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New dimensions of connectomics and network plasticity in the central nervous system

DOI 10.1515/revneuro-2016-0051

Received August 9, 2016; accepted September 20, 2016; previously published online December 28, 2016

Abstract: Cellular network architecture plays a crucial role as the structural substrate for the brain functions. Therefore, it represents the main rationale for the emerging field of connectomics, defined as the comprehensive study of all aspects of central nervous system connectivity. Accordingly, in the present paper the main emphasis will be on the communication processes in the brain, namely wiring transmission (WT), i.e. the mapping of the communication channels made by cell components such as axons and synapses, and volume transmission (VT), i.e. the chemical signal diffusion along the interstitial brain fluid pathways. Considering both processes can further expand the connectomics concept, since both WT-connectomics and VT-connectomics contribute to the structure of the brain connectome. A consensus exists that such a structure follows a hierarchical or nested architecture, and macro-, meso- and microscales have been defined. In this respect, however, several lines of evidence indicate that a nanoscale (nano-connectomics) should also be considered to capture direct protein-protein allosteric interactions such as those occurring, for example, in receptor-receptor interactions at the plasma membrane level. In addition, emerging evidence points to novel mechanisms likely playing a significant role in the modulation of intercellular connectivity, increasing the plasticity of the system and adding complexity to its structure. In particular, the roamer type of VT (i.e. the intercellular transfer of RNA, proteins and receptors by extracellular vesicles) will be discussed since it allowed us to introduce a new concept of ‘transient changes of cell phenotype’, that is the transient acquisition of new signal release capabilities and/or new recognition/decoding apparatuses.

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Keywords: brain network; microvesicles; neuroanatomy; volume transmission; wiring transmission.

Dedicated to: This article is dedicated to Kjell Fuxe, Tomas Hökfelt and Sylvester Vizi for their pioneering findings on transmitter-identified neuronal systems and innovative views on intercellular communication processes in the brain.

Introduction

From a general point of view, the types of dynamics a biological system exhibits can be classified into two broad classes (see Anderson et al., 2012). In component-dominant dynamics, behavior is the product of a rigidly delineated architecture of modules, each with predetermined functions. In contrast, interaction-dominant dynamics is crucially based on the plasticity of the system components and on the network of communication processes among these components. The central nervous system (CNS) can be better described as an interaction-dominant dynamics system where interaction processes alter the integrative action of the single components and where it is difficult, and sometimes impossible, to assign tightly defined and unique roles to each specific component. The CNS is in fact a huge network of cells, regions and systems whose interconnections determine virtually all aspects of its integrative function. Thus, the key role played by the network architecture as a structural substrate for the CNS functions represents the main rationale for the emerging field of connectomics, the comprehensive study of all aspects of CNS connectivity (Sporns, 2012). This idea has a quite long history behind it. As pointed out by Schmähmann and Pandya (2007), early neuroanatomists were aware of the inappropriateness of their anatomical techniques to unravel the complex brain organization, and mapping the connections within the CNS has been a scientific goal for centuries. As an example, the 1685 Steno’s prescient lecture ‘On the anatomy of the brain’ (see Steno, 1965) deserves a mention. It emphasized the need of a program aimed at detailing brain anatomy in particular for what it concerns the fibers course through the white matter, since ‘it is impossible to explain the movements of a machine if the contrivance of its parts is unknown’. The characterization of inter-neuronal pathways, however, had to wait quite a long time until new methods able to stain

and trace neuronal connections became available [Flechsig, 1901; see Lanciego and Wouterlood (2011) for a review on more recent methods]. These studies very rapidly provided increasing evidence highlighting the important role of structural connectivity in shaping CNS physiological responses (see Sporns, 2013, for a thoughtful historical summary on this topic). A further significant advancement in the field occurred in the 1990s with the development of noninvasive diffusion imaging methods (see Le Bihan and Johansen-Berg, 2011, for a review) and the related computational techniques for inferring anatomical inter-neuronal pathways. Thus, the notion that brain function could be understood on the basis of the structural features of neuronal networks became generally accepted and the idea of creating a comprehensive map of the brain's structural connections emerged, also leading to the definition of the NIH 'Human connectome project' (www.humanconnectomeproject.org) aimed at providing an unparalleled compilation of neural data and the opportunity to achieve never before realized conclusions about the living human brain (Van Essen et al., 2012).

Although networks of neurons interconnected by synapses represent the fundamental structural substrate of the CNS function, they do not deal exhaustively with the issue. In fact, a broadened view on the connectivity in the CNS came with the proposal (Agnati et al., 1986) that a spectrum of intercellular communication processes is exploited in the system. They can be classified according to a dichotomous criterion: wiring transmission (WT: point-to-point communication via private channels, e.g. synaptic transmission) and volume transmission (VT: communication in the extracellular fluid and in the cerebrospinal fluid [CSF]). Subsequent experimental evidence suggested that these communication processes involve not only neurons but also other types of cells in the CNS (Syková and Chvátal, 2000; Färber and Kettenmann, 2005), allowing the formation of 'complex cellular networks' (CCN) including neurons, astrocytes, microglial cells, oligodendroglial cells, ependymal cells, pericytes and mast cells, in addition to the extracellular matrix (Agnati et al., 2014a,b). These cell assemblies operate as a plastic network by exchanging signals in a certain volume of brain tissue and, thanks to this cross-talk, integrating their activity (Agnati and Fuxe, 2000). More recently, data have also been provided (see Simons and Raposo, 2009; Agnati et al., 2014a) indicating the existence of VT processes aimed at modulating the intercellular connectivity. They involve, for instance, the intercellular transfer of elements of the recognition/decoding apparatus (e.g. receptors) by microvesicles (MV), leading to a transient phenotypic change in the target cell (Agnati et al., 2014a).

All these aspects, significantly related to the connectome concept and strongly increasing the degrees of freedom available to the whole system, will be the main focus of the present review article, since they indicate possible stimulating new challenges for the study of CNS organization.

The connectome concept

The term 'connectome' was first defined by Sporns et al. (2005) as 'a comprehensive structural description of the network of elements and connections forming the human brain'. At the same time and independently the term 'connectomics' was proposed by Hagmann (2005) to describe the study of the structural connections in the brain. Despite the differences in the proposed experimental approaches to fulfill the task, both the above-mentioned proposals agreed on the connectome as a concept primarily grounded in the brain structure.

