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On the role of the extracellular space on the holistic behavior of the brain

Abstract: Multiple players are involved in the brain integrative action besides the classical neuronal and astrocyte networks. In the past, the concept of complex cellular networks has been introduced to indicate that all the cell types in the brain can play roles in its integrative action. Intercellular communication in the complex cellular networks depends not only on well-delimited communication channels (wiring transmission) but also on diffusion of signals in physically poorly delimited extracellular space pathways (volume transmission). Thus, the extracellular space and the extracellular matrix are the main players in the intercellular communication modes in the brain. Hence, the extracellular matrix is an 'intelligent glue' that fills the brain and, together with the extracellular space, contributes to the building-up of the complex cellular networks. In addition, the extracellular matrix is part of what has been defined as the global molecular network enmeshing the entire central nervous system, and plays important roles in synaptic contact homeostasis and plasticity. From these premises, a concept is introduced that the global molecular network, by enmeshing the central nervous system, contributes to the brain holistic behavior. Furthermore, it is suggested that plastic 'brain compartments' can be detected in the central nervous system based on the astrocyte three-dimensional tiling of the brain volume and on the existence of local differences in cell types and extracellular space fluid and extracellular matrix composition. The relevance of the present view for neuropsychiatry is discussed. A glossary box with terms and definitions is provided.

Keywords: astrocytes; brain compartments; extracellular matrix; intercellular communication; neuropsychiatry; tetra-partite synapse.

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General premises

A basic aspect of the integrative action of the central nervous system (CNS; see Table 1 for the list of abbreviations) to be investigated is the morphological and functional organization that allows the holistic behavior of such a heterogeneous system. Actually, in the brain, different types of cells (neurons, astrocytes, oligodendrocytes, microglia, ependymal cells, pericytes) and liquids (extracellular fluid, cerebro-spinal fluid, blood) operate as a synergic contraption to fulfill different tasks such as the homeostasis of the interstitial space fluid (ISF) of the brain and its handling of information. It was clear from the beginning of the last century, mainly thanks to the work of Golgi and Cajal, that one and likely, the first fundamental issue to be investigated is the process of intercellular communication. In fact, this is a prerequisite for the synergic behavior of the system and hence a basic step toward the understanding of the brain integrative action. For recent

Table 1: Abbreviations used.

AIS	Axon initial segment
ALS	Amyotrophic lateral sclerosis
AQPs	Aquaporins
BBB	Blood brain barrier
BCs	Brain compartments
BD	Bipolar disorder
CAMs	Cell adhesion molecules
CCNs	Complex cellular networks
CNS	Central nervous system
CSF	Cerebrospinal fluid
ECF	Extracellular fluid
ECM	Extracellular matrix
ECS	Extracellular space
GM	Gray matter
GMN	Global molecular network
ISF	Interstitial space fluid
LTP	Long-term potentiation
MMPs	Metallo-proteases
NVU	Neuro-vascular unit
PAPs	Perisynaptic astrocyte processes
RT-VT	Roamer-type of VT
SCs	Synaptic clusters
VT	Volume transmission
WM	White matter
WT	Wiring transmission

proposals on the relevance but also on the limits of the classical views of the neural circuitry for brain integrative functions, see Agnati and Fuxe (2000), Agnati et al. (2007a), Fuxe et al. (2007b), and Cook et al. (2014).

Let us briefly discuss this aspect and a possible perspective that has also been previously introduced on the roles of the extracellular matrix (ECM) and the extracellular space (ECS) in the integrative actions of the CNS (Nicholson and Sykova, 1998; Agnati and Fuxe, 2000; Agnati et al., 2002, 2004b, 2005c, 2006a; Rauch, 2004; Kamali-Zare and Nicholson, 2013).

Intercellular communication modes in the brain: wiring transmission and volume transmission

Brain is a highly effective integrator and processor of sensory-motor signaling, which results from the synergistic and coordinated actions of neuronal networks, and, as Faingold and Blumenfeld (2014) point out, an understanding of the brain's neural networks is a critical requirement for understanding normal brain function, as well as CNS disorders. From a very general standpoint, networks are basically formed by nodes (recognizing and decoding signals) and connections among nodes

(transmitting signals). Thus, the functional organization of the nodes and of the connecting pathways as well as the type of exchanged signals has to be considered to describe networks.

Our group has suggested a dichotomy classification of the intercellular communication modes (Agnati et al., 1986, 2010a; Agnati and Fuxe 2000):

– The wiring transmission (WT; see Table 2 for Glossary: terms and definitions) mode that is characterized by signal transmission via physically well-delimited communication channels. This is the case of synaptic contacts and gap junctions.

– The volume transmission (VT) mode that is characterized by the diffusion of signals in physically poorly delimited ECS pathways. This type of communication mode has been observed, e.g., for monoamines in various brain areas (Fuxe et al., 2010). VT signals move from the source to the target cells by diffusion and convection along energy gradients allowing a widespread intercellular communication that occurs in the ECS through the meshes of the ECM of the brain as well as in the cerebrospinal fluid (CSF) (Agnati and Fuxe 2000; Agnati et al., 2010a).

As far as neuronal networks are concerned, brain neurons communicate via chemical and electrical synapses, and, to some extent, through ephaptic transmission (i.e., electrical coupling and potential fields among closely apposed bundles of nerve fibers or neuronal membranes) (Arvanitaky, 1942; Kamermans and Fahrenfort, 2004). As far as astrocyte networks are concerned, nodes are astrocytes (or possibly clusters of astrocyte processes) and connecting pathways are astrocyte VT signals (i.e., gliotransmission) and gap junctions (Nagy et al., 2004; Parpura and Zorec, 2010).

WT and especially VT allow the assemblage of complex cellular networks (CCNs), while the newly discovered roamer-type of VT can allow the emergence of phenotype plastic CCNs

The concept of VT has implied a revision of how the brain carries out its integrative action. In particular, it has allowed us to introduce the concept of CCNs (Agnati and Fuxe, 2000), i.e., the set of cells of any type, which by exploiting the entire spectrum of intercellular communication modes are capable of integrating multiple inputs to give out appropriate outputs and to support each other's

survival (Agnati and Fuxe, 2000; Agnati et al., 2014). It can be surmised that an interplay between astrocytes and neurons occurs in organizing the CCNs especially via astrocytic release of gliotransmitters that are similar to known neurotransmitters and interact with target receptors similar to those targeted by neurotransmitters. Thus, as recently proposed (see Araque et al., 2014), astrocytes represent an additional neuromodulatory system that acts in complement to the neuronal ones, but with its own time and space domains based upon the particular intrinsic properties of Ca^{2+} signaling that encode and integrate incoming inputs from neurons and other environmental sources. According to such a view, Ca^{2+} signaling provides an intermediate regulation between the direct neuronal modulation and the slow but long-lasting 'hormonal-like' regulation carried out by astrocytic VT signaling. Araque and collaborators suggest that astrocyte processes in close proximity to synapses provide a balanced and easily tunable feedback or feed-forward response that regulates neuronal communication in a different time domain with respect to the more classical pre- and postsynaptic controls. Thus, astrocytes and neurons are structurally and functionally strictly interconnected since astrocytes enwrap neuronal processes, and it has been shown that at an ultra-structural level, the astrocytic process is opposed to the pre- and postsynaptic neuronal compartments of most synapses. Actually, an astrocyte can contact about 100,000 synapses. In agreement with these morphological data, it has been demonstrated that astrocytes are essential for a number of supportive functions, such as synaptic glutamate uptake, glutamate recycling, K^+ buffering, and lactate secretion (Halassa et al., 2014). In addition, astrocytes are capable of releasing gliotransmitters such as Adenosine triphosphate (ATP), D-serine, glutamate, brain-derived neurotrophic factor, and Neuropeptide Y (NPY) in a vesicular manner. Notably, D-serine released by astrocytes acts as a co-agonist of synaptic N-methyl-D-aspartate (NMDA) receptors to boost NMDA-mediated currents; in addition to D-serine, astrocytes release glutamate that can act on both pre- and postsynaptic metabotropic and ionotropic glutamate receptors.

