



Review article

Gap junctions and communication within the nervous systemAdriano T. Ramos¹, Paulo C. Maiorka², Maria L. Z. Dagli², Dominguita L. Graça³¹Veterinary Medicine Graduate Studies Program (PPGMV), Veterinary Pathology Laboratory (LPV), Federal University of Santa Maria (UFSM), Rio Grande do Sul, Brazil.²Toxicology and Pathology Department (VPT), Veterinary Medicine and Zootechny School (FMVZ) University of São Paulo (USP), São Paulo, Brazil.³Veterinary Pathology Laboratory (LPV), Federal University of Santa Maria (UFSM), Rio Grande do Sul, Brazil.***Corresponding author:** Paulo César Maiorka, Pathology and Toxicology Department. Veterinary Medicine and Zootechny School, São Paulo University, Av. Orlando Marques de Paiva, 87, 05508-270, São Paulo, Brazil. Phone: 11 3291 1375. E-mail: maiorka@usp.brSubmitted November 14th 2007, Accepted March 3rd 2008**Abstract**

Gap junctions are sites on the cellular membrane with intercellular channels build up by twelve protein subunits called connexins. Each connected cell contributes with a hemichannel made up by six connexins subunits. This kind of connection represents an efficient way of intercellular communication in most tissues, including the nervous system. It works as a passage for ions, secondary messenger and metabolites exchange between the cells. In a complex tissue like the nervous tissue they are particularly important because they connect the various cellular types composing a panglial syncytium that performs neuronal protection and tissue homeostasis. The expression of connexins and the intercellular communication through gap junctions are crucial to regulate vital functions as cellular motility, proliferations and survival; changes in the conformational expression of connexins may be involved in diseases as Alzheimer's disease, neoplasms, bacterial and parasitic infections, or even affect cellular groups when they occur as genetic mutations leading to functional defects of the nervous system as demyelination in the PNS (Charcot-Marie-Tooth disease), hereditary epilepsy, non-syndromic deafness and senile cataract.

Key Words: gap junctions, connexins, diseases of the nervous system**Introduction**

At the first attempts to study the nervous system glial cells were interpreted as the neuronal glue, "*Nervenkitt*" as was named by Virchow in 1859, but it was beyond knowledge that other than maintaining the neurons together, the glia would be responsible by intercellular communication (13). Nowadays basic functions of each glial type are well documented, yet, it will be shown the subtle cell-to-cell relationship via gap junctions, an open field of discovery.

During the embryogenesis of the central nervous system (CNS) the first step is the formation of the notochord and the paraxial mesoderm with eventual

evolution to the neural tube. These procedures take place along many transformations including the proliferation of neuroepithelial germ cells that delimit the neural tube where neuronal and glial cells precursors will be generated. The organization of the CNS ends after the migration of the progenitor cells to the germ zone to form the gray columns of the spinal cord, nuclei of the brain stem, nuclei and cortex of cerebrum and cerebellum. Full development of the CNS takes place after birth (49).

Many factors may affect the complex CNS maturation leading to defective circuitry among different cellular types; these interactions are of vital importance for the correct development and function of the tissue. Gap junctions are an essential molecular component for the

integration of CNS cells functions that command intercellular signals either by net transmission or by volumes (9,13) which are transmitted either by gap junctions or through the synapses where they pass as a chemical (neurotransmitter) or electrical (ion) signal (32).

Neuroglial integration

For a very long time it was accepted that synaptic neuronal connections alone guaranteed the precision of information processing. In the last few years, however, research point to neuronal-glial cells interactions as the promoter of CNS functioning (16).

The number of glial cells is defined by the neurons that signal apoptosis regulation. Out of six glial progenitor cells only three or four survive until the end of embryogenesis (23). Glial cells are specialized cells many of which have to migrate long distances to reach their final

destination during nervous system development. Cell motility is a hallmark of glial cells; nonetheless, their migratory abilities combined with an uncontrolled cell division may be lethal as seen in gliomas (16,56). The main function of glial cells consists of modulation and controlling of neuronal growing and to build up the nervous circuitry during development. Developing axons and dendrites are not fit to navigate freely within the tissues thus, they ought to be instructed to change directions as they grow. This critical function would be performed by guidance glial cells, such as the segmental limiting cells described within the embryonic nervous system of the locust. Another cell type with reported similar function is the limiting external glia located at the transitional zone between the central and peripheral nervous systems and that defines the tract to be followed by motor and sensory axons (53). (Figure 1. limiting glia).