As pointed out by Sporns (2013), the emphasis on structure is important because anatomically determined connections among CNS elements embody a large but finite set of relations that (at least in principle) can be objectively mapped and represented by appropriate network models characterized by well-defined geometrical and biophysical features, such as spatial trajectories, metric length, conduction delays and physiological strength.

Thus, defining the connectome involves a careful analysis of the communication processes existing between CNS elements and of the pathways they exploit.

Connectomics and the dichotomous classification of communication strategies

About 30 years ago, our group and other groups (see Agnati et al., 2000; Fuxe et al., 2007; Vizi et al., 2010) provided new data on the communication modes in the CNS that, while not dismissing the fundamental relevance of synaptic contacts, have broadened the field. In particular, they allowed us to propose (Agnati et al., 1986) the existence of two main modes of intercellular communication in the CNS: WT and VT. This proposal was influenced by previous important contributions on communication in the CNS (Golgi, 1914; Guillemin, 1978; Nicholson, 1979; Schmitt, 1984; Vizi, 1984) and based on a number of observations, especially on the central monoamine neurons (see Fuxe and Agnati, 1991; Agnati et al., 2000).

The criteria characterizing WT and VT and their subclasses can be deduced (Agnati et al., 2010a, 2014a,b; Fuxe et al., 2013) not only from structural and neurochemical findings, but also from concepts and from the lexicon offered by informatics (Hopcroft and Ullman, 1979; Le Boudec and Thiran, 2001). In this respect, it has to be observed that the main criteria that allow the differentiation of WT from VT are the characteristics of the communication channel and, more precisely, the physical boundaries of the channel, which are well delimited for WT but not for VT. The classification can be further detailed by taking into account other signal features, some of them already briefly mentioned here:

- Signal privacy: a signal is characterized by high privacy (i.e. a ‘reserved signal’) if only cells endowed with a specific recognition-decoding apparatus (e.g. specific receptors) can have access to it. In contrast, we are dealing with a low-privacy signal (i.e. a ‘broadcast signal’) when any cell reached by the signal can have access to it.
- Signal safety: as far as safety is concerned, we are dealing with a ‘safe signal’ if it is not altered during its conduction from the source to the target cell, and with an ‘unsafe signal’ if it can be altered during its pathway. This occurs, for instance, with some VT signals that can be broken down or modified (e.g. by enzymes) or be trapped in a cul de sac in the extra cellular space (ECS) pathways.
- Plasticity of the connection: if the connections between cells can be rapidly formed or removed, they provide a ‘dynamic network’. In contrast, the structure of a communication network is ‘static’ when the pattern of connections is mostly stable in time.

Thus, based on these concepts, a unitary scheme could be devised for a more detailed characterization of WT and VT and of their subtypes (see Table 1). They will be briefly examined in the sections that follow.

Wiring transmission

The specific feature of this type of communication is the existence of a virtual wire connecting the source of the signal with the target. Such a specific anatomical link, therefore, can identify relationships closely matching the above-mentioned geometrical and biophysical requirements for their inclusion in the connectome.

It is beyond question that the most important example of WT is classic synaptic wiring representing the primary mechanism through which neurons transmit information

Table 1: Different types of wiring transmission and volume transmission modes (Agnati et al., 2014b).

	Network	Privacy	Safety
Wiring transmission	Synaptic transmission	Reserved	Safe
	Gap junctions	Broadcast	Safe
	Tunneling nano-tubes	Broadcast	Safe
Volume transmission	Long distance VT	Reserved (common)	Unsafe
		Broadcast (rare)	
	Ephaptic transmission	Broadcast	Safe
	Perisynaptic VT	Reserved	Unsafe
	Roamer-type VT	Reserved or broadcast	Safe

and control behavior. Actually, a small gap (the synaptic cleft < 50 nm) is present between the site of release of the transmitter and the site where the receptors capable of detecting and transducing the signal are located. However, in the ‘classical synaptic transmission’ it is assumed that this gap does not interfere with the continuity of the channel (Savtchenko and Rusakov, 2007; Tang et al., 2016) and with the ‘safety’ of the message. Moreover, as mentioned before, the signal can be classified as ‘reserved’ since specific receptors are needed to decode the message.

A further form of WT is represented by gap junctions (GJ). They are channels providing a direct pathway for electrical and metabolic signals between cells. Each GJ channel (characterized by an inner pore diameter of 10–12 Å) is composed of two hemi-channels (connexons, one on each adjacent cell), which in turn are composed of six subunits of the protein connexin (Oviedo-Orta and Evans, 2004). GJ have been found between many types of neurons, between glial cells and in few somewhat controversial instances between astrocytes and neurons (Nagya et al., 2004). GJ seem to play a particular role in astrocytes, where their main function is to minimize the differences for substrates such as glucose (Rouach et al., 2008) and to dissipate extracellular K^+ or glutamate, whose extracellular accumulation can be detrimental for proper neuronal functions. It should be underlined, in addition, that GJ also allow the coupling of astrocytes to each other to form a network (Carmignoto, 2000) in which cells can exchange signals mediated by calcium waves (Pereira and Furlan, 2010). Since the GJ channel can be regulated by extra- and intracellular signals (Glaume, 2010), it has been suggested that astrocyte networks could implement computational processes (Pereira and Furlan, 2010; Guidolin et al., 2011) and participate in higher brain functions (Robertson, 2002; Volterra and Meldolesi, 2005).

GJ can also be observed in chemical synapses. Hence, ‘mixed synapses’ are possible (Rash et al., 1996), which may have a significant modulatory role on the neural networks,

imparting fundamental alterations to their properties. It has to be pointed out, however, that in the mammalian CNS, the physiological roles and distributions of mixed synapses are still to be clearly established (Fuxe et al., 2007).

A recently proposed form of WT (see Agnati et al., 2010a) is tunneling nano-tubes (TNT). These structures, involved in intercellular communication, have been discovered by means of *in vitro* studies (Rustom et al., 2004). In a transient way, they connect two cells forming a ‘private’ direct channel that has no gaps and resembles the plasmodesmata of plant cells. These transcellular channels, with a diameter of 50–200 nm and a length of up to several cell diameters, could lead to the formation of syncytial cellular networks (Gerdes and Carvalho, 2008). As far as animal cells are concerned, they have been identified in a variety of cultured cell systems, including cells of the immune system, kidney cells, PC12 cells and human glioblastoma cells (Rustom et al., 2004; Onfelt et al., 2006; Sowinski et al., 2008; Agnati et al., 2010b). It is still unclear, however, whether this mode of communication is really used *in vivo* by the cells in the CNS. Furthermore, being transient structures not displaying relatively stable anatomical distribution, connections by TNT cannot be included in the definition of the connectome (Friston, 2011). Should their existence in CNS tissue be proved, however, TNT could represent a mechanism able to

modulate cell connectivity (see the ‘Mechanisms modulating intercellular connectivity’ section), since it has been demonstrated that receptors can move from one cell to the plasma membrane of another cell by exploiting this type of intercellular channels (Agnati et al., 2011; Guescini et al., 2012).