A peculiar type of CCN is the neuro-vascular unit (NVU) that has been defined as the assembly of elements of a certain brain region involved in the cell survival, allowing optimal interactions between that brain volume and capillaries. Thus, NVU was defined by Harder et al. (2002) as a structure formed by neurons, inter-neurons, microglia, astrocytes, basal lamina covered with smooth muscular cells and pericytes, endothelial cells, and the ECM. Each component fulfills its own special task but

thanks to strong and reciprocal interactions among the components, the NVU operates as an anatomical and functional device allowing a highly efficient regulation of the local cerebral blood flow (Muoio et al., 2014). This interplay between different cell types is made possible by gap junctions, adhesion molecules (such as cadherins and integrins), and ionic channels (such as Ca^{2+} and K^+ channels), as well as VT signals (such as glutamate and ATP) (Duchemin et al., 2012). In view of the high energy and sophisticated homeostatic requirements of neurons, these cells likely operate as pacemaker of the NVU (Koehler et al., 2006; Banerjee and Bhat, 2007). In fact, neurons detect very small variations in the supply of nutrients and oxygen and transform these signals into electrical and chemical messages to adjacent inter-neurons and/or astrocytes (Figley and Stroman, 2011). Anatomically, neurovascular communication takes place mainly through the astrocyte endfoots that are specialized astrocyte extensions in contact with the surface of the smooth muscular cells and pericytes (Kacem et al., 1998). Thus, the endfoots provide a broad surface contact with both these cell types, wrapping them and acting as a fast and efficient surface for the exchange of signals. Therefore, astrocytes could act as versatile organizers of the NVU allowing the proper VT and WT communications between neurons and other NVU components, in particular, blood vessels (see references in Muoio et al., 2014). As Nedergaard points out, arrays of astrocyte-delimited micro-domains along the capillary microvasculature allow the formation of higher order gliovascular units, which serve to match local neural activity and blood flow while regulating neuronal firing thresholds through coordinative glial signaling (Nedergaard et al., 2003). On the other hand, pericytes and endothelial cells seem mainly act as effectors of the NVU and their role is still matter of investigations. In particular, pericytes are rounded and isolated cells that are in close anatomical and functional contact with endothelial cells that contract in response to ATP increases and secrete adhesion molecules. There is also evidence that the pericytes constrict in a synchronized manner when calcium waves spread across the NVU, leading to a constriction on their contiguous capillaries (Fernandez-Klett et al., 2010). Pericytes and endothelial cells also play a fundamental role in the blood-brain barrier and produce both vasodilators (e.g., nitric oxide) and vasoconstrictors (e.g., endothelin and thromboxane) (Furchgott and Zawadzki, 1980; Emanueli et al., 2003; Duchemin et al., 2012). In addition, it should be underlined that these cells also produce trophic factors that play a role in angiogenesis (Armulik et al., 2010). Thus, the NVU by means of WT and VT communication

modes among its components maintains the homeostasis of composition and volume of the external milieu (i.e., ECS plus ECM) playing a crucial role for brain cell survival and cell functions in a certain brain region.

As mentioned above, the main criterion which allows differentiating WT from VT are the characteristics of the communication channel, especially the absence or the presence of well-defined physical boundaries of the channel, which are well delimited for WT (axons and their chemical synapses, gap junctions) but not for VT (the fluid-filled channels of the ECS and the CSF). It should be noted that the concept of VT gave a new unitary functional meaning to the ECS and the ventricular system (i.e., the CSF) pointing out that they work as important channels for chemical transmission in the CNS. Furthermore, VT signals could not only interconnect any cells of CCNs of the brain but also allow brain-body crosstalk (Agnati et al., 2012b; see also Bechter, 2011), especially thanks to lipophilic blood-born signals as well as to brain regions outside the blood brain barrier (BBB). The basic dichotomous classification of intercellular communication in the brain proposed almost three decades ago (Agnati et al., 1986; see also Agnati and Fuxe, 2000) can also easily accommodate recent evidence concerning the existence of other specialized structures for intercellular communication, such as micro-vesicles for a well-demonstrated VT type (Agnati et al., 2010a) and tunneling nanotubes for a WT that is not yet *in vivo* demonstrated in the brain (Rustom et al., 2004). Thus, recently a more complete classification has been suggested for both VT and WT (see Agnati et al., 2010a, 2014). As far as the VT is concerned, the distinction has been proposed between 'classical VT' (migration of chemical and physical signals) and roamer type of VT (RT-VT) (migration of micro-vesicles, see below). Both types of VT imply the diffusion in the brain mass, in the ECS pathways as well as in the CSF (Agnati et al., 2014). The RT-VT is mediated by exosomes, shedding vesicles, exosome-like vesicles, and apoptotic bodies. These membrane-bound structures can transfer not only a single message but even a set of messages. It has been shown that plasma membrane receptors (Canonico et al., 2012; Guescini et al., 2012; Luchetti et al., 2012), mRNA (Skog et al., 2008), miRNA (Valadi et al., 2007), DNA (Balaj et al., 2011), and mtDNA (Guescini et al., 2010a,b) can be sent via microvesicles (acting as protective containers) that by diffusing into the ECS can reach the proper targets (Simons and Raposo, 2009; Agnati et al., 2010a).

The following energy gradients allow the migration of VT and RT-VT signals in the ECS of the brain: concentration gradients (diffusion of uncharged signals), gradients of electrical potentials (for charged signals), thermal gradients, and pressure gradients (vector-mediated

migration for charged and uncharged signals). It should be noted that electrical, thermal, and pressure gradients can operate both as VT signals producing 'waves' in the ECS which can affect brain cell function sensitive to these physical signals and energy gradients favoring other VT signal migration (Agnati et al., 1995b). In addition, VT does not need a dedicated channel; hence, it represents a space sparing intercellular communication mode. In fact, VT takes advantage of the ECS and CSF that accomplish other functional roles especially related to the brain cells' internal medium homeostasis. Thus, VT signaling in the brain has the important feature of an optimal use of the energy gradients and of the ECS.

Furthermore, two important aspects differentiate the brain from the other body organs as far as diffusion of VT signals in the mass of the organ is concerned:

– The existence of the BBB that tends to confine hydrophilic signals in the ECS of the brain. Thus, the BBB, until a certain extent, prevents the migration of hydrophilic VT signals into the capillaries, favoring their migration toward target cells within the CNS.
– The pulses in the cerebral arteries (see above) that induce a piston-like movement of the brain toward the occipital foramen (Greitz, 1993; Agnati et al., 1995a). This phenomenon induces movements in the extracellular fluid (ECF) and hence VT-signal migration. It could be interesting to evaluate possible differences in these movements in the case of clinostatic vs. orthostatic posture of the subject as well as in the aged subjects in view of the alterations that have been detected in the Young's modulus (describing a material resistance to be deformed under mechanical stress) in some brain areas during the aging processes (Sack et al., 2011; Tyler, 2012).