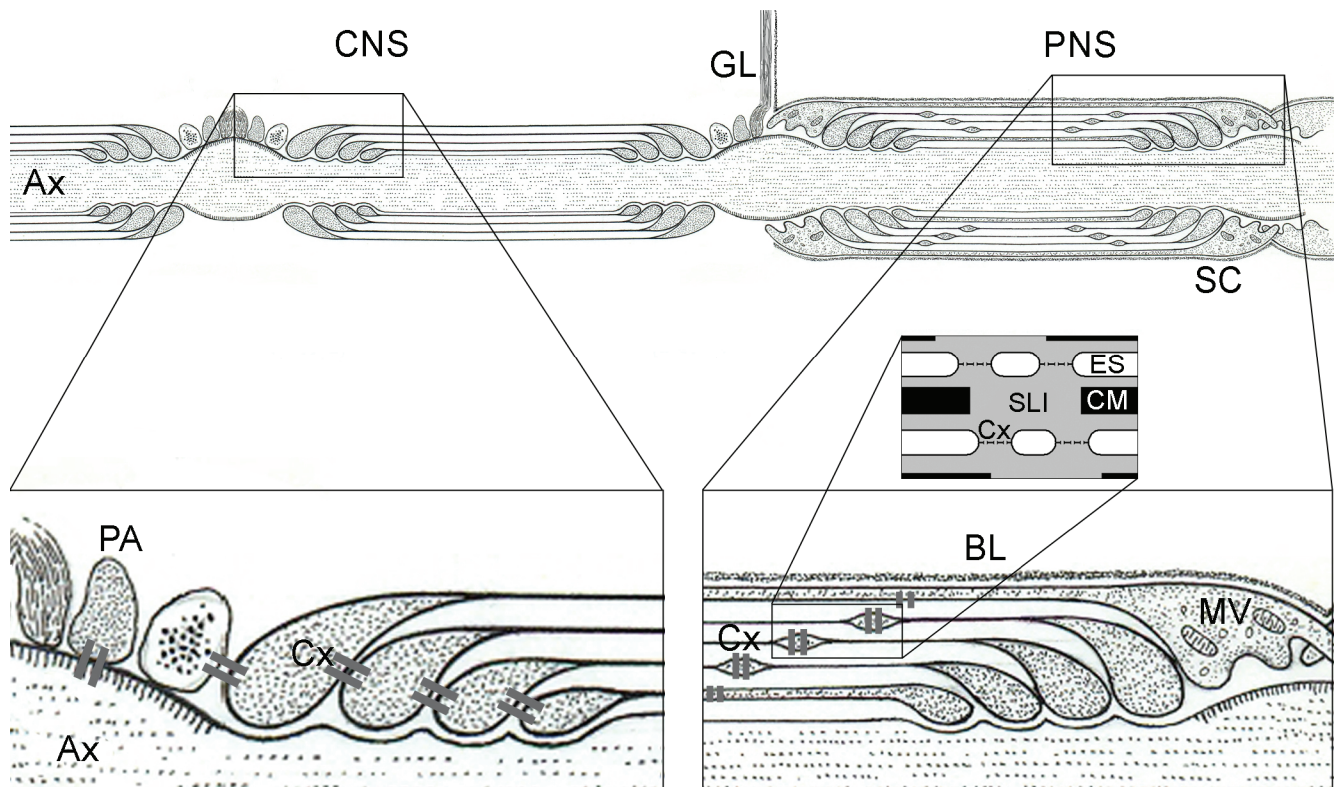


Figure 1. Representation of the myelin sheaths in the CNS, made up by oligodendrocytes and in the PNS where Schwann cells (SC) perform myelination. GL: glial limiting membrane formed by astrocytic processes (AS) separates the CNS from the PNS. In the inset depicting CNS internode, connexins (Cx) communicate the axon (Ax) with the perinodal astrocyte (PA). In the second inset a PNS myelin sheath has Schmidt-Lanterman incisures (SLI) with connexins (cx) on the cell membrane intercalated with extracellular spaces (ES) and compact myelin (CM). Schwann cells have also microvilli (Mv) and a basal

A straight pathway to build up the functional neuronal circuits is based upon glial cells ability to modify the efficiency of synaptic connections. The clearance of the neurotransmitter L-glutamate, mediated by glutamate

transporters expressed by a specific glia is necessary to prevent neuropil oxidative stress and degeneration (46,53)

The most powerful example of neuroglial integration can be seen after axonal trimming from the

synaptic tree, a process studied in arthropods in which the exuberant neuronal projections are removed from the target regions by glial cells. Another example are the glial sheath perineuronal cells that cover neuronal processes to avoid their contact with the hemolymph as observed in *Drosophila* and locust (16).

Glial cells contacts are characterized by regular arrays in electron dense septa named *folded septa junctions*, that turn round the inter-membranous space. A few proteins have been identified as expressed within those junctions and found to be necessary to maintain trans-epithelial seal integrity. Some of these proteins are expressed by glial cells. Molecular data indicate that mammal paranodal junctions which insure electric conductivity at the nodes of Ranvier are homologous to those junctions present in insects nervous system (16).

Connexins

Gap junctions are sites at the cell membrane with intercellular channels made up by twelve protein subunits (connexins). Each connected cell contributes with one connexon or hemichannel, build up by six connexin subunits (48). This kind of junction represents an efficient way of intercellular communication in many tissues, including the nervous tissue (12), and works as a passage for ions, secondary messengers and metabolites exchange between the cells (12). Gap junctions allow the passage of molecules of up to 1 kDa (8,12,25,48,54), and they are present in all cell types except circulating erythrocytes, spermatozoa and mature innervated skeletal muscle cells (48). Connexin expression and intercellular communication across gap junctions are important to regulate cell motility, proliferation and survival (27,56).

So far 20 connexins have been identified in rodents and 21 in human beings (55). In mice, connexins are divided into three groups: group I (β) includes connexins mCx26, mCx30, mCx30.2, mCx30.3, mCx31, mCx31.1 and mCx32; within group II (α) are located connexins mCx33, mCx37, mCx40, mCx43, mCx46, mCx50 and mCx57; group III (γ) includes the connexins mCx36 (48) and mCx45 (60). Connexins mCx29, mCx39 and mCx47 are not currently grouped in any category. In humans, group I (β) includes connexins hCx26, hCx30, hCx30.3, hCx31, hCx31.1, hCx31.9, and hCx32. Within group II (α) are included connexins hCx43, hCx46, hCx50 and hCx62. There is no defined group for connexins hCx25, hCx30.2, hCx36, hCx37, hCx40, hCx40.1, hCx47 and hCx58 (60).

Members of the connexin family are highly homologous and have approximately 50% of identical amino acid sequences; they also show a diversified pattern of tissue distribution. Structurally each connexin gets through the membrane four times and have the carboxy and amino terminals on the cytoplasmic face of the channel. The two extracellular loops are highly conserved

and have 3 cysteins within each loop located in identical form in all 12 connexins (12,48) (Figure 2A).