Volume transmission

Studies on monoaminergic (Ungerstedt et al., 1969; Geffen et al., 1976) and peptidergic (Hokfelt et al., 1975; Fuxe et al., 1977; Ljungdahl et al., 1978; Bloom and Segal, 1980; Burbach, 1982; De Wied and Jolles, 1982) neurons and on the modulation of cortical release of acetylcholine by noradrenaline (Vizi, 1980a) provided support to the functional assumption of a diffuse, non-synaptic (Vizi, 1980b), mode of intercellular communication and led to the definition of VT (Agnati et al., 1986).

VT is characterized by the absence of any wire-like channel connecting the source of the signal with its own targets. This communication mode, in fact, uses several often spatially divergent tortuous channels made by the clefts (about 20 nm in diameter; Chen and Nicholson, 2000) between cells and filled with extracellular fluid and extracellular matrix (Figure 1). VT is primarily mediated

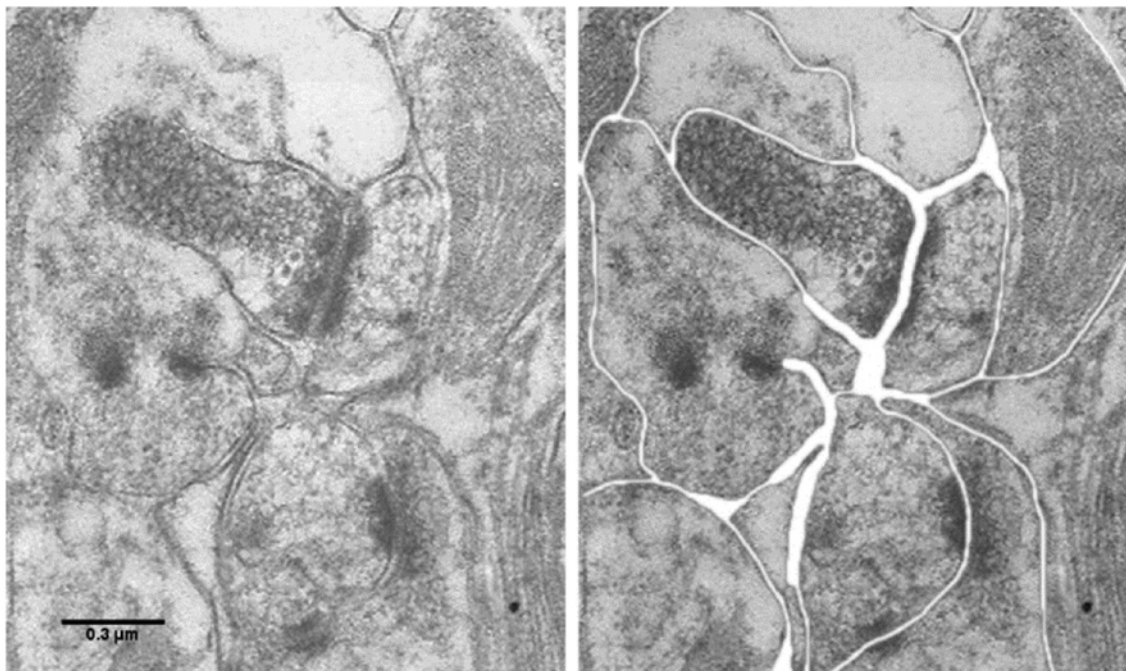


Figure 1: Left panel: transmission electron micrograph of rat hippocampal neuropil. Right panel: the ECS is outlined in white. As pointed out by Nicholson (Syková and Nicholson, 2008), it is a well-connected foam-like structure formed from the interstices of simple convex cell surfaces. Even though the ECS is probably reduced in width due to the fixation procedure, it is still evident that it is not completely uniform in width.

by simple diffusion but also by pressure waves due to the arterial pulses in the cerebral arteries and by thermal and electrical gradients (Agnati et al., 1994, 2005a). Since signals migrate in the ECS, they may be stopped if they reach a blind alley (a cul de sac) (Hrabetová et al., 2003), inactivated by enzymes or cleared over the brain capillaries (Jansson, 2000), taken up into cells via transporters (Rice and Cragg, 2008). Thus, VT is in general characterized by a low signal safety. As summarized in Table 2 (see Agnati et al., 2010a, 2014a,b; Vizi et al., 2010, for more complete and detailed reviews), available evidence supports the assumption that VT mainly employ the same set of signals as WT, namely, transmitters, peptides, ions and gases. An important finding was that non-synaptic receptors are characterized by high affinity (Vizi, 2000) and can represent relevant targets of drug treatment in clinical practice (Vizi et al., 2010). Other types of signals, however,

were suggested to be exchanged by VT (Agnati et al., 1994; Fuxe et al., 2010). They include physical signals, such as field potentials (ephaptic transmission or electrical VT; Arvanitaki, 1942) and thermal waves, especially affecting neurons endowed with high Q10 values (i.e. sensitivity to temperature elevation), such as those present in particular hypothalamic regions (Rivera et al., 2006).

VT signals can be released from any type of brain cells. They can be released from neurons (in particular from dendrites), from soma and axon terminals (varicosities), entirely lacking synaptic membrane specializations (Descarries et al., 2008), and from astroglial, microglial, oligodendroglial, mast and ependymal cells as well as pericytes. Being able to reach a variety of targets within entire brain regions (Jansson et al., 1999), VT signals are potentially characterized by a quite high divergence. Of particular importance for our discussion, however, is

Table 2: Chemical mediators involved in volume transmission.