A schematic representation of the main steps in the evolution of the concepts on intercellular communication modes is given in Figure 1.

The main functional characteristics of the ECM and of the ECS

In the brain, ECM molecules are synthesized by neurons, glia, and non-neural cells, and are secreted into the ECS where they could associate with cell surface receptors and form heterogeneous aggregates that regulate diverse cell functions. These ECM molecules included reelin, tenascin R, tenascin C, chondroitin sulfate proteoglycans and laminins, and the major ECM receptors, integrins. They play a role in long-term potentiation (LTP) and long-term
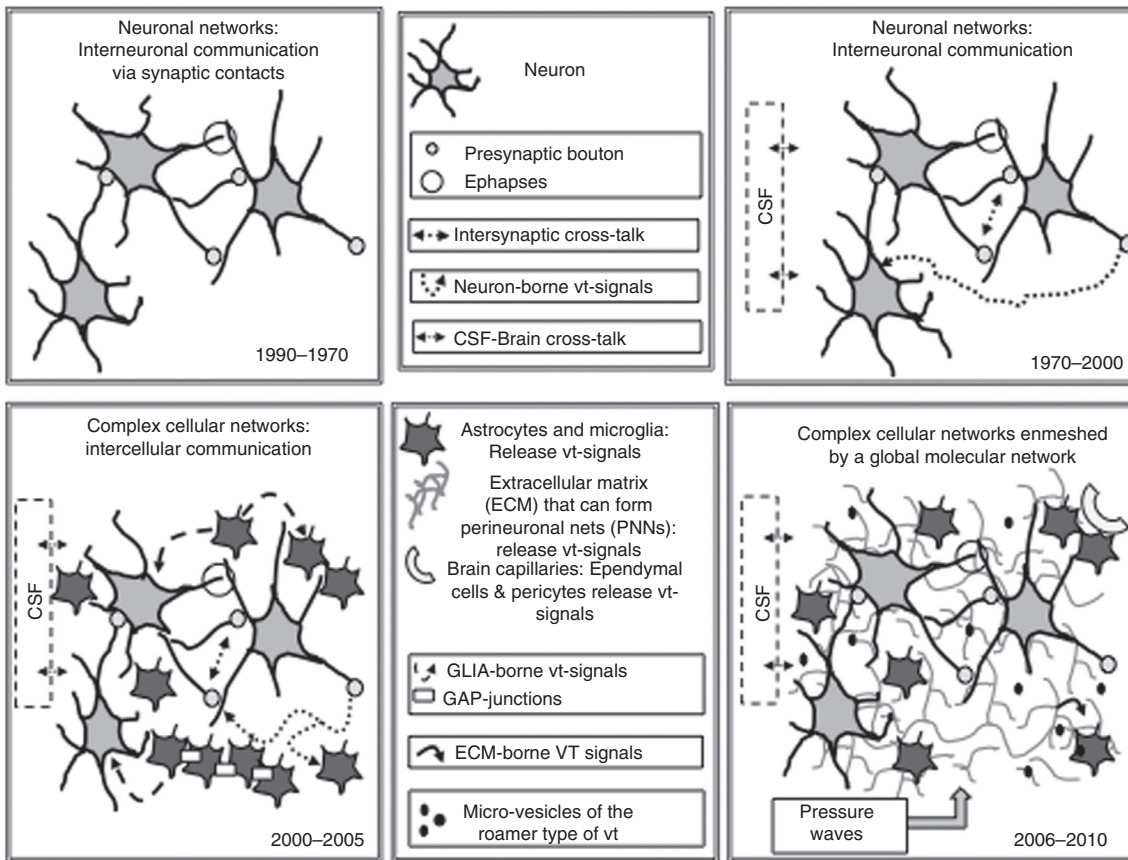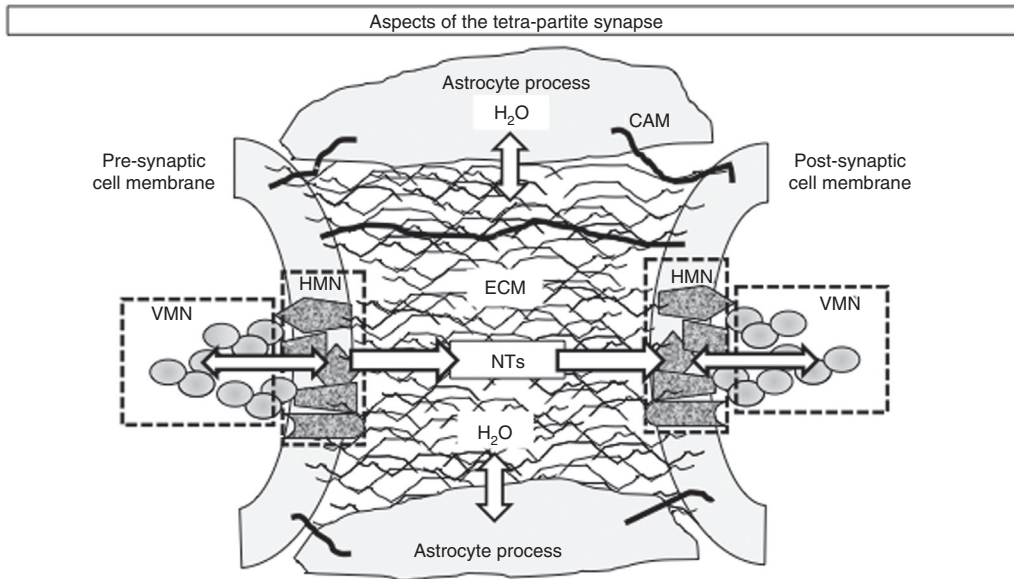
Figure 1: Schematic representations of the historical development of the proposals of brain network organization. For further details, see the text. CSF, cerebrospinal fluid; VT, volume transmission.

depression (LTD) at excitatory glutamatergic synapses in the hippocampus (Dityatev et al., 2010a). Specific enzymes are constantly remodeling the ECM in particular metallo-proteases (MMPs) that are a major class of protein enzymes (24 different versions) that can cut the ECM proteins producing signals that can influence many processes, such as differentiation of stem cells and cell death pathways (Scemes and Spray, 2012). In the MMP family, MMP9 is the best-characterized member and it has been shown that it is involved in activity-dependent structural and functional changes at glutamatergic synapses especially related to learning and memory since increased neuronal activity enhances MMP9 expression in various experimental paradigms, from the cellular level to animal models of learning and memory. In agreement with the ECM actions on glutamatergic synapses, it has been shown that effects on learning are inhibited by blocking NMDA receptors or MMP9 expression or activity, demonstrating the essential role of MMP9 in establishing and maintaining LTP and learning (Thalhammer and Cingolani, 2014).