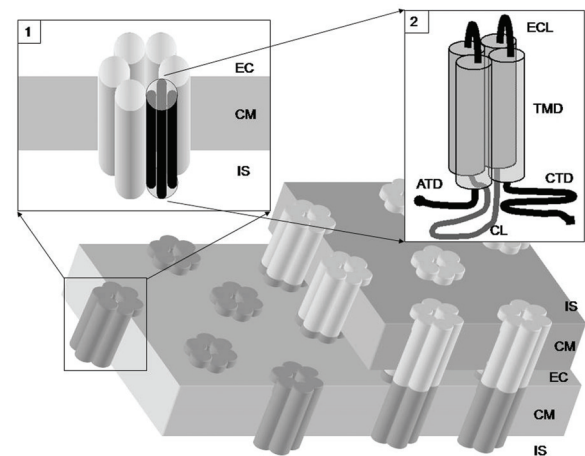


Figure 2 - A gap junction between two cells: the channels link two intracellular spaces (IS) through two membranes (CM). In inset 1: a connexon traverses the CM of one cell forming a pore with 6 connexins. In inset 2 a connexin formed by 4 transmembrane domains (TMD), two extra cytoplasmic loops (ECL), a cytoplasmic loop (CL), an aminoterminal domain (ATD) and a carboxiterminal domain (CTD).

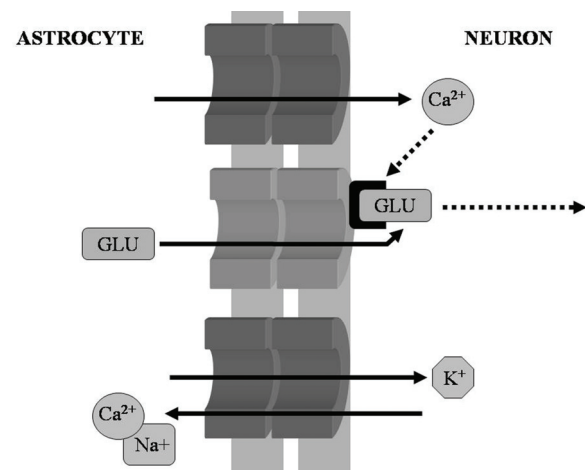


Figure 3 - Ion transportation between astrocytes and neurons. Ions are transported across gap junctions. Calcium ions act on glutamate receptors to free glutamate within neurons.

Connexins are expressed in an overlapped pattern of tissue distribution, as more than one type of connexin may be expressed by a single cell. Each cell contributes with a hemichannel or connexon with selective affinity for each other, and that may form homotypic, heterotypic or heteromeric junctions. Homotypic junctions are composed of 12 identical connexin subunits. For the heteromeric-homomeric channels all connexins within a connexon are identical, but differ the connexon connexins of the other cell. In heteromeric channels there is more than a connexin type in each connexon (48). The complexity of heteromeric and heterotypic channels is limited by the different connexin affinities for other connexins either of

the same or different type. Some connexins only form functional channels if connected with specific connexin types, i.e. Cx40 only relates with itself; Cx43 may connect with other connexin types. An interesting example is that of Cx31.1 and Cx33 that do not form functional channels with each other or with other connexins. Cx33 may block other connexin oligomers acting as either an anti-connexin or a dominant negative connexin (13).

Gap junctions are found in most tissues and they are particularly important in the central (CNS) and peripheral (PNS) nervous system, where tissue structure is more complex and more connected than in other tissues. Eleven connexins have been characterized in gap junctions of mammals nervous system (Cx26, Cx29, Cx30, Cx31, Cx32, Cx36, Cx37, Cx43, Cx45, Cx47 and Cx57) in neurons, glial cells and meninges (32) (Table 1).

Connexins and the nervous system

Cells	Cx26	Cx29	Cx30	Cx31	Cx32	Cx33	Cx36	Cx37	Cx40	Cx43	Cx45	Cx46	Cx47	Cx57
Leptomeningeals	(12,48,55)		(55)							(27,55)				
Ependimal	(12,48)									(27,55)				
Neurons	(9,48)					(43,48)	(15,48)	(48)	(48)	(9,48)				
Astrocytes	(8,32)		(8,9,11,32,36)						(11)	(9,32,48)	(8,11,48)	(50)		
Oligodendrocytes		(32,34,55)			(9,37,48)						(9,10)		(32,34,37)	
Microglia							(15,55)			(18,25,55)	(15)			
Schwann cells					(47)							(6,7)		
Anomalies	Non-syndromic recessive deafness (47)			(51)*	Charcot-Marie-Tooth disease (47)		Hereditary epilepsies (47)			Non-syndromic deafness (22), astrocytomas (22)		Senile cataracts (47)		(51)*

Table 1 - Connexins in CNS cells and the disease or anomaly induced by the occurrence of mutations. *Non cell linked protein and anomaly.

The main functions of mammal nervous system connexins involve synchronized relationship of specific neuronal subpopulations and the maintenance of glia-glia communication (9).

Considering the different functional properties of gap junctions (permeability, voltage sensitivity and conductivity unity) formed by varied connexons types, their expression in specific cell types and changes in expression during development appear to be vital for the physiological brain functions (48).

Glia represents the main connected cell population via gap junctions in the CNS. The amount of glial cell gap junctions if compared to that of neurons is greater and persists in the adult. For astrocytes and oligodendrocytes (macroglia) tens of connexins have been mapped (48).

Glial cell gap junctions may be homologous (astrocyte-astrocyte, A/A), heterologous (astrocyte-oligodendrocyte A/O), or even autologous (homocellular, O/O) between structures of the same cell (myelin lamellae) (36).

Cx43 is the most conspicuous protein in mammal gap junctions and it is found in most tissues (60). It is the main constituent of astrocytes gap junctions (48), and is found in neuroectodermal cells at the beginning of embryonic development (47).

Intriguing facts are the changes in connexin expression during the development of the nervous tissue. Cx 43 and Cx26 are the predominant proteins in embryonic nervous tissue cells; with time Cx26 is confined to a small cell population, within the leptomeninges, ependyma and pineal cells in the adult brain (12,48). Cx43 increased expression takes place simultaneously with Cx 26 decrease. Disappearance of Cx26 immunoreactivity within the developing brain coincides with Cx32 expression and a critical event in brain development, i.e. the conversion to the adult form of N-CAM (neuronal cell adhesion molecule); that event gives an appropriate arrangement of nervous tissue cells and stabilizes neuronal connections (14).