Signaling molecule	Brain region	Animal	References
Dopamine	Globus pallidus	Primates	Eid and Parent, 2016
		Rat	Floresco et al., 2003
	Substantia nigra (compacta)	Rat	Rice and Cragg, 2008
	Ventral tegmental area	Rat	Rice and Patel, 2015
	Ventral subiculum	Mouse	Goto et al., 2007
	Pedunculopontine nucleus	Mouse	Floresco, 2007
Serotonin	Retina	Mouse	Hirasawa et al., 2015
	Globus pallidus	Primates	Eid and Parent, 2016
	Hippocampus	Rat	Bunin and Wightman, 1999
	Frontal cortex	Rat	Ridet and Privat, 2000
	Cerebellum	Rat	Doly et al., 2004
	Dorsal raphe nucleus	Rat	Jennings, 2013
Noradrenaline	Substantia nigra (reticulata)	Rat	Ciranna, 2006
	Spinal cord	Rat	Bunin and Wightman, 1998
	Cerebral cortex	Rat	Descarries et al., 1977
	Prefrontal cortex	Mouse	Mundorf et al., 2001
Glutamate	Hippocampus	Rat	Kullmann et al., 1996
			Diamond, 2001
GABA	Cerebellum	Rat	Szapiro and Barbour, 2007
	Retina	Mouse	Hirasawa et al., 2015
	Cerebral cortex	Rat	Rózsa et al., 2015
Acetylcholine	Cerebral cortex	Rodents	Ovsepian et al., 2016
	Parietal cortex	Rat	Lendvai and Vizi, 2008
	Hippocampus	Rat	Descarries and Mechawar, 2000
β-Endorphin	Midbrain	Rat	MacMillan et al., 1998
	Cerebral cortex		
Galanin	Raphe	Rat	Fuxe et al., 1998
	Amygdala		
Nitric oxide	Brainstem	Rat	Steinert et al., 2008
			Garthwaite, 2016
Ca ²⁺	Hippocampus	Rat	Yano et al., 2004
	Neocortex		
CO ₂	Hippocampus	Rat	Sun and Alkon, 2002

to underline that the ECS also fulfills active tasks in the transmission process, since it may direct the diffusion of electrochemical messages in an anisotropic fashion favoring or preventing the communication between two brain areas. This may be due, in part, to the fact that the extracellular matrix is not an amorphous filling and can also differ among the various cell types of the CNS (Agnati et al., 2000; Marcoli et al., 2015). In this respect, Nicholson's work (see Nicholson, 2001; Syková and Nicholson, 2008) provided a deep characterization of the biophysical features of VT. Diffusion in the interstitial space was accurately modeled with appropriate modifications of classical equations and parameters describing the structure of the ECS (e.g. volume fraction and tortuosity) or the direction, metric length and conduction delay of the signal migration (e.g. effective diffusion coefficients) were derived. Moreover, measurement techniques to assess these diffusion characteristics were analyzed and proposed (see Syková and Nicholson, 2008, for a review). Examples include radiotracer techniques (see Patlak et al., 1998), the tetramethylammonium method (Nicholson and Phillips, 1981) and methods using real-time iontophoresis or pressure ejection. Thus, VT pathways connecting specific areas in the CNS could be identified and characterized, opening the concrete possibility that also VT processes could contribute to the definition of the brain connectome. Interestingly, these diffusive pathways appear significantly hindered and the volume of the ECS reduced in many neuropathological states that are associated with cellular edema and ischemia (Syková et al., 2000).

Summing up, intercellular communication occurs via both WT and VT for most of the CNS components, with some of them using mainly (or even only) one of the two modes. In the case of neurons, the ratio between WT and VT can vary from one neuron system to the other and with the structural and functional state of each neuron. Both processes, therefore, seem to contribute to the structure of the brain connectome. In this respect, a point deserves some more consideration. As emphasized by Sporns (2013), there is a natural affinity between connectomics and network science. In fact, although it may seem rather abstract representing the connectome as a network or graphical model, appropriately chosen concepts of network science appear well suited to capture real neurobiological structures and processes. In this context, network models combining WT and VT connections have been proposed to describe 'correlation learning' in hippocampal neurons (Gally et al., 1990) and 'temporal difference learning' in mesencephalic dopamine neurons (Guidolin et al., 2007). Thus, the possibility exists to describe a brain connectome involving both WT and VT pathways by using

suitable techniques from network theory. In the Appendix, an abstract model of neuron implementing both communication strategies is briefly illustrated. It is based on the 'neurotransmitter field theory' originally proposed by Greer (2007).

Connectomics refers to a hierarchical network structure

It is well known that all anatomic systems exhibit the pivotal property to form multiscale structures (Jacob, 1970) each of which forms 'a whole in relation to its parts and is simultaneously part of a larger whole' (Grizzi and Chiriva-Internati, 2005). This feature is of particular relevance in the CNS, whose architecture extends over a range of up to five orders of magnitude of scales: from microns for cell structures at one end to centimeters for inter-areal neuronal connections at the other. A hierarchical or nested architecture has been suggested as a suitable model providing a unified view of the different spatial scales characterizing the brain network organization (Agnati and Fuxe, 1984; Sporns et al., 2005; Sporns, 2013; Guidolin et al., 2016). According to this concept, smaller network communities can be further connected to form larger and larger assemblies. From the connectomics point of view this structural feature poses a significant challenge (see Zalesky et al., 2010). It concerns the unambiguous identification of the significant levels of organization that closely relates to the problem of 'node definition', i.e. the identification of the basic network's elements at each level. In fact, the graph theory-derived topological properties of a connectome strongly depend on how the brain is parceled out into elements.

In this respect, an almost general consensus exists (see Agnati and Fuxe, 1984; Sporns et al., 2005) in targeting at least three levels of organization (see Figure 2).

Macroscale

At this level brain areas and neuronal populations represent the basic elements. The human cerebral cortex, for instance, could be arranged in a number of anatomical regions on the order of 100 or more (Van Essen et al., 1998; Glasser et al., 2016). Parcellation can be performed on the basis of several different criteria (see Wig et al., 2011), but no single universally accepted procedure still exists. The most productive approach is likely to define brain regions and their boundaries according to multiple structural and functional criteria (Nelson et al., 2010;

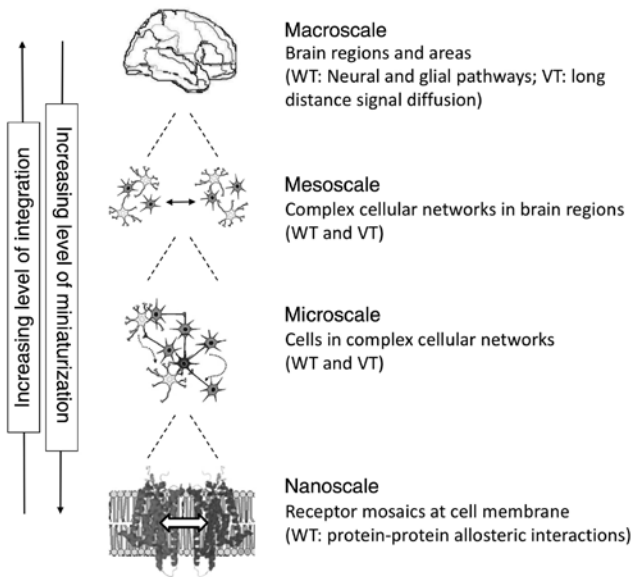


Figure 2: Schematic view of the CNS connectivity organized according to a hierarchical principle (see the text). At each level the nodes and the connection modes they can exploit are indicated.