A great relevance for synaptic function has the area between the presynaptic and postsynaptic neurons, the synaptic cleft, which contains a variety of cleft proteins, including proteins of the ECMs, receptor ectodomains, and cell adhesion molecules (CAMs) (see Figure 2). Focalized proteolysis of synaptic ECMs and CAMs by serine proteases and MMPs might participate in a mechanical and signaling conversion system during neural plasticity (Yoshida and Shiosaka, 1999; Shiosaka, 2004; Shiosaka and Ishikawa, 2011). The advantage of this system is the quick and local activation of extracellular zymogen, fine-tuning of cellular responses by altering protease/inhibitor balance, and, in some cases, simultaneous induction of multiple signals (Tamura et al., 2013). Actually, pre- and postsynaptic sides get in direct contact with each other via CAMs that can regulate synaptic efficacy, by recruiting scaffolding proteins, neurotransmitter receptors, and synaptic vesicles in response to the binding of counter-receptors across the synaptic cleft. Furthermore, there are CAMs that mediate neuron-astrocyte interactions at the synapse. Although adhesion complexes between astrocytes and nerve terminals do not cross the synaptic cleft, and are therefore not strictly synaptic, they are likely to be as important as trans-synaptic CAMs in determining synaptic structure

Figure 2: Schematic representation of the tetra-partite synapse formed by pre- and postsynaptic membranes but also the ECM molecules and the astrocyte processes. The tetra-partite synapse operates as an inter-neuronal communication device endowed with a high plasticity hence capable of multiple integrative functions. For further details, see the text and Figure 3. CAM, cell adhesion molecules; ECM, extracellular matrix; HMN, horizontal molecular network; NTs, neurotransmitters; VMN, vertical molecular network.

and function (Volterra and Meldolesi, 2005; Thalhammer and Cingolani, 2014). The neuronal CAM Thy-1, interacting with integrins in astrocytes and triggering neurite retraction and inhibition of axonal growth, affects cell-cell or cell-matrix interactions; although lacking a cytoplasmic domain, it affects intracellular signaling cascades through interactions with molecules within lipid raft micro-domains (Leyton and Hagood, 2014). It can, therefore, be surmised that direct interactions via CAMs may inform presynaptic terminals of changes in postsynaptic efficacy and initiate presynaptic homeostatic adaptations (Thalhammer and Cingolani, 2014). The term 'tetra-partite synapse' has been introduced to underlie the importance of the ECM in shaping synaptic function by mediating interaction and signaling between neurons and astrocytes (see Figure 2). Thus, the concept of chemical synapses has evolved from a two-component structure playing a key role in the information transfer and storage of information to a complex multi-component device. In fact, it was realized that a tri-partite model that involves the intimate association of astroglial contacts could better explain the experimental data; now, in agreement with our proposal of the fundamental role of ECM in intercellular communication modes, recent evidence suggests the more complex model of a 'tetra-partite synapse' that also includes synaptic and perisynaptic ECM complexes as schematically illustrated in Figure 2 (Agnati et al., 2006a,b; Dityatev and Rusakov, 2011; Guidolin et al., 2013).

Aim of the present paper: an integrative view of the brain morpho-functional organization

In this paper, the role of the ECM for the creation of plastic compartments that can be visualized in the brain is discussed. In agreement with this proposal, a recent paper shows that the distribution of the ECM components marked the territories of the circumventricular organs (see Pócsai and Kálmán, 2014).

Cell networks and the extracellular environment cooperate in the brain holistic behavior

Astrocytes, ECM, and ECS characteristics give rise to plastic brain compartments (BCs): tetra-partite synapses and synaptic clusters (SCs) are basic components of BCs

A BC can be defined as a brain volume that is relatively isolated and differs from the neighboring tissue for the composition of CCNs (relative density of different cell types), ISF (volume and ions composition), and ECM (different molecules present and their peculiar interactions). The BC is a plastic and sometimes rapidly changing

functional structure since it can open up its boundaries exchanging signals with the neighboring tissue.

A peculiar type of BC is formed by the astrocyte morphological and functional organization (Robertson, 2013). Thus, recent anatomical studies show that human protoplasmic astrocytes form innumerable uniform polyhedral tessellating domains (i.e., compartments) that are arranged three-dimensionally and individual protoplasmic astrocytes occupy separate three-dimensional not overlapping domains. Each domain subtends approximately 2,000,000 human tetra-partite synapses that signal to perisynaptic astrocyte processes, which encode and integrate synaptic information allowing a neuron to astrocyte rapid and reciprocal signaling (Robertson, 2013). In principle, astrocyte-tessellating domains can exchange signals being interconnected not only by VT signals but also by gap junctions that allow the exchange of molecules that can transiently affect cell phenotypes. This adds an additional level of complexity to interactions between astrocyte domains that may extend over large areas including the entire neocortex. Thus, these domain-domain interconnections can lead to the formation of different and very large BCs, with the emergence of new integrative actions of these 'contraptions' (see Jacob, 1977; Anderson, 2010; Agnati et al., 2013). It has been pointed out that astrocytes receive signals from synaptic contacts, and also receive multiple signals and homeostatic information from different cellular sources, including neurons, vascular cells, other astrocytes, and even different types of glial cells (see the concept of NVU). Thus, it can be surmised that astrocytes play fundamental roles in the BC homeostasis by linking neuronal metabolic requirements and supply, sensing neuronal activity. In particular, astrocytes provide energy support to neurons through the glucose/glycogen pathways (Pellerin and Magistretti, 1994; Magistretti et al., 1999) and regulate blood flow (Zonta et al., 2003; Mulligan and MacVicar, 2004; Haydon and Carmignoto, 2006; Takano et al., 2006; Gordon et al., 2008; Attwell et al., 2010; Araque et al., 2014). In addition, astrocytes exchange information concerning immune state with microglia and detect local pH and osmolality changes allowing the proper control of breathing (Gourine et al., 2010) and water homeostasis, respectively (Haj-Yasein et al., 2011; Araque et al., 2014). As a whole, astrocytes act as integrators of metabolic, neuronal, and other cell signals but with different characteristics according to the organization and the metabolic conditions of that brain volume, i.e., of that plastic BC. The role of astrocytes in the plastic assembly of BCs is markedly affected by the ECM that actually also controls the polarized localization of ion channels and transporters in astrocytic

membranes. Astrocytic processes contact neurons, blood vessels, and other astrocytes with a polarized distribution of membrane proteins, a feature that is considered essential for astrocytic function. For example, inwardly rectifying K^+ channels (Kir) and aquaporins (AQPs) are enriched in astrocytic endfeets that contact blood vessels, and the local ECM controls their expression levels. Thus, ECM components influence astrocytic function and regulate water and K^+ homeostasis by affecting the density of astrocytic membrane proteins. As mentioned above, the heterogeneity of the ECM throughout the brain and during different developmental stages is one of the features that demark the BCs; hence, the ECM serves not only as an extracellular scaffold but also as a barrier for reducing the diffusion of soluble and membrane-associated molecules between neighboring BCs. The ECM, therefore, contributes to the clustering of signaling molecules in functional micro-domains in neurons and glial cells (Dityatev et al., 2010b). Axons also harbor several highly specialized sub-compartments, such as the axon initial segment (AIS), and the juxta-paranode and internode segments of the nodes of Ranvier, which are covered by specific ECM components. The formation of most of these axonal sub-compartments is dependent on interactions with other cells (e.g., myelinating glia). However, the AIS is unique in that the recruitment and retention of AIS proteins is mainly specified intrinsically via axonal cytoskeletal and scaffolding proteins (Dityatev et al., 2010b).