Recent findings point out that qualitative and quantitative protein composition of gap junctions during development and differentiation is peculiar to each cellular

type (14). When gap junction communication takes place between two different cell types, each cell contributes with a hemichannel made up of specific connexins. These channels are proper for molecules transportation necessary for the perfect communication between the connected cells. When gap junctions form with different connexin classes there is the possibility that different regulatory factors act on each side of the channel (12).

Most oligodendrocytes gap junctions, up to 97%, are made with astrocytes. Those gap junctions mediate the communication between successive oligodendrocytes and allow distant oligodendrocytes to participate of the intercellular communication forming a broad panglial syncytium (45).

It is likely that over 80% of oligodendrocyte-astrocyte (O/A) junctions contain Cx30 and Cx43 on the astrocytic side and that more than 70% of O/A contain Cx26 and Cx43; this suggests that astrocytic portion of at least 56% of all O/A junctions have all three astrocytic connexins (32). Thus, astrocytes form two different gap junction classes, one of them contains Cx43 and Cx30 and the other contains Cx26; both of them form O/A heterotypic channels and A/A homotypic channels. When heterotypic junctions occur astrocytic Cx26 associates mainly with oligodendrocyte Cx32 and astrocytic Cx43 and Cx30 associate with oligodendrocyte Cx47 (1).

Connexins in astrocytes and microglia

Astrocytes are responsible for building up structural barriers along vascular surfaces, wrapping neurons and isolating regions of different or oscillating ionic composition in order to separate CNS physiological and metabolic different neuronal groups (36). Metabolically astrocytes contribute to extracellular K^+ homeostasis through a special buffering process (36) that involves a cell to cell redistribution of extracellular K^+ excess along a large gap junctions interconnected astrocytic network (33). Since perineuronal K^+ buffer is made up by astrocytes, gap junctions provide a straight pathway within perivascular compartments to the disposability of K^+ (38) and water (44) within this system.

Within the mature CNS the more prevalent connexin is Cx43, expressed in astrocytes and endothelial cells, ependymal cells (27), leptomeningeal cells (27,55) and microglia (55). Cx43 regulates proliferation and migration of various cell types. A key point to that function is its relationship with β -actin²⁷(27).

Astrocytes express Cx43 and under certain conditions are connected *in vivo* and in culture. Nonetheless the strength of that connection and the degree of Cx43 expression by astrocytes varies according to the brain regions; the strength is bigger in the hypothalamus than in the striatum; those differences may also occur according to the astrocytic cell type (12,48).

Astrocytes express another connexins in a lower degree as Cx26 and Cx30 related to Cx43 expression.

Cx26 is found in low amounts within the adult brain where it is confined to the leptomeningeal, ependymal and pineal cells (12,48) and neurons(48) (Table 1). Cx43 expression by ependymal cells and microglia increase the amount of Cx43 within the CNS. Cx30 was found in ependymal and leptomeningeal cells close to blood vessels. Leptomeningeal cells also express Cx43 and Cx26. Microglia communicate to each other via gap junctions that contain Cx43. These junctions are useful to mount the inflammatory response in the brain and are stimulated by inflammatory cytokines (55). In the brain of normal rats 5% of microglia express Cx43; after an injury this number increases and up to 60% of microglia express this connexin (18,25). Cx36 has been detected in amoeboid and ramified microglia(15,55) (Table 1). Cx36 may take part in the communication between microglia and neurons representing a fundamental connection in neuropathology. A third connexin, Cx45 was detected in mice microglia (15)

Cx43 expression in astrocytes may be affected in both brain ischemia and Alzheimer's disease which exhibit a large number of microglia-derived macrophages. It may be concluded that activated microglia inhibits the communication across gap junctions and Cx43 expression by astrocytes and therefore they can modulate glial communication in the CNS (25). In activated microglia the communication via gap junctions transmit neuroinflammatory signals, maximizing the attack against a bacterial agent; in other way this communication may spray proinflammatory cytotoxic factors that shorten cellular viability (20).

Initially some studies described Cx26 presence only in the developing brain whereas other reports that involved advances in the molecular biology of connexins state that it may be expressed in the mature brain (31,40). During brain ontogeny connexins are expressed in a peculiar way (48): Cx26 and Cx43 are highly expressed in the early stages of development; Cx26 expression decreases during the process with a concomitant increase of Cx32 formerly expressed in very low amounts(14).

Morphological studies show that astrocytes have autologous junctions, also called reflexive junctions that take place among membranous processes of the same cell probably serving for a synaptic monitoring. However it is necessary a deeper investigation to find out the role of this type of communication (48). These reflexive junctions are also found in oligodendrocytes and Schwann cells (36,61).

Ca^{2+} signaling between astrocytes and neurons in mixed cell culture was described as unidirectional, from astrocytes to neurons. On the other hand the propagation of Ca^{2+} waves from astrocytes to neurons is suggested to be dependent on astrocytic glutamate release which requires calcium and neuronal glutamatergic receptors activation; all that ion exchange takes place across gap junctions (48) (Figure 2B).

Astrocytic gap junctions have been investigated regarding neuroprotection by comparing neuronal

vulnerability with and without junctional astrocytic communication (48). In those environments where astrocytes are disconnected, neurons exposed to oxidative injuries have an increase of peroxide counting and high cell death. In the same oxidative stress state with disconnected astrocytes there is a sudden increase of Ca^{2+} indicating astrocytic gap junctions participation on Ca^{2+} homeostasis (48). Cx43 levels are elevated in either reactive astrocytes or round kainic acid injections. Nonetheless the degree of connection of these glial cells along the progression from normal to reactive states remains obscure. Cx43 is increased after mild to moderate ischemic brain lesions of the striatum, although it is reduced in areas with few neurons (36). Many regulatory processes carried out by Cx43, including dephosphorylation, membrane dispersion and internalization, remain active in areas of neuronal damage according to the degree of the injury (36).

It has been suggested that astrocytic gap junctions must be remodeled after injury to drive ions and metabolites flux to protect the surviving tissue (36). This phenomenon signals a neuroprotective astrocytic function and establishes a relationship between the status of the astrocytic gap junctions and neuronal vulnerability to oxidative damage (3). On the other hand, it has been reported that the diffusion and amplification of cellular death signals take place through astrocytic gap junctions and may configure a secondary propagation of an encephalic injury (29).

