junction channel*, which connects two cells with a large, unselective pore (about 1.5 nm in diameter) that allows ions and small molecules as large as 1 kDa to pass between cells. These gap junction channels (GJCs) interconnect hepatocytes of the liver, cardiac muscle fibers of the heart and smooth muscle of the gut, β cells of the pancreas, epithelial cells in the skin, and even in the cornea of the eye, to name just a few. GJCs provide pathways for chemical communication and electrical coupling between cells (which are probably also morphofunctional substrat for acupuncture meridians and their reflexogenic points [15-19]).

In one mode of regulation, increases in $[Ca^{2+}]_i$ can cause GJCs to close, but in addition, gating of GJCs can be regulated by the voltage difference between the coupled cells, as well as by phosphorylation. For Ca^{2+}-depending gating, in the absence of Ca^{2+}, the pore is in an open configuration and the connexin subunits are tilted 7 to 8 degrees from an axis perpendicular to the plane of the membrane; after the addition of Ca^{2+}, the pore closes and the subunits move to a more parallel alignment. The gating of the GJC may thus correspond to a conformational change that involves concerted tilting of the six connexin subunits to widen (open) or constrict (close) the pore, which can be studied by measuring electrical currents through GJCs, using two patch electrodes simultaneously placed in a pair of coupled cells.

Voltage-gated cation channels. The superfamily of *voltage-gated channels* consists of K^+, Na^+, and Ca^{2+} channels that have a common structural motif. The main part of each of these channels consists of four subunits or domains, each of which containing six transmembrane segments denoted as S1 through S6. *K^+ channels* are believed to represent the evolutionary precursor form of these channels because their pore-forming subunit contains only one S1 through S6 domain; several types of voltage-gated K^+ channels are tetramers of these simple subunits. The pore-forming subunits of *Na^+* and *Ca^{2+} channels*, both comprise four domains (I, II, III, and IV), referred to as *pseudosubunits*; each of these four domains contains the S1 through S6 structural motif that is homologous to the basic K^+ channel subunit. Members of the voltage-gated superfamily of channels are also recognized by a characteristic structure of the S4 domain in which four to seven positively charged residues (lysine or arginine) are located at every third position. This unique S4 domain appears to function as the protein's voltage sensor.

Voltage-gated Ca^{2+} channels also illustrate another feature of some ionic channels: they are multisubunit complexes consisting of *accessory proteins* in addition to the channel-forming subunits. For example, voltage-gated α_2 are composed of a large pseudotetrameric α_1 subunit with domains I through IV that form the pore, plus four additional structurally unrelated subunits known as α_2, β, γ, and δ. Like the homologous α subunit of Na^+ channels, the large α_1 subunit of Ca^{2+} channels specifies most of the basic channel functions, while functional role of β, γ, and δ subunits is largely unknown.

Cyclic nucleotide-gated channels. Several other types of channels that are not considered voltage gated, are nevertheless clearly evolutionarily related to the voltage-gated superfamily. One example is a family od cation-selective channels that are directly activated by intracellular cGMP or cyclic adenosine 3'5'-monophosphate (cAMP), with important role in visual and olfactory sensory transduction. The cyclic nucleotide-gated channels have the same basic S1 through S6 motif as K^+ channels, but they contain a unique cyclic nucleotide binding domain at the C terminus.

Ca^{2+}-activated K^+ channels. A second family of K^+ channels, large conductance Ca^{2+}-activated K^+ channels, is also related to the superfamily of voltage-gated channels. The pore-forming subunit of these Ca^{2+}-activated K^+ channels has the basic S1 through S6 structure; however, it also has a transmembrane segment named S0 at the N terminus, plus a large C-terminal region that contains binding sites for Ca^{2+}.

Inward rectifier K^+ channels. A third family of K^+ channels consists of the inward rectifier K^+ channels. The basic pore-forming subunit of this family of channels contains only two membrane-spanning segments, structurally related to the S5-S6 portion of the S1 through S6 domain of the voltage-gated K^+ channels. It appears that inward rectifier K^+ channels contain the minimal structural unit required for forming a K^+-selective pore.