The tetra-partite synapse and the synaptic clusters can be considered basic brain compartments. In particular, several synapses can interact directly or through soluble signaling molecules, with the ECM that can incorporate molecular signatures of both glial and synaptic elements and ECM molecules modulate activities of pre- and post-synaptic receptors and ion channels (see Figure 2). The ECM can respond to network activity either by incorporating secreted molecules and shed extracellular domains of transmembrane molecules, or by freeing products of its activity-dependent proteolytic cleavage as signaling messengers. As mentioned above, these observations have suggested that the ECM is a fourth essential element of what has been termed the 'tetra-partite synapse'. Theoretically, including the ECM as a fourth player increases the number of interaction pathways in a synapse (Dityatev and Rusakov, 2011) and give further evidence to the concept of basic BCs made by tetra-partite synapses and likely at a higher integrative level by SCs.

It should be underlined that enwrapping of synapses by perisynaptic astrocyte processes (PAPs) is an important feature that allows high efficiency and privacy of the transmission (see Figure 3). These astrocytes' fine
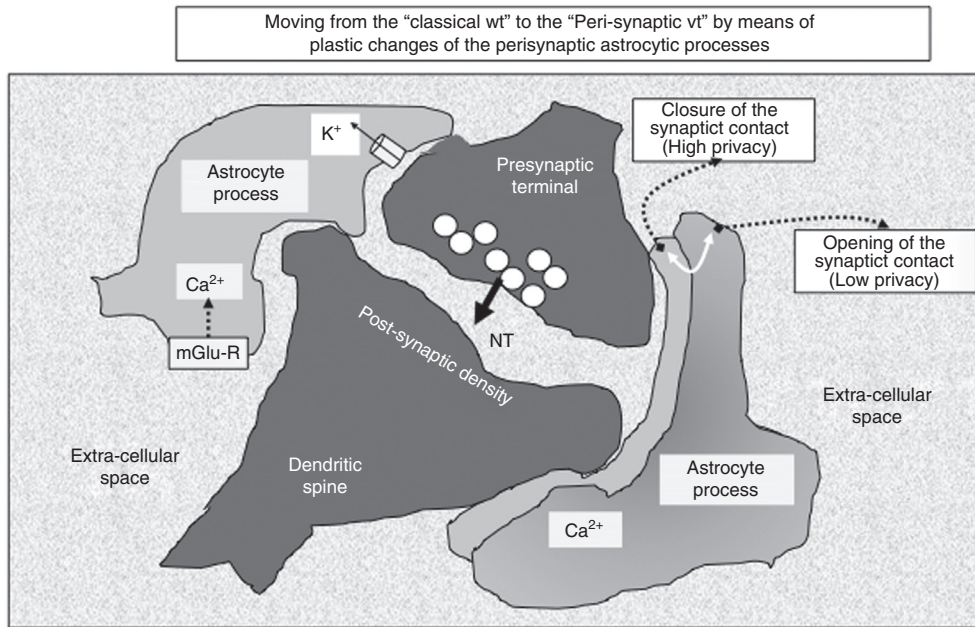
Figure 3: The tetra-partite synapse can operate both according to the 'classical WT mode' and (following plastic changes of the perisynaptic astrocytic processes) as a device that by means of a 'perisynaptic VT' can affect extra-synaptic, neighboring receptors. NT, neurotransmitter; VT, volume transmission; WT, wiring transmission.

ramifications account for 70–80% of the astrocytic plasma membrane and often surround spine synapses, sometimes completely encapsulating them; however, even if PAPs are found in all brain regions, the proportion of synapses having them and the level of synaptic coverage vary significantly. Thus, the function and efficacy of synaptic transmission are determined not only by the composition and activity of pre- and postsynaptic components but also by the features of the PAPs that enwrap the synapse. In fact, astrocytes can sense neuronal activity by elevating their intracellular Ca^{2+} in response to neurotransmitters and may communicate with neurons even if a detailed model of the role of astrocytes in synaptic function and plasticity remains to be established (see also above). A further functionally important aspect is the PAPs' ability to rapidly restructure their thin-branched processes modifying their coverage of the synaptic elements (Bernardinelli et al., 2014). PAPs have been described by several studies as plastic structures able to change their morphology within minutes, thus modifying their coverage of pre- and postsynaptic elements (Reichenbach et al., 2010; Bernardinelli et al., 2014). In other words, astrocytic PAP insulation of the synapses constitutes a plastic physical barrier controlling the transmitter spillover from the synaptic cleft. The opening of this barrier allows extra-synaptic diffusion and hence hetero-synaptic signaling that can lead to the

transient formation of a crosstalk between neighboring synapses and hence to a functional cluster of synaptic contacts especially at the level of dendritic spines (Agnati et al., 2014). In agreement with the concept of plastic BCs, it could be surmised that PAPs' structural changes can give rise to different BCs each of which composed of a different number and/or location of tetra-partite synapses and hence of SCs that are confined in an astrocyte three-dimensional domain. Light and EM immunochemistry have shown that PAPs surrounding excitatory spine synapses have an important role not only on synaptic privacy but also on the functional characteristics of the tetra-partite synapse. In fact, PAPs can express several proteins that deeply affect morphology and function of the quadripartite synapse such as glutamine synthetase, astrocytic glutamate transporters, metabotropic glutamate receptors, CAMs, K^+ channels, and aquaporins that may regulate 'adaptive' swelling of PAPs (Bernardinelli et al., 2014). Thus, by enwrapping a synapse, astroglia not only constitutes a physical barrier for transmitter diffusion and helps to retain a high level of neurotransmitter around that synapse but also, through the expression of specific proteins in perisynaptic processes, can sense and modulate synaptic activity.

In view of these data, we propose (see Figure 3) that a sophisticated control of the PAPs' plasticity (especially at excitatory synapses) allows moving from a high
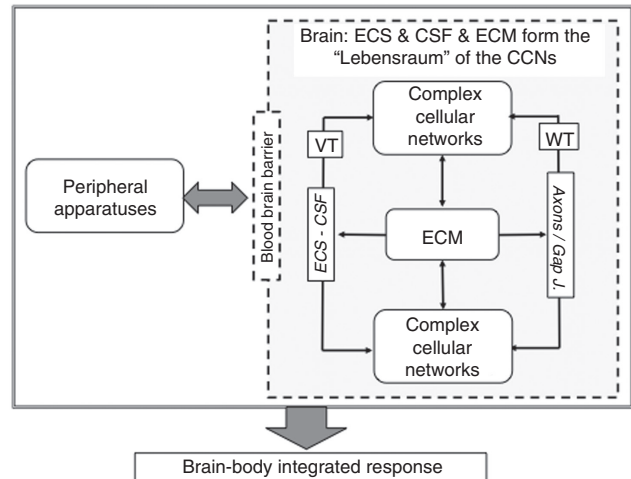
privacy of the synaptic transmission (close enwrap of the tetra-partite synapse) to a more or less broad opening of the enwrapping. This leads to transmitter diffusion (i.e., extra-synaptic VT) with diffusion to neighboring synapses especially along dendrites allowing integrative activity of SCs and hence the formation of BCs that have been supposed to play a crucial role in the vertical elaboration of the information in a functional module (Agnati et al., 2012a; Bernardinelli et al., 2014). It could also be surmised that neurotransmitter spillover out of the tetra-partite synapse leads to the formation of local transmitter clouds (see Greer's proposal; Greer, 2007) that allows a complex activation in the neighboring tissue of receptor mosaics formed by heteromers (see Agnati et al., 2005a,b, 2007b; Fuxe et al., 2009). This view broadens up Lehre and Rusakov's (2002) proposal that some synapses can undergo transitory morpho-functional changes that allow a large spillover of neurotransmitters hence a VT inter-synaptic crosstalk. The possible overlaps of transmitter clouds in different functional conditions and their overlapping actions on the cognate receptors sometimes forming receptor mosaics (Agnati et al., 2010b) could be suggested as an interesting field of investigation.