Glasser et al., 2016; Sporns and Betzel, 2016). Despite these limitations, the macroscale level is considered the most feasible organizational level for a first draft of a connectome (Sporns et al., 2005) and the analysis of datasets obtained in various mammalian species (Hilgetag et al., 2000) revealed a number of common network characteristics. As far as the neuronal networks are concerned, the connections between different cortical areas were shown to possess an organization in the form of ‘small-world networks’ (Watts and Strogatz, 1998), forming clusters of nearby cortical areas with short links, which in turn have long-range connections to other clusters (Sporns and Zwi, 2004; Stam and Reijneveld, 2007). Within clusters, functional magnetic imaging identified a network topology of the type called ‘scale-free’ (Eguiluz et al., 2005), in which some nodes (hubs of connectivity) have a high number of connections to other nodes, whereas most nodes have just a handful.

VT pathways at macroscale were also proposed (see Fuxe et al., 2013). They are based on the hypothesis that cyclic pressure oscillations (linked to intracranial arterial pulses) exist in the subarachnoid space which induces ‘tide’ movements (Agnati et al., 2005a) in the fluid of the Virchow-Robin spaces. Such convective movements would provide long-distance VT signals (Picard and Zanardi, 2015). Peptide neurons likely operate via long-distance VT with distances in the range of millimeters involving also flow in the CSF (Jansson et al., 2002). One

of the best examples is CSF-delivered β -endorphin which accumulates in nerve cell subpopulations all over the paraventricular hypothalamus as seen 15 min after the CSF injection (Bjelke and Fuxe, 1993). In the experiments by Duggan et al., after 60–90 min of stimulation of the β -endorphin secreting neurons in the arcuate nucleus, the peptide was detected at a distance of several millimeters in midbrain and cerebral cortex areas (MacMillan et al., 1998), indicating that β -endorphin could migrate for long distances after its release from nerve terminals.

Mesoscale

Within each brain region, functional subdivision or segregated subcircuits can be recognized. A classic example is provided by cortical columns (Lorente de Nò, 1938) consisting of an array of cooperating neuronal groups extending radially across the cortical cellular layers and representing units of operation (DeFelipe et al., 2002; Rakic, 2008). As far as the anatomical boundaries of these units are concerned, they appear loosely delimited in morphological terms, since they are dynamic entities changing according to functional needs. A role in delimiting them is probably played by astrocytes, since the astroglial cells, especially in mammalian brains, define the microarchitecture of the parenchyma by dividing the gray matter into relatively independent structural units through the process known as ‘tiling’ (Bushong et al., 2004).

A broadening of this view is needed when VT-based intercellular communication processes are taken into account. In fact, this signaling backbone involves not only neurons, but also other types of cells in the CNS, such as astrocytes and microglial cells (Syková and Chvátal, 2000; Färber and Kettenmann, 2005). Hence, the concept of ‘complex cellular networks’ has been introduced to indicate the set of cells of any type that exchanging signals in a certain volume of brain tissue are capable not only of integrating multiple inputs to give out appropriate outputs but also of supporting each other’s survival (Agnati et al., 2000). In this context, the relationship between neurons and astrocytes is the best studied (see Fellin and Carmignoto, 2004) and evidence exists highlighting the involvement of ‘neuron-astroglial interactions’ in the higher brain functions (Schipke et al., 2008; Pereira and Furlan, 2010). As a matter of fact, the concept of ‘tripartite synapse’ has been introduced, since in most glutamatergic central synapses, the extremity of a protoplasmic astrocyte process wraps the synaptic cleft. Since astrocytes express membrane receptors to neurotransmitters and can release their own chemical messengers

(gliotransmitters), this arrangement allows them to establish a cross-talk with both pre- and postsynaptic neurons. As discussed in the ‘Connectomics and the dichotomous classification of communication strategies’ section, several astrocytes participate in this functional organization, coupled with each other by GJ, leading to the formation of real neuroastroglial networks.

On this basis, it can be proposed that the basic network elements at mesoscale should be defined by considering not only neuronal networks but also whole compartments of brain tissue where different cell types and the extracellular matrix work as an integrated ‘functional module’ (Agnati et al., 2009; Meunier et al., 2010; Bassett et al., 2011).

Microscale

At this scale, single cells and synapses can be found. Of particular interest at this level are the so-called synaptic clusters (SC), in which multiple synapses act cooperatively to modulate their strength (Shepherd, 1979; Golding et al., 2002). SC are often organized around the dendritic spines and partially isolated from the surrounding environment by glial cells (Golding et al., 2002; Grillner and Graybiel, 2004; Cutsuridis et al., 2009).

As pointed out by Sporns et al. (2005), drawing the connectome at microscale is infeasible, at least in the near future. Even taking into account the single neurons as the basic element, the connections to map would be in the order of 10^{15} , a technically impossible task. If we also consider other cell types and VT connections, the connectome size would become even greater. However, it has to be said that such a level of structural detail may be unnecessary, since cognitive functions emerge from the activity of large and distributed cell populations (Mountcastle, 1998). Thus, at microscale, the simple characterization of plastic changes and of mechanisms remodeling connectivity could represent a sufficiently significant dataset for a deeper description of CNS functions. In this context, the study of the balance between WT and VT in neurons and the changes it undergoes in different physiological or pathological conditions could provide information useful to understand their functional state at a single-cell level (Agnati et al., 2014a,b).