Agonist activated channels. In addition to the voltage-gated superfamily, the agonist activated channels as an example of *ligand-gated channels* are also represented by two large and diverse gene superfamilies. These receptor channels are gated by the binding of ACh, serotonin, GABA, glycine, and glutamate.

As a representative, the *acethylcholine receptor* (AChR) is a channel that is a pentamer which comprises four different homologous subunits. The α subunit is represented twice; therefore, the pentamer has a subunit composition of $\alpha_2\beta\gamma\delta$. The nicotinic AChR channel (nAChRC) is located in a specialized region of the skeletal muscle membrane, at the postsynaptic nerve terminal. The receptor responds to ACh released from the nerve terminals by opening and allowing cations to flow through its pore. Images of the AChR show a pentameric radial symmetry that corresponds to a rosette-like arrangement of the five subunits, as an extracellular entrance to the cation-selective channel. The structural changes induced by ACh binding that control opening and closing of the channel appear to occur in a central region of the protein that lies within the plane of the lipid bilayer.

Others. Other important channels are two types of membrane channels selective for Cl^- (anion channels): the cystic fibrosis transmembrane conductance regulator (CFTR) and ClC (transport Cl- and, to a lesser extent bicarbonate ion [HCO_3^-], can take place). Amiloride-sensitive Na^+ channels are prominent in Na^+-transporting epithelia. Finally, there are two types of Ca^{2+} release channels: one is present in the endoplasmic reticulum membrane and is gated by the intracellular messenger inositol 1,4,5-triphosphate (IP_3), and the second is located in the sarcoplasmic reticulum membrane of muscle and plays a critical role in the release of Ca^{2+} during muscle contraction.

This rich variety of ionic channels in different types of cells may make it possible to develop drugs that can activate or block channels in selected regions of the neuromuscular system. Such drugs would, in principle, have maximum therapeutic effectiveness with a minimum of side effects. That detailed knowledge of the genetic basis of channel structure and function may one day make it possible to devise new pharmacological therapies for specific neurological and psychiatric disorders or other diseases.

Channelopathies

Cells cannot survive without functional ionic channels. The single genetic abnormalities that cause over 600 human diseases have now been identified [2]. Many of the diseases are rare, but others are more common and some cause conditions that are severe and eventually fatal. Examples include variety of *channelopathies*, diseases that mostly affect muscle and brain tissue and produce episodic paralyses or convulsions.

There is a number of different mechanisms by which this may occur [20]: (1) Mutations in the coding region of ionic channel genes may lead to gain or loss of channel function, either of which may have deleterious consequences; (2) Mutation in the promoter region of the gene may cause under- or over-expression of a given ionic channel; (3) Other diseases result from defective regulation of channel activity (by cellular constituents or extracellular ligands), which may be caused by mutations in the genes encoding the regulatory molecules themselves or defects in the pathways leading to their production; (4) Autoantibodies to channel proteins may cause disease by down-regulating channel function - often by causing internalization of the channel protein itself; (5) Finally, a number of ionic channels are secreted by cells as toxic agents, being inserted into the membrane of the target cell and forming large nonselective pores, leading to cell lysis and death.

Traumatic brain injury (*traumatic depolarization*) also causes massive amino-acid-mediated ionic fluxes across the membrane of neurons [20], as well as *ischemic brain injury* (*anoxic depolarization*) which causes neuronal cell damage by initiating massive ionic fluxes. These two forms of brain injury may account in part for the injured-brain's increased susceptibility to hypoxia. The excitatory amino acids appear to play a vital role in these processes, as they transport glutamate into glia rather than neurons. Glutamate uptake into neurons and glia is important because glutamate is an excitotoxin that kills cells by overstimulating them. There is evidence that during ischemia and anoxia, loss of neurons is increased because glutamate reuptake is inhibited [2].

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