Figure 4: Proposal that ECS, CSF, and ECM form the 'Lebensraum' (vital space) of the CCNs. Thus, these components not only represent the combined environment allowing the survival of the brain cells but also are media for their communication processes. For further details, see the text. CSF, cerebrospinal fluid; ECM, extracellular matrix; ECS, extracellular space; VT, volume transmission; WT, wiring transmission.

The concept of 'Lebensraum' and the role of astrocytes in its homeostasis – focus on the extracellular GMN as an 'intelligent glue' that fills up the ECS

In agreement with the hypothesis of a global molecular network (GMN) enmeshing the CNS, it has been demonstrated that neurons, astrocytes, microglia, and macrophages secrete ECM molecules that can interact with each other and have major effects on neural and astrocyte networks' topological organization and functions (Agnati et al., 2006b, 2007a). For instance, hyaluronan membrane receptors (such as CD44) can trigger cellular responses to changes in the external matrix composition (Ghosh and Guidolin, 2002), and, similarly, a major receptor on the membrane, integrin, interacts with the matrix molecules reelin, tenascin, chondroitin proteoglycans, and laminins (Arnoys and Wang, 2007).

As illustrated in Figures 3 and 4, the ECS may play a central role in the intercellular communication processes not simply thanks to the ECS pathways but also in view of the actions of chemical (ECM molecules and ions) present in this space and its interconnections with the CSF. Thus, ECS and ECM are active players of the integrative action of the brain and there are reciprocal interactions between these two systems and the CCNs that, in turn, control both the ECS volume and geometry and secrete the ECM.

In broad terms, it can be stated that the ECS together with the ECM (Agnati et al., 2009) forms the 'Lebensraum' of the CCNs (see Figure 4). In particular, it should be pointed out that many other unique large molecules such as trophic factors (e.g., IGFs, FGFs, TGF-βs, and HGF) have been found to be associated with ECM proteins or heparin sulfate (Taipale and Keski-oja, 1997). Thus, the ECM is not a simple amorphous filling between the different cell types of the CNS. On the contrary, it is characterized by three-dimensionally organized peculiar molecules, which determine the geometry of the ECS pathways, and their chemical interactions with the VT signals. For example, a VT peptide can be modified or even split by ecto-enzymes (Konkoy and Davis, 1996) with the possible formation of different sets of fragments from the same parent peptide that can differentially modulate the various cells of CCNs eliciting different types of integrative responses (Agnati and Fuxe, 2000; Agnati et al., 2004a, 2005c; Fuxe et al., 2007a). Fuxe et al. (2012) suggested that galanin (1–15) can play a specific role in depression in view of its high diffusion capabilities [lower molecular weight with respect to galanin (1–29)] and hence its possible extra-synaptic modulatory actions on GalR-5-HT1A heteroreceptor complexes. Such a view underlines our proposal that GMN by regulating diffusion of VT signals in the ECS has a fundamental

role in the brain holistic behavior. Furthermore, the GMN contributes to the structural and functional organization of the brain since the ECM component of the GMN has both a scaffolding role enabling the appropriate location of the CNS components and a functional role in cooperating to the maintenance of the microenvironment around cells and in providing a specific trophic support to the CCNs. As mentioned above, an increasing number of growth factors have been found to be associated with ECM proteins or heparin sulfate (Taipale and Keski-oja, 1997). Summing up, the GMN, thanks to its ECM component, can regulate the geometry/viscosity of the diffusion pathways in the ECS and could be the storage of growth factors that, via a proteolytic release and activation, generate rapid and highly localized trophic signals.

The ECS is in contact with CSF at the ventricles, and ECF and CSF have a similar composition that plays a role in allowing neuronal electrical and chemical activity as well as a fundamental role in the extracellular signaling via VT. As far as the ions present in the ECS are concerned, they should be mentioned for both their effects on local field potential diffusion and cell membrane electrical potential. In fact, external cations, particularly divalent ones, attracted by negative membrane surface potential, form a thin, diffuse double ionic layer near the membrane surface that acts as an electric screen reducing the cell surface potential and affect the charged chemical VT diffusion. Furthermore, at chemical synapses, an increase in $[Ca^{2+}]$ in the ECF facilitates, but an increase in $[Mg^{2+}]$ inhibits, neurotransmitter release. Thus, physiological concentrations of Mg^{2+} in the ECF (about 1 mM) play an important role in the regulation of neurotransmitter release, neuronal excitability, and electrolyte kinetics (Rausche et al., 1990). It should also be mentioned in view of the relevance for protein conformations and for the ASIC 1a receptor activation that glia cells play an important role in maintaining the ECF pH since transporters for H^+ and HCO_3^- are found on the astrocyte membrane (see also above). As a matter of fact, composition and abnormalities in the function of astrocytes are associated with certain neurological disorders such as neuropathic pain, epilepsy, Alzheimer's disease, schizophrenia, and depression.

Nicholson's group has given fundamental contributions to a better understanding of the multi-facet basic role of the ECS in the brain (Kamali-Zare and Nicholson, 2013). It should be noticed that the ECS represents up to 20% of the total brain volume and provides an essential medium for the transport of nutrients, ions, and oxygen. It should be underlined that the entire vascular system in the brain occupies only about 3% of the brain volume but with a much higher turnover of the fluid (i.e., the blood)

flowing in small-diameter channels (i.e., the capillaries) that respond to a regional control (see above NVU).

Summing up, besides the energy gradients the key players involved in VT signaling are as follows (Kamali-Zare and Nicholson, 2013):

– ECS pathway size that has a width of about 20–60 nm (Thorne and Nicholson, 2006);
– ECS pathway geometry in particular 'tortuosity'. Tortuosity of the ECS pathways describes hindrance posed to the diffusion process by a geometrically complex medium in comparison to an environment free of any obstacles (for a detailed study and for a discussion of its importance, see Tao and Nicholson, 2004; Kamali-Zare and Nicholson, 2013).
– ECM composition. As a matter of fact, the matrix may increase local viscosity or act more specifically on molecules that undergo steric or electrostatic binding with some molecules of the matrix. As pointed out above, this gives ECS the potential to regulate diffusion of signals and vesicles.

In this respect, it is interesting to note that there is a functional relationship between neuronal network activity and ECS/ECM characteristics since at sites of high neuronal activity, ECS shrinks by more than 30% (Kamali-Zare and Nicholson, 2013). This use-dependent change in the ECS volume could result from the glial response to the focal extracellular K ion accumulation by a depolarization of the exposed membrane and its propagation along the glial syncytium to sites where the extracellular K ion concentration has not yet increased. In other words, a focal increase in the ECF $[K^+]o$ increases K^+-influx into glial cells and causes an osmolality decreases of ECF, and hence a shrinkage of the ECS volume. At remote sites, K^+-efflux and the consequent increase of the ECS volume are expected.