Connexins in oligodendrocytes and Schwann cells

Oligodendrocytes and Schwann cells express a different gap junction protein, Cx32, although in a minor degree than astrocytic Cx43 expression (12,48). Oligodendrocytes Cx32 is located in the plasma membrane at the nodes of Ranvier (32), in compact myelin of Schmidt-Lanterman incisures and in internodal myelin where it forms autologous (reflexive) gap junctions between turns and adjacent myelin layers of the same cell (Figure 1), and allow the existence of short cuts of gap junctions between these structures similar to those found in the PNS (10,51,57). Recent investigations using several antibodies against Cx32 state that this connexin is associated with myelin sheaths (32), mainly in larger fibers and also that another connexin Cx29 is present in small diameter fibers (55).

Oligodendrocytic Cx29 is found round the cell body and initial segments of the cell processes. Cx29 occurs along oligodendrocytes development although in a lesser degree than Cx32 (32). Cx47 is another oligodendrocytic connexin abundantly expressed in adult mice CNS (32). Experiments using knockout mice for Cx32 and Cx47 indicate that these animals have myelination defects and a more severe demyelination (37). Cx32 and Cx47 are also located on the body and initial processes; this location is more conspicuous in gray than in

white matter (1). CNS myelinic gap junctions connect the non-compacted cytoplasmic compartments of external myelin layers and astrocytic processes that wrap internodal myelin compartments (34). (Figure 1). The existence of gap junctions between astrocytes and oligodendrocytes is well established and includes Cx43 participation on the astrocytic side of the junction (34).

Schmidt-Lanterman incisures consist of small circumferential dilations of Schwann cells cytoplasm that invade all the extension of the internodal myelin sheath. Considering that myelin lamellae form water-proof barriers to the diffusion of substances, the Schmidt-Lanterman incisures supply a metabolic route for myelin support; on those incisures gap junctions are reflexive between internal and external cytoplasmic membranes which provide a pathway formed by successive communicating channels for the radial diffusion of ions and metabolites (34).

Homologous junctions between oligodendrocytes (O/O) are quite rare. Gap junctions between astrocytes and oligodendrocytes have Cx32 and Cx47 and possibly Cx29 (24). Yet, most heterologous junctions (O/A) have Cx47 (1,24). Autologous myelin gap junctions, precisely at the Schmidt-Lanterman incisures and between the paranodal turns have almost exclusively Cx32 (24). Cx47 participates on gap junctions between myelin layers and astrocytes (24,28). (Figure 1). In the PNS autologous communication between myelin layers involve Cx29 and Cx32, located at the paranodal loops and the Schmidt-Lanterman incisures (34).

Pathophysiology of connexins

Gap junctions role has been well evaluated concerning cell to cell interaction. There are two effects derived from gap junctions function that may determine life and death of the connected cells (2).

The *bystander effect* promotes the death of normal cells adjacent to an apoptotic cell by diffusing toxic metabolites through gap junctions. In the same way there is the *good Samaritan effect* that allows a condemned cell to live by draining the toxic metabolites to adjacent cells and maintaining all cells alive and thus tissue homeostasis. In this way gap junctions perform a dual function either saving or killing interconnected cells (2,19).

Some pathological conditions are directly related to gap junctions or to their altered function (47). Some human diseases are caused by mutated connexins (47). Mutations on Cx32 induce a peripheral neuropathy named Charcot-Marie-Tooth disease. The many conductivity changes observed in this disease may be caused by altered protein traffic to the junctions, altered channel permeability and, sometimes, altered conformation of heterotypic channels (13). Mutations of Cx36 may lead to the most common hereditary non-syndromic deafness.

Cx43 structure may be altered in some forms of human epilepsy where Cx43 mRNA expression may or may not be altered. High Cx43 levels have been detected

in β -4 positive amyloid plaques of Alzheimer's disease (35,36), indicating either astrocytes invasion of the plaques or increased Cx43 expression by astrocytes, as observed in PC12 cells (cells from a rat pheochromocytoma) with increased expression of carboxy-terminal portions of amyloid precursor protein (30,33,36). However a higher Cx43 expression in that area may reflect the existence of many activated macrophages/microglia. The decrease of Cx43 within an inflammatory focus suggest that factors as IL-1 β are involved in astrocytic connectivity decrease as observed in autoimmune experimental encephalitis. This change of Cx43 expression may modulate those processes involved in inflammatory demyelination as the observed in multiple sclerosis (4). In certain conditions astrocytes may induce active microglia to become quiescent through anti-inflammatory cytokines such as TGF- β (21,52).

Areas of the cerebral cortex submitted to hypoxia followed by reoxygenation may or may not show alteration of Cx43 levels as detected by immunolabelling, whereas Cx43 mRNA is increased. Changes of Cx32 and Cx36 also take place whereas their mRNA expression does not change (39,47).

A sudden increase of extracellular glutamate levels may have severe consequences, one of them is to accelerate cell death by excitotoxicity hyperstimulating ionotropic glutamate receptors. This situation is seen in degenerative diseases, i.e. multiple sclerosis, and soon after traumatic spinal cord injuries and can be avoided by astrocytes connected by functional gap junctions (41).

Intracellular parasitic infections such as those caused by *Trypanosoma cruzi* and *Toxoplasma gondii* induce a diminished intercellular communication. This decrease takes place in the absence of connexins changes on expression or transcription. The lack of organization of gap junctions may happen because of an altered connexons structure (5). Independently of the acting mechanism, Cx43 changes affect astrocytic function dramatically, disturbing K⁺ balance on glial cells and interfering with Ca⁺⁺ waves propagation between glia and neurons (47). *Staphylococcus aureus* is also capable of modulating connexin expression and inhibiting the formation of functional gap junctions (17).