To better capture properties concerning the strength and plasticity of synapses and VT connections a ‘nanoscale’ level (nano-connectomics) should also be considered. At this further level of miniaturization, molecular networks can be found. They are made of molecules (in particular proteins) that function as a metabolic

and/or regulatory signaling pathway in a cell (Bhalla and Iyengar, 1999). For our discussion of particular interest are the ‘receptor mosaics’, i.e. macromolecular complexes formed at the membrane level by G protein-coupled receptors (GPCR) (Agnati et al., 1982, 2005b; Fuxe et al., 1983) as a consequence of direct allosteric receptor-receptor interactions (RRI). The term RRI refers to an interaction requiring a direct physical contact between the involved receptor proteins leading to the formation of receptor complexes (dimers or high-order oligomers) at the cell membrane (see Kenakin et al., 2010, for the definition, as assessed by a specific international consensus workshop). The basic biochemical mechanism leading to the formation of these receptor assemblies are allosteric interactions, and, as recently outlined by Changeux and Christopoulos (2016), the cooperativity that emerges in the actions of orthosteric and allosteric ligands of the GPCR forming the assembly provides the cell decoding apparatus with sophisticated dynamics (see Guidolin et al., 2015, for a recent review) in terms of modulation of recognition, G-protein signaling and selectivity, receptor desensitization (Gainetdinov et al., 2004; Plested, 2016) and switching to β -arrestin signaling (Smith and Rajagopal, 2016). Thus, the formation of the receptor mosaics allows an integration of the incoming signals already at the plasma membrane level and can significantly contribute to set and tune the efficiency of the connections between cells and, in particular, the synaptic strength (Agnati et al., 1982, 2003). Interestingly, methods from graph and network theory appear appropriate also to describe the dynamic behavior of interacting receptors (Guidolin et al., 2007), further suggesting the possibility of including a nanoscale level in the context of connectomics. In particular, the possible existence of receptors acting as ‘hubs’ in the receptor assembly has been suggested (Agnati et al., 2008, 2016). Due to their position in the network of interactions, hub receptors could play a key role in the integrative action of the assembly and represent a target of primary importance from the pharmacological perspective.

Diverse methods are presently available for identifying direct interactions between GPCR. They involve not only binding (Wreggett and Wells, 1995) and functional studies but also the use of bivalent ligands (Akgün et al., 2013) and biophysical proximity assays, such as resonance energy transfer methods (Marullo and Bouvier, 2007) and line-scan fluorescence cross-correlation spectroscopy (Herrick-Davis et al., 2013). Biochemical techniques, such as co-immunoprecipitation (Skieterska et al., 2013) and proximity ligation assay (Borrito-Escuela et al., 2011), provide further experimental support. In the last decade, they allowed the demonstration of an increasing number

of interactions among GPCR, leading to the identification of quite a large number of macromolecular complexes they can form.

Mechanisms modulating intercellular connectivity

The experience-based reshaping of the brain structures (McEwen, 2010) is a general feature that deeply characterizes the nervous system. Regional networks can be redeployed (or reused) for multiple functions, as recently shown by Anderson (2010) following a careful survey of neuroimaging data. Changes in connectivity are well known at the level of local circuits and neurons. They include changes in dendritic branches (Bennett et al., 1964) and in synaptic strength (Holtmaat and Svoboda, 2009), the creation of new connections (see, for instance, the neural circuitry responsible for seasonal breeding in several species; Adams et al., 2006) and the reactivation of ‘silent’ synapses when needed (Kerchner and Nicoll, 2008). At the molecular level, a key mechanism regulating synaptic efficiency is receptor trafficking composed of lateral (between synaptic and non-synaptic compartments) and vertical (between intracellular stores and cell membrane) mobility of receptors (see Vizi et al., 2010, for a review). Moreover, the existence of RRI can lead to the assembly of oligomeric receptor complexes with different properties even if formed by the same types of monomers (Agnati et al., 2010c).

Emerging evidence, however, points to novel mechanisms likely playing a significant role in the modulation of intercellular connectivity. In particular, it indicates that the cross-talk between perisynaptic astrocytes and neurons can mediate synaptogenesis, synapse elimination and structural plasticity through a variety of secreted and contact-dependent signals (Stevens 2008; Theodosis et al., 2008). Furthermore, a specific form of VT, involving the transfer of receptors and receptor complexes by MV, could lead to transient changes in the recognition/decoding apparatus of the target cells (Agnati et al., 2014a). These processes will be the focus of the sections that follow.

Astrocyte processes and modulation of the synaptic transmission

Astroglia constitutes the major glial population of the brain. Highly branched and ramified protoplasmic astrocytes are the predominant form in gray matter and are

found in almost all regions of the CNS. They are characterized by a polarized orientation of their processes (Reichenbach et al., 2010). While one or only few processes have contacts with CNS boundaries such as capillaries and pia, an overwhelming number of thin filopodia- and lamellipodia-like process terminals contact and enwrap synapses, the sites of neuronal communication. These astrocytes’ fine ramifications account for 70%–80% of the astrocytic plasma membrane and often surround spine synapses, sometimes completely encapsulating them. Even if perisynaptic astrocyte processes (PAP) are found in all brain regions, however, the proportion of synapses having them and the level of synaptic coverage vary significantly and it should be underlined that enwrapping of synapses by PAP is an important feature that allows high efficiency and privacy of the transmission (see Figure 3). 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 PAP that enwrap the synapse.

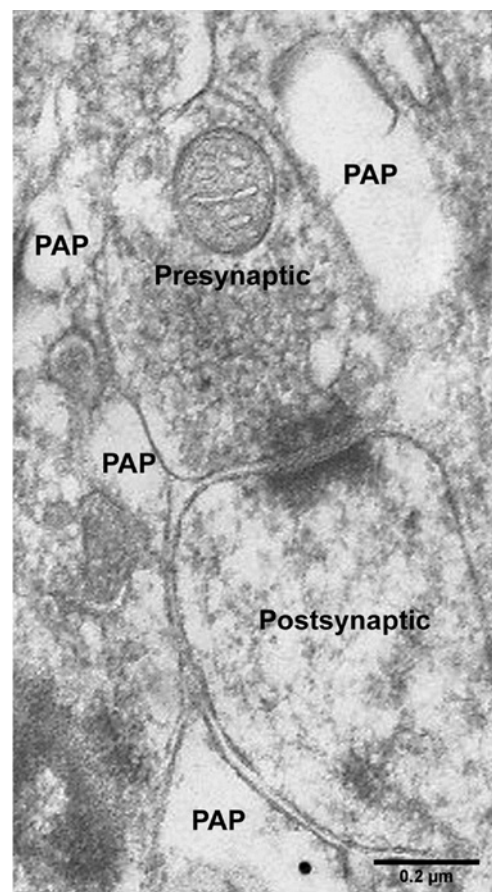


Figure 3: Transmission electron micrograph of a rat hippocampal synapse illustrating the relationship between the pre- and postsynaptic elements and the perisynaptic astrocyte processes (PAP).