Thus, a complex interplay between the molecular and topological organization of ECS&ECM and the intercellular WT and VT occurs with the optimal tuning of the CNS integrative actions. It should be noticed that our proposal has some common aspects with Beaumont's hypothesis, in particular on the possible role of the ECF as a medium playing a fundamental role for the integrative action of the brain. In fact, Beaumont suggests that the brain ECF could be considered "a sponge-like 'inverse cell' that surrounds all the cells. During neuronal resting and action potentials, sodium and potassium ions shuttle into, and out of, this "Reciprocal Domain" within the brain. This localised flux of ions is the counterpart to all the neuronal electrochemical activity, so a complementary version of all that potential information is integrated into this space within

the brain" (Beaumont, 2014). However, our proposal points out that there are plastic BCs that can also be a result of the local brain cell activity in that brain region creating local compartments of the reciprocal domain. Furthermore, the RT-VT and likely the tunneling-nanotube WT can be intercellular communication modes not directly affected by the reciprocal domain.

Concluding remarks and perspectives

The active role of the GMN and of the ECS pathways in the holistic behavior of the brain

Because of these data and of the evidence of PAPs' plasticity, we introduce a hypothesis that during sleep there is an 'opening of the basic BCs' (i.e., tetra-partite synapses and SCs), namely an increase in the perviousness of VT pathways across the BC boundaries essentially based on PAPs' plastic changes (Bernardinelli et al., 2014), with a dramatic change in some aspects of synaptic functions and of the VT signaling. This hypothesis is based on a very important paper published by Xie et al. (2013). These authors have demonstrated that natural sleep or anesthesia is associated with a 60% increase in the interstitial space, resulting in a striking increase in convective exchange of CSF with ISF favoring the clearance of waste products (e.g., amyloid β) during sleep. This process depends on the glymphatic system that has one of the main molecular effectors in AQP4 water channels. Xie and collaborators report interesting data on the ISF volume and tortuosity of the diffusion pathways. Thus, the cortical interstitial volume fraction has been evaluated to be between 13% and 15% in the awake state as compared to 22% and 24% in sleeping or anesthetized mice. The tortuosity of the interstitial space did not differ significantly according to changes in the state of brain activity; awake, sleeping, and anesthetized mice all exhibited a λ value in the range of 1.3–1.8. (The dimensionless parameter tortuosity, λ, may be used to characterize the hindrance to diffusion where $\lambda=(D/D^*)^{1/2}$. The magnitude of D^* reflects the hindrance imposed by the geometry of the path; therefore $D^*<D$, where D is the free diffusion coefficient. In addition to being affected by the geometry, the diffusing molecule may also interact with the matrix; this too can be incorporated into the tortuosity; Nicholson et al., 2011). Our hypothesis maintains that λ does not decrease during sleep notwithstanding the increase in the ECS for the opening of excitatory synapses enwrapped by PAPs with the creation of a number of 'local voids', that is the synaptic gaps, which increase the tortuosity of the ECS. As far as the temporal aspects of these phenomena are concerned, it has already been pointed out that PAPs have the ability to rapidly restructure their thin-branched processes modifying their coverage of the synaptic elements (Bernardinelli et al., 2014). Thus, PAPs are plastic structures which can change their morphology within minutes, thus modifying their coverage of pre- and postsynaptic elements (Reichenbach et al., 2010). According to our hypothesis, these plastic changes can create new 'voids'; hence, they lead to a tortuosity increase that does not occur since it is compensated by the ECS increase.

The possible relevance of the brain GMN in psychiatry and neurology

Early investigations in psychiatric neurobiology, carried out from the vantage point of therapeutics, first associated some clear-cut mechanisms of effective psychotropic drugs with psychopathological domains, and lately raised awareness that during effective treatment of psychiatric conditions the expected changes of monoaminergic neurotransmission are paralleled by profound changes in brain metabolism, neural responses to stimuli, sleep architecture, biological rhythms, and, at the intracellular level, neuronal signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms (Millan, 2006; Benedetti and Smeraldi, 2009). Taking advantage of functional and structural brain imaging methods, in the last decade consistent findings associated major psychoses with distinctive patterns of disruption of gray matter (GM) and white matter (WM) integrity which, notwithstanding regional brain areas differences between the illnesses, progressively spread in the whole brain (Honea et al., 2005; Benedetti and Bollettini, 2014). A reduced timing and synchrony in the modular relationships between the component processes of the human brain (Gazzaniga, 1989), possibly due to altered myelination and GM microstructural changes (Bartzokis, 2012), could in turn lead to abnormal functional connectivity and altered mood and cognition (Benedetti and Bollettini, 2014; Vai et al., 2014). Notwithstanding classical findings about single neurotransmitters and regional abnormalities, a research focus on brain holistic behavior, and considering the key role of GMN and ECM in structuring the brain tissue microenvironment, might help to

better frame these recent perspectives about the biological underpinnings of psychiatric disorders.

Bipolar disorder (BD) offers a good example of the above. Signs of disruption of WM integrity spread in most tracts contributing to alterations in the functional integrity of the brain. Thus, *in vivo* diffusion tensor imaging suggests an increased space between fibers due to demyelination or dysmyelination (Benedetti and Bollettini, 2014) as well as a 40% reduction of the GM mainly in hippocampus (Yildiz-Yesiloglu and Ankerst, 2006) and in subgenual and orbitofrontal cortex. It should be pointed out that the number of neurons is preserved but there is a marked reduction of glial cell number and a reduction of synapses (Drevets et al., 2008). The imbalanced network biomarkers which have been identified up to now share the unique common feature of paracrine VT signaling. Both the neurotransmitters serotonin (5-HT) and noradrenaline, functionally associated with depressive states (Millan, 2006; Sharp and Cowen, 2011), and dopamine, hyperactive in mania (Cousins et al., 2009), are modulating the wired brain via VT (Fuxe et al., 2010) and are the target of treatment options aimed at modifying mood states, which could then be dependent on signal-receptor coupling through the ECM. Neurotrophic signaling cascades, which have been associated with cell atrophy and loss in BD (Shaltiel et al., 2007), require the diffusion in the ECM of neurotrophins (Lessmann et al., 2003). An increased systemic cortisol metabolism, coupled with altered brain glucocorticoid sensitivity (Spijker and van Rossum, 2012), has been associated with pathophysiology, stress vulnerability, and progressive allostatic load in BD (Steen et al., 2011; Grande et al., 2012). A substantial fraction of the transcriptome is under circadian regulation (Koike et al., 2012), with evidence in patients with BD suggesting a high dependence of behavior and psychopathology on the characteristics of the biological clock (Harvey, 2008; Benedetti and Terman, 2013; McClung, 2013): both neurons and glia express cellular circadian rhythms, coordinated in the brain by the suprachiasmatic nuclei by a diffusible signal (Slat et al., 2013), and with a thermolabile circulating factor influencing the endogenous rhythm of the clock molecular machinery to produce circadian behaviors (Pagani et al., 2011). An activated monocyte pro-inflammatory state, with a high inflammatory set point of circulating monocytes at the transcriptome level (Padmos et al., 2008) and *in vivo* activation of brain microglia (Haarman et al., 2014), has been associated with BD and parallels development in the offspring of patients with BD (Padmos et al., 2009; Mesman et al., 2015); again, pro-inflammatory cytokines diffuse into the brain via VT in the ECM. Lithium, the mainstay for the long-term treatment of BD and the only drug able to specifically counteract the signs of both WM and GM disruption

associated with the illness (Benedetti et al., 2013, 2015), has major effects on water homeostasis and increases the water content of the brain (Phatak et al., 2006; Regenold, 2008; Benedetti et al., 2015), thus probably also modifying the structure of the ECM and influencing the metabolite clearance which is coupled with changes of the interstitial fluid volume (Xie et al., 2013).