The expression of purinergic receptors P2Y is altered in spinal cord astrocytes of knockout mice for Cx43 (47,59) due to the interaction between P2Y receptors and Cx43 in intercellular Ca⁺⁺ transmission. In Cx43 depleted astrocytes there is an increase on P2Y receptors as well as on exchange of adenosine-sensitive P2Y receptors for uridine-sensitive P2Y receptors (59).

Changes in connexin expression are responsible for the above mentioned anomalies; yet, in neoplastic changes as gliomas and glioblastomas connexin types are not altered albeit the relationship between the expression of connexin and the malignancy of the neoplasm are inversely related (47,56). A low Cx43 expression was also found in astrocytomas (22).

Cx43 has been shown to have an important role in tumoral growth suppression, independently of the formation of gap junctions, by means of a reduction of cell proliferation without intensifying linking through gap junctions (37).

Cx43 expression was recently connected to programmed cell death through the altered expression of the antiapoptotic protein bcl-2 with formation of non junctional hemichannels or other mechanisms (22). On the other hand Cx43 may protect cells in culture against injuries; this protective effect is not removed when the cells are separated in order to limit gap junction formation or by blocking connexin channels (58). This protective effect has been observed also *in vivo* (26). Many studies link the augmented resistance to injuries mediated by connexins to the cytoskeleton reorganization and the fast normalization of cytotoxic calcium levels within the cells (58).

Conclusion

The identification of proteins with specific cell functions is a priority to understand the correct cell functioning and a great advance to unravel the etiology and pathogenesis of neurological diseases. The evaluation of mutations on specific sites will also be of great help to understand degenerative and regenerative processes within the CNS and constitutes the base for the identification and modulation of those processes that impair the functioning of brain circuitry after damage.

The complexity of the CNS is observed during ontogeny and continues in adulthood, mainly during the development and recovery from various diseases. Many investigations focus on gap junctions to understand the mechanisms involved in the physiology of normal tissue and in the pathogenesis of chronic progressive neurodegenerative diseases.

One of the ways of studying those diseases is the use of genetically modified laboratory animals, i.e. knockout mice for specific connexins of the CNS (42), to get an insight within the core of degeneration and regeneration or the lack of it as seen in diseases as multiple sclerosis.

References

1. ALTEVOGT, BM, PAUL, DL. Four classes of intercellular channels between glial cells in the CNS. *J Neurosci.*,2004, 24, 4313-23.
2. ANDRADE-ROZENTAL, AF, ROZENTAL, R, HOPPERSTAD, MG, WU, JK, VRIONIS, FD, SPRAY, DC. Gap junctions: the "kiss of death" and the "kiss of life". *Brain Res. Brain Res. Rev.*,2000, 32, 308-15.
3. BLANC, EM, BRUCE-KELLER, AJ, MATTSO, MP. Astrocytic gap junctional communication decreases neuronal vulnerability to oxidative stress-

- induced disruption of Ca^{2+} homeostasis and cell death. *J. Neurochem.*,1998, 70, 958-70.
4. BRAND-SCHIEBER, E, WERNER, P, IACOBAS, DA, IACOBAS, S, BEELITZ, M, LOWERY, SL, SPRAY, DC, SCEMES, E. Connexin43, the major gap junction protein of astrocytes, is down-regulated in inflamed white matter in an animal model of multiple sclerosis. *J Neurosci. Res.*,2005, 80, 798-808.
 5. CAMPOS DE CARVALHO, AC, ROY, C, HERTZBERG, EL, TANOWITZ, HB, KESSLER, JA, WEISS, LM, WITTNER, M, DERMIETZEL, R, GAO, Y, SPRAY, DC. Gap junction disappearance in astrocytes and leptomeningeal cells as a consequence of protozoan infection. *Brain Res.*,1998, 790, 304-14.
 6. CHANDROSS, KJ, KESSLER, JA, COHEN, RI, SIMBURGER, E, SPRAY, DC, BIERI, P, DERMIETZEL, R. Altered connexin expression after peripheral nerve injury. *Mol. Cell Neurosci.*,1996, 7, 501-18.
 7. CHANDROSS, KJ, SPRAY, DC, COHEN, RI, KUMAR, NM, KREMER, M, DERMIETZEL, R, KESSLER, JA. TNF alpha inhibits Schwann cell proliferation, connexin46 expression, and gap junctional communication. *Mol. Cell Neurosci.*,1996, 7, 479-500.
 8. CONTRERAS, JE, SANCHEZ, HA, VELIZ, LP, BUKAUSKAS, FF, BENNETT, MV, SAEZ, JC. Role of connexin-based gap junction channels and hemichannels in ischemia-induced cell death in nervous tissue. *Brain Res. Brain Res. Rev.*,2004, 47, 290-303.
 9. DERMIETZEL, R. Gap junction wiring: a 'new' principle in cell-to-cell communication in the nervous system? *Brain Res. Brain Res. Rev.*,1998, 26, 176-83.
 10. DERMIETZEL, R, FAROOQ, M, KESSLER, JA, ALTHAUS, H, HERTZBERG, EL, SPRAY, DC. Oligodendrocytes express gap junction proteins connexin32 and connexin45. *Glia*,1997, 20, 101-14.
 11. DERMIETZEL, R, GAO, Y, SCEMES, E, VIEIRA, D, URBAN, M, KREMER, M, BENNETT, MV, SPRAY, DC. Connexin43 null mice reveal that astrocytes express multiple connexins. *Brain Res. Brain Res. Rev.*,2000, 32, 45-56.
 12. DERMIETZEL, R, SPRAY, DC. Gap junctions in the brain: where, what type, how many and why? *Trends Neurosci.*,1993, 16, 186-92.
 13. DERMIETZEL, R, SPRAY, DC. From neuro-glue ('Nervenkitt') to glia: a prologue. *Glia*,1998, 24, 1-7.
 14. DERMIETZEL, R, TRAUB, O, HWANG, TK, BEYER, E, BENNETT, MV, SPRAY, DC, WILLECKE, K. Differential expression of three gap junction proteins in developing and mature brain tissues. *Proc. Natl. Acad. Sci. U. S. A.*,1989, 86, 10148-52.
 15. DOBRENIS, K, CHANG, HY, PINA-BENABOU, MH, WOODROFFE, A, LEE, SC, ROZENTAL, R, SPRAY, DC, SCEMES, E. Human and mouse microglia express connexin36, and functional gap junctions are formed between rodent microglia and neurons. *J Neurosci. Res.*,2005, 82, 306-15.
 16. EDENFELD, G, STORK, T, KLAMBT, C. Neuron-glia interaction in the insect nervous system. *Current Opinion in Neurobiology*,2005, 15, 34-9.
 17. ESEN, N, SHUFFIELD, D, SYED, MM, KIELIAN, T. Modulation of connexin expression and gap junction communication in astrocytes by the gram-positive bacterium *S. aureus*. *Glia*,2007, 55, 104-17.
 18. EUGENIN, EA, ECKARDT, D, THEIS, M, WILLECKE, K, BENNETT, MV, SAEZ, JC. Microglia at brain stab wounds express connexin 43 and in vitro form functional gap junctions after treatment with interferon-gamma and tumor necrosis factor-alpha. *Proc. Natl. Acad. Sci. U. S. A.*,2001, 98, 4190-5.
 19. FARAHANI, R, PINA-BENABOU, MH, KYROZIS, A, SIDDIQ, A, BARRADAS, PC, CHIU, FC, CAVALCANTE, LA, LAI, JC, STANTON, PK, ROZENTAL, R. Alterations in metabolism and gap junction expression may determine the role of astrocytes as "good samaritans" or executioners. *Glia*,2005, 50, 351-61.
 20. GARG, S, MD, SM, KIELIAN, T. Staphylococcus aureus-derived peptidoglycan induces Cx43 expression and functional gap junction intercellular communication in microglia. *J Neurochem.*,2005, 95, 475-83.
 21. HINKEROHE, D, SMIKALLA, D, HAGHIKIA, A, HEUPEL, K, HAASE, CG, DERMIETZEL, R, FAUSTMANN, PM. Effects of cytokines on microglial phenotypes and astroglial coupling in an inflammatory coculture model. *Glia*,2005, 52, 85-97.
 22. IACOBAS, DA, SCEMES, E, SPRAY, DC. Gene expression alterations in connexin null mice extend beyond the gap junction. *Neurochem. Int.*,2004, 45, 243-50.
 23. JACOBS, JR. The Midline Glia of *Drosophila*: a molecular genetic model for the developmental functions of Glia. *Progress in Neurobiology*,2000, 62, 475-508.
 24. KAMASAWA, N, SIK, A, MORITA, M, YASUMURA, T, DAVIDSON, KG, NAGY, JI, RASH, JE. Connexin-47 and connexin-32 in gap junctions of oligodendrocyte somata, myelin sheaths, paranodal loops and Schmidt-Lanterman incisures: implications for ionic homeostasis and potassium siphoning. *Neuroscience*,2005, 136, 65-86.
 25. KIELIAN, T, ESEN, N. Effects of neuroinflammation on glia-glia gap junctional intercellular communication: a perspective. *Neurochem. Int.*,2004, 45, 429-36.