In fact, increasing evidence suggests the existence of bidirectional interactions between neurons and astrocytes at excitatory synapses, leading to the concept of ‘tripartite synapse’ (Araque et al., 1999). Astrocytes display activity-mediated Ca^{2+} responses *in vitro* (Pasti et al., 1997) and *in vivo* (Hirase et al., 2004) and Ca^{2+} signals can trigger the release of gliotransmitters (glutamate, D-serine, ATP), which in turn regulate synaptic transmission (Volterra and Meldolesi, 2005). PAP can also express several proteins that deeply affect the morphology and function of the tripartite synapse such as glutamine synthetase, astrocytic glutamate transporters, metabotropic glutamate receptors, cell adhesion molecules, K^+ channels and aquaporins that may regulate ‘adaptive’ swelling of PAP (Bernardinelli et al., 2014).

In this respect, a functionally important aspect is the motile nature of PAP that have the ability to rapidly restructure their thin-branched processes modifying their coverage of the synaptic elements (Bernardinelli et al., 2014). Several studies describe them as plastic structures able to change their morphology within minutes, thus modifying their coverage of pre- and postsynaptic elements (see Reichenbach et al., 2010; Bernardinelli et al., 2014). As a consequence, astrocytic PAP insulation of the synapses can act as a plastic physical barrier controlling the transmitter spillover from the synaptic cleft. In view of these data, it has been proposed (Marcoli et al., 2015) that a sophisticated control of the PAP’s plasticity could allow moving from a high privacy of the synaptic transmission (close enwrap of the tripartite synapse) to a more or less broad opening of the enwrapping. This would lead to transmitter diffusion (by extra-synaptic VT; Agnati et al., 2014a,b) to neighboring synapses especially along dendrites and to a modulation of the integrative activity of SC and CCN at microscale (Grillner and Graybiel, 2004).

Intercellular transfer by MV of recognition/decoding apparatuses

In the last decade, evidence was obtained that cells can exchange a set of chemical messages via extracellular vesicles (acting as protective containers) (Février and Raposo, 2004; Simons and Raposo, 2009). Different types of MV have been described (see Lakkaraju and Rodriguez-Boulan, 2008; Agnati et al., 2014a). For the purposes of the present discussion, however, two types of vesicles deserve a particular attention:

Exosomes are vesicles (40–100 nm in diameter) contained in the so-called early, late or recycling endosomes, a type of multivesicular bodies (MVB). Endosomes usually transport newly synthesized material from the Golgi

complex and endocytosed material from the plasma membrane to various intracellular destinations. An alternative fate of MVB, however, is their exocytotic fusion with the plasma membrane leading to the release of exosomes into the extracellular milieu both constitutively and in a regulated manner (van Niel et al., 2006; Lakkaraju and Rodriguez-Boulan, 2008).

Extracellular vesicles can also be formed from lipid raft domains of the plasma membrane, and are then called shedding vesicles (Smalheiser, 2007). The shedding of vesicles is preceded by the budding of small cytoplasmic protrusions, which then detach by fission of their stalk. Thus, shedding vesicles show surface markers that are largely dependent on the composition of the membrane of origin and constitute a larger and more heterogeneous population of extracellular vesicles, ranging from 100 to 1000 nm in diameter.

As amply supported by experimental findings, both exosomes and shedding vesicles can deliver a variety of chemicals (including mRNA, miRNA and proteins) to recipient cells (Lee et al., 2012). Furthermore, data exist indicating that, probably owing to the membrane deformability of these structures, their diffusion in the ECS is quite extended and often anisotropic (Agnati et al., 2014a). Thus, according to the previously stated basic criterion (see the ‘Volume transmission’ section), this class of intercellular communication belongs to the VT mode of communication (virtually no continuous channel). This peculiar class of VT has been called ‘roamer type of VT’ (Agnati et al., 2010a, 2014a), based on an analogy between vesicles and itinerant workers who move away from the source of production and roam from cell to cell with their set of products.

The possible relevance of this type of VT for the intercellular connectivity can be appreciated in the light of several experimental findings. A number of receptors were found to be transferred from one to another cell type via the exosome pathway; for example, bystander B cells acquire antigen receptors from activated B cells by a membrane transfer (Quah et al., 2008) and tumor cells induce apoptosis of activated T cells through the transfer of MV-mediated Fas ligand (Kim et al., 2005). In addition, transferrin receptor 2 (TfR2), the tetraspanin CD81, and to a lesser extent caveolin-1 were found to be part of the exosomal budding vesicles in erythroleukemic and hepatoblastoma cells (Calzolari et al., 2006). In particular, these authors demonstrated that TfR2 localizes in low-density plasma membrane microdomains, where it promotes cell signaling, and is exported out of the cells through exosomes, where it acts as an intercellular messenger.

For what it concerns GPCR involved in the recognition/decoding of signals between CNS cells recent data were obtained demonstrating that they can be transported by MV and in particular by exosomes (Agnati et al., 2011). In order to provide further details on the exchange of GPCR among cells by means of MV, Guescini et al. (2012) created two populations of cells, the first transfected with a CFP-labeled dopamine D2 receptor (D2R-CFP) and the second with a YFP-labeled adenosine A2A receptor (A2AR-YFP).

These two types of cells were co-cultured, and acceptor photobleaching fluorescence resonance energy transfer (FRET) analysis demonstrated cells positive to both D2R-CFP and A2AR-YFP. Furthermore, recipient cells preincubated for 24 h with A2AR positive MV were treated with the adenosine A2A receptor agonist CGS-21680. The significant increase in cAMP accumulation clearly demonstrated that A2AR were functionally competent in target cells. These findings (summarized in Figure 4) demonstrated