It has been suggested that together with neurotrophins, cytokines and other factors could be stored in the ECM which might serve as a natural, intermittent-release matrix for their delivery (Brightman, 2002). Beyond this possible mechanism, in more general terms the definition of the structure of the GMN in psychiatric conditions could help to understand the relationship between pathophysiological mechanisms globally affecting the brain, and regional changes possibly related to the boundaries of plastic brain compartments limiting the diffusion of toxic or trophic substances in the diseased brain. Still in its infancy, the computational investigation of subject-specific cerebrospinal fluid flow (Kurtcuoglu et al., 2007) could provide hints about the preferential diffusion of compounds in the ECM of specific brain regions, by combining data on cerebrospinal fluid circulation (Bechter, 2011) and preferential ways for diffusion in the GMN. Accordingly, it has been shown that a progressive alteration in the GMN could be a necessary correlate of the specific progression of damage typical of psychiatric illnesses, such as the temporal and spatial progression of regional GM shrinking during the transition to psychosis and the lifetime course of schizophrenia (Farrow et al., 2005; Honea et al., 2005; Takahashi et al., 2009), or the progressive volume reduction of hippocampus and subgenual cortex in BD. The definition of these mechanisms could then lead to new insights about the still unexplained course of illness progression, and provide unexpected targets to treat and prevent the detrimental effects of psychiatric illnesses on the structure and function of the brain.

Not limited to psychiatric diseases, a research perspective focusing on the analysis of subtle alteration of GMN might help to better conceptualize early changes in magnetic resonance imaging measures and histopathology to detect, differentiate, and individually quantify axon injury/loss, demyelination, and inflammation, which parallel onset and course of multiple sclerosis (Wang et al., 2015), to better understand the pathophysiology of the early stages of the illness and identify biomarkers and targets for treatment.

Age-related changes might also affect the ECM composition and the perviousness of the extracellular pathways, with remarkable consequences on the onset and

development of the most common neurodegenerative disorder, Alzheimer's disease. In fact, β-amyloid, like other proteins linked to neurodegenerative diseases, is released in the interstitial space (Cirrito et al., 2005), and can be cleared from the brain by the convective exchange of CSF and interstitial fluid through the ECM (Malkki, 2013) according to a circadian pattern. Thus, it has been shown that in animal models increased convective fluxes of interstitial fluid during sleep favor an increased rate of β-amyloid clearance (Xie et al., 2013). In humans poor sleep quality in older adults is associated with increased brain levels of β-amyloid (Spira et al., 2013), while unfragmented sleep could reduce the risk of AD and attenuate age-related cognitive decline and development of neurofibrillary tangles in individuals at genetic risk (Lim et al., 2013). These findings point toward the circadian β-amyloid clearance and hence to an important role of the ECM structure and topology in affecting resilience to Alzheimer's disease. Actually, several data support a complex and important role of the extracellular part of GMN in neurodegeneration. In this context, the abnormal deposition of sulfated proteoglycans in the formation of the histopathological lesions characterizing Alzheimer's disease should be mentioned (Bruinsma et al., 2010; Morawski et al., 2014).

Furthermore, a balance between MMPs and the tissue inhibitor of MMPs plays a pivotal role in the maintenance of the normal structure and function of the CNS (Wright et al., 2002). Chronic activation of MMPs has also been implicated in stroke, multiple sclerosis, and Alzheimer's disease (Wright et al., 2002). As far as amyotrophic lateral sclerosis (ALS) is concerned, important recent findings (Kaplan et al., 2014) demonstrate that the matrix MMP9 is expressed only by fast motor neurons, which are selectively vulnerable. In ALS model mice expressing mutant superoxide dismutase (SOD1), reduction of MMP9 function using gene ablation, viral gene therapy, or pharmacological inhibition significantly delayed muscle denervation.

A focus on the GMN structure and its subtle changes could then become a new, yet unexploited basis for prevention and potential therapeutic options in psychiatric and neurologic diseases.

Table 2: Glossary: terms and definitions.

Brain compartment (BC)	Brain volume differing from the neighboring tissue in terms of cellular, interstitial fluid and extracellular matrix composition. The BC is a plastic and sometimes rapidly changing functional structure since it can open up its boundaries exchanging signals with the neighboring tissue. Basic examples of BC are tetra-partite synapses and synaptic clusters, which in turn are assembled in higher level integrative structures in the CCNs. As mentioned in the text, when an increase in the perviousness of VT pathways across the BC boundaries takes place an opening of the BC can occur.
Complex cellular network (CCN)	A set of cells of any type exploiting the entire spectrum of intercellular communication modes to coordinate their outputs and to support each other's survival.
Global molecular network (GMN)	Three-dimensional molecular network filling up the intra- and extracellular spaces of the central nervous system (CNS) and interacting with the molecular networks located at the plasma membrane. It is composed of proteins, carbohydrates, lipids, whose physical interactions often lead to high molecular weight complexes.
Lebensraum	Literally 'vital space'. In the CNS, the morphological structure of the extracellular space together with the ion composition of the interstitial space fluid (ISF) and the molecular arrangement of the extracellular matrix form the 'Lebensraum' of the system.
Perisynaptic astrocyte processes (PAPs)	Astrocyte processes enwrapping pre- and postsynaptic sides of most synapses. They modulate the synaptic activity by providing a balanced and easily tunable feedback or feed-forward response. Thus, astrocytes and neurons become structurally and functionally strictly interconnected. The synaptic wrapping is highly dynamic and activity-dependent.
Synaptic clusters (SCs)	The term 'synaptic cluster' classically describes brain environments in which the density of synapses is relatively high when compared to other close brain regions. Thus, a crosstalk of neighboring synapses is possible. SCs are especially present on the same dendritic branch.
Tetra-partite synapse	Basic integrative structure formed by four interacting components: (i) the presynaptic side; (ii) the postsynaptic side; (iii) the PAPs; (iv) the extracellular matrix (ECM) molecules.
Volume transmission (VT)	Intercellular communication mode characterized by the diffusion of signaling molecules in the extracellular space (ECS) of the brain and/or in the cerebro-spinal fluid (CSF). A particular example is the so-called roamer type of VT (RT-VT), characterized by the intercellular exchange of micro-vesicles.
Wiring transmission (WT)	Intercellular communication modes characterized by signal transmission via physically well-delimited communication channels (a 'virtual wire'). Examples include synaptic contacts and gap junctions. The concept can be extended to molecular networks (systems of interacting proteins) to describe a signal progressing through transient changes in the conformation of the downstream proteins.

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