26. LEE, IH, LINDQVIST, E, KIEHN, O, WIDENFALK, J, OLSON, L. Glial and neuronal connexin expression patterns in the rat spinal cord during development and following injury. *J Comp Neurol*, 2005, 489, 1-10.
27. LI, W, HERTZBERG, EL, SPRAY, DC. Regulation of connexin43-protein binding in astrocytes in response to chemical ischemia/hypoxia. *J. Biol. Chem.*, 2005, 280, 7941-8.
28. LI, X, IONESCU, AV, LYNN, BD, LU, S, KAMASAWA, N, MORITA, M, DAVIDSON, KG, YASUMURA, T, RASH, JE, NAGY, JI. Connexin47, connexin29 and connexin32 co-expression in oligodendrocytes and Cx47 association with zonula occludens-1 (ZO-1) in mouse brain. *Neuroscience*, 2004, 126, 611-30.
29. LIN, JH, WEIGEL, H, COTRINA, ML, LIU, S, BUENO, E, HANSEN, AJ, HANSEN, TW, GOLDMAN, S, NEDERGAARD, M. Gap-junction-mediated propagation and amplification of cell injury. *Nat. Neurosci.*, 1998, 1, 494-500.
30. LYNN, BD, MAROTTA, CA, NAGY, JI. Propagation of Intercellular Calcium Waves in Pc12 Cells Overexpressing A Carboxy-Terminal Fragment of Amyloid Precursor Protein. *Neuroscience Letters*, 1995, 199, 21-4.
31. MIRAGALL, F, HWANG, TK, TRAUB, O, HERTZBERG, EL, DERMIETZEL, R. Expression of Connexins in the Developing Olfactory System of the Mouse. *Journal of Comparative Neurology*, 1992, 325, 359-78.
32. NAGY, JI, DUDEK, FE, RASH, JE. Update on connexins and gap junctions in neurons and glia in the mammalian nervous system. *Brain Res. Brain Res. Rev.*, 2004, 47, 191-215.
33. NAGY, JI, HOSSAIN, MZ, HERTZBERG, EL, MAROTTA, CA. Induction of connexin43 and gap junctional communication in PC12 cells overexpressing the carboxy terminal region of amyloid precursor protein. *Journal of Neuroscience Research*, 1996, 44, 124-32.
34. NAGY, JI, IONESCU, AV, LYNN, BD, RASH, JE. Connexin29 and connexin32 at oligodendrocyte and astrocyte gap junctions and in myelin of the mouse central nervous system. *J. Comp Neurol.*, 2003, 464, 356-70.
35. NAGY, JI, LI, W, HERTZBERG, EL, MAROTTA, CA. Elevated connexin43 immunoreactivity at sites of amyloid plaques in Alzheimer's disease. *Brain Research*, 1996, 717, 173-8.
36. NAGY, JI, RASH, JE. Connexins and gap junctions of astrocytes and oligodendrocytes in the CNS. *Brain Res. Brain Res. Rev.*, 2000, 32, 29-44.
37. NAKASE, T, NAUS, CC. Gap junctions and neurological disorders of the central nervous system. *Biochim. Biophys. Acta*, 2004, 1662, 149-58.
38. NEWMAN, EA. High Potassium Conductance in Astrocyte Endfeet. *Science*, 1986, 233, 453-4.
39. OGURO, K, JOVER, T, TANAKA, H, LIN, Y, KOJIMA, T, OGURO, N, GROOMS, SY, BENNETT, MV, ZUKIN, RS. Global ischemia-induced increases in the gap junctional proteins connexin 32 (Cx32) and Cx36 in hippocampus and enhanced vulnerability of Cx32 knock-out mice. *J. Neurosci.*, 2001, 21, 7534-42.
40. ORSINO, A, TAYLOR, CV, LYE, SJ. Connexin-26 and connexin-43 are differentially expressed and regulated in the rat myometrium throughout late pregnancy and with the onset of labor. *Endocrinology*, 1996, 137, 1545-53.
41. PITT, D, WERNER, P, RAINE, CS. Glutamate excitotoxicity in a model of multiple sclerosis. *Nat. Med.*, 2000, 6, 67-70.
42. RAMOS, AT, GRAÇA, DL. O modelo desmielinizante do brometo de etídio (BE): estudos morfológicos em camundongos c57bl/6 normais e knockout para conexina 32 (Projeto de pesquisa CNPq :305756/2-6). 2006,
43. RASH, JE, STAINES, WA, YASUMURA, T, PATEL, D, FURMAN, CS, STELMACK, GL, NAGY, JI. Immunogold evidence that neuronal gap junctions in adult rat brain and spinal cord contain connexin-36 but not connexin-32 or connexin-43. *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 7573-8.
44. RASH, JE, YASUMURA, T. Direct immunogold labeling of connexins and aquaporin-4 in freeze-fracture replicas of liver, brain, and spinal cord: factors limiting quantitative analysis. *Cell and Tissue Research*, 1999, 296, 307-21.
45. RASH, JE, YASUMURA, T, DUDEK, FE, NAGY, JI. Cell-specific expression of connexins and evidence of restricted gap junctional coupling between glial cells and between neurons. *J. Neurosci.*, 2001, 21, 1983-2000.
46. RIVAL, T, SOUSTELLE, L, STRAMBI, C, BESSON, MT, ICHE, M, BIRMAN, S. Decreasing glutamate buffering capacity triggers oxidative stress and neuropil degeneration in the *Drosophila* brain. *Current Biology*, 2004, 14, 599-605.
47. ROZENTAL, R, CAMPOS DE CARVALHO, AC, SPRAY, DC. Nervous system diseases involving gap junctions. *Brain Res. Brain Res. Rev.*, 2000, 32, 189-91.
48. ROZENTAL, R, GIAUME, C, SPRAY, DC. Gap junctions in the nervous system. *Brain Res. Brain Res. Rev.*, 2000, 32, 11-5.
49. SADLER, TW. Embryology of neural tube development. *Am. J. Med. Genet. C. Semin. Med. Genet.*, 2005, 135, 2-8.
50. SCMES, E, DERMIETZEL, R, SPRAY, DC. Calcium waves between astrocytes from Cx43 knockout mice. *Glia*, 1998, 24, 65-73.