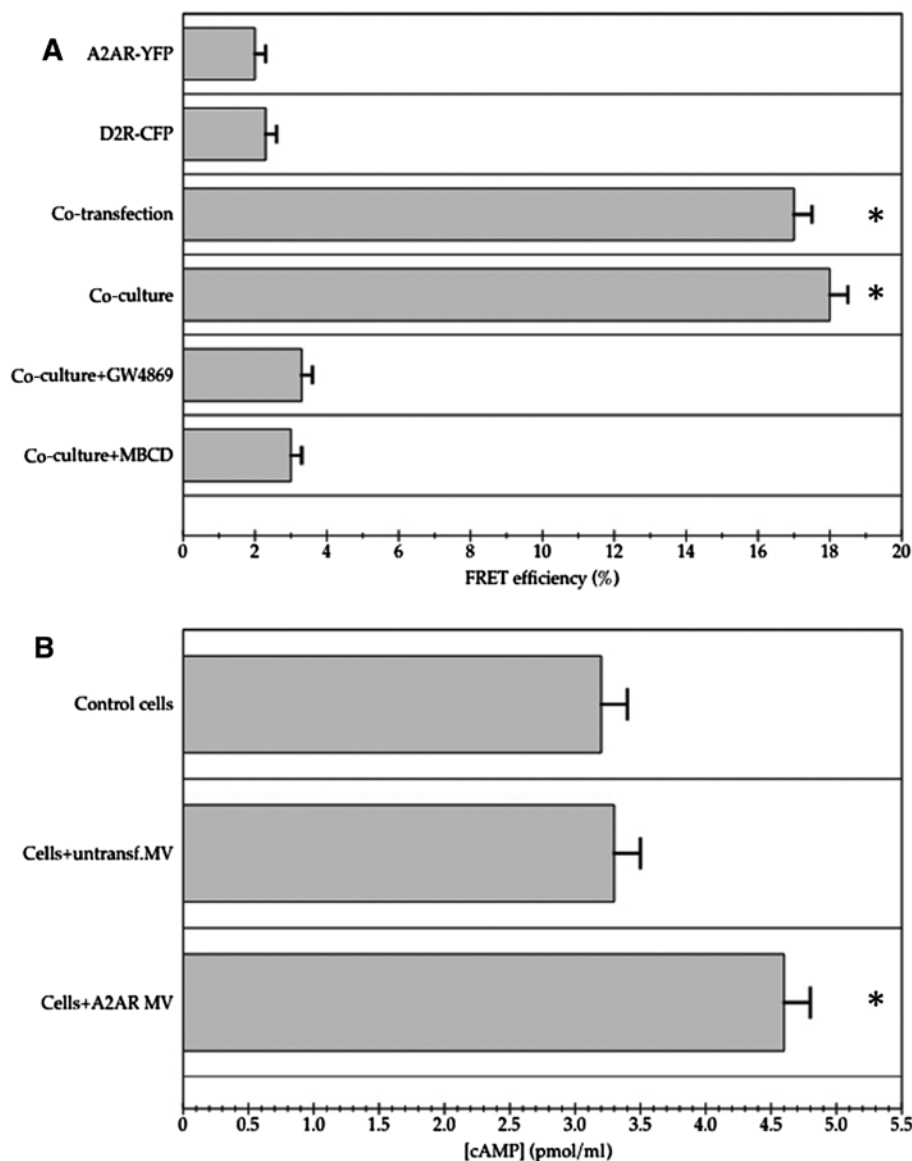


Figure 4: Analysis of GPCR exchange by means of MV, as reported by Guescini et al. (2012).

Two populations of cells were considered: COS-7 cells transfected with A2AR-YFP and HEK293T cells transfected with D2R-CFP. (A) After 24-h co-culture the FRET analysis showed positive cells for either A2AR-YFP and D2R-CFP. Treatment with two inhibitors of MV release (2.5 mM methyl- β -cyclodextrin or 10 μ M GW4869) abolished the effect. This result indicated that receptor transfer by MV occurred between the two cell populations. (B) When cAMP accumulation was evaluated after treatment with CGS (200 nM for 2 h) a significant increase was observed in cells exposed to MV carrying A2AR, indicating that the transferred receptors were also functionally competent in target cells. Each bar is the mean \pm SEM of three independent experiments. * $p < 0.05$.

that A2AR were not only safely transferred via MV from source to target cells, but in the target cells they were also capable of recognizing and decoding their signal.

Thus, the available experimental evidence suggests that this mode of intercellular communication can lead to the transient acquisition by the target cell of a new phenotype, enabling it to recognize/decode transmitters and/or modulators for which the cell does not express the pertinent cognate receptors. MV release in the roamer type of VT can therefore represent a novel mechanism for the modulation of neuron-neuron and glia-neuronal connectivity.

Concluding remarks

Connectomics, i.e. the comprehensive study of the structural connections in the brain, is a nascent, data-driven field with parallels to the far more developed biological discipline of genomics (Lichtman and Sanes, 2008). The rapid increase of studies addressing connectomics, however, clearly indicates that the anatomical mapping of the relationship among CNS components can represent a significant advance to reach a deeper level of understanding of CNS functions. In fact, the brain is believed to accomplish its activity mainly through the integrative actions of networks in which functions emerge from collections of elementary units (nodes) that are linked by connections and bound together dynamically (Bullmore and Sporns, 2012). Thus, drawing the connectome is far more than collecting a large dataset. It strongly implies the adoption of network models for brain function, including but not limited to the quantitative methods offered in abundance by network science (Sporns, 2013).

This research effort has been so far focused mainly on neuronal connectivity at a macroscale level, exploiting the possibilities offered by magnetic resonance imaging to evaluate the inter-regional structural and functional connectivity patterns (Mori and van Zijl, 2002; Hagmann et al., 2007; Gong et al., 2009). The obtained data have demonstrated a number of nontrivial topological features in adult human neuronal networks, including their efficient small-world architecture, prominent modular structure and highly connected and centralized network hubs (He and Evans, 2010; Stam, 2010; Bullmore and Bassett, 2011; Van den Heuvel and Sporns, 2013). Furthermore, connectomics-based studies concerning typical and atypical development of human brain neuronal networks from birth to early adulthood become increasingly available (see Cao et al., 2016, for a review).

Experimental and theoretical limitations of the present approach, however, exist and some of them should be carefully considered. The major limitation of many connectomics research programs rely to the assumption that all functionality of neuronal circuits and systems can be derived once the complete pattern of synaptic connections has been recorded (see Sporns, 2013, for a critical analysis). Accordingly, interneural connections at macro- and mesoscale have been the focus of most investigations. Brain integrative actions, however, depend mainly, but certainly not only, on the wiring diagram of neurons, since additional processes and networks exist modulating neuronal activity and brain functions (Brezina, 2010). In this respect, two aspects can be highlighted.

The first concerns the scales at which the mapping of the inter-node communications should be performed (Sporns et al., 2005). In particular, the term ‘nano-connectomics’ has been used here to emphasize a nanometer spatial resolution that allows us to capture protein-protein allosteric interactions and hence the integrative actions of molecular networks. Such interactions can be of the greatest importance at the cell plasma level where direct (structural) RRI could play a role in resetting the synaptic efficacy and in memory processes (Agnati et al., 1982; Guidolin et al