51. SCHERER, SS, DESCHENES, SM, XU, YT, GRINSPAN, JB, FISCHBECK, KH, PAUL, DL. Connexin32 is a myelin-related protein in the PNS and CNS. *J. Neurosci.*,1995, 15, 8281-94.
52. SCHILLING, T, NITSCH, R, HEINEMANN, U, HAAS, D, EDER, C. Astrocyte-released cytokines induce ramification and outward K⁺ channel expression in microglia via distinct signalling pathways. *Eur. J Neurosci.*,2001, 14, 463-73.
53. SEPP, KJ, SCHULTE, J, AULD, VJ. Peripheral glia direct axon guidance across the CNS/PNS transition zone. *Developmental Biology*,2001, 238, 47-63.
54. SOHL, G, MAXEINER, S, WILLECKE, K. Expression and functions of neuronal gap junctions. *Nat. Rev. Neurosci.*,2005, 6, 191-200.
55. SOHL, G, ODERMATT, B, MAXEINER, S, DEGEN, J, WILLECKE, K. New insights into the expression and function of neural connexins with transgenic mouse mutants. *Brain Res Brain Res Rev.*,2004, 47, 245-59.
56. SOROCEANU, L, MANNING, TJ, JR., SONTHEIMER, H. Reduced expression of connexin-43 and functional gap junction coupling in human gliomas. *Glia*,2001, 33, 107-17.
57. SPRAY, DC, DERMETZEL, R. X-linked dominant Charcot-Marie-Tooth disease and other potential gap-junction diseases of the nervous system. *Trends Neurosci.*,1995, 18, 256-62.
58. STOUT, C, GOODENOUGH, DA, PAUL, DL. Connexins: functions without junctions. *Curr. Opin. Cell Biol.*,2004, 16, 507-12.
59. SUADICANI, SO, DE PINA-BENABOU, MH, URBAN-MALDONADO, M, SPRAY, DC, SCEMES, E. Acute downregulation of Cx43 alters P2Y receptor expression levels in mouse spinal cord astrocytes. *Glia*,2003, 42, 160-71.
60. WILLECKE, K, EIBERGER, J, DEGEN, J, ECKARDT, D, ROMUALDI, A, GULDENAGEL, M, DEUTSCH, U, SOHL, G. Structural and functional diversity of connexin genes in the mouse and human genome. *Biol. Chem.*,2002, 383, 725-37.
61. WOLFF, JR, STUKE, K, MISSLER, M, TYTKO, H, SCHWARZ, P, ROHLMANN, A, CHAO, TI. Autocellular coupling by gap junctions in cultured astrocytes: A new view on cellular autoregulation during process formation. *Glia*,1998, 24, 121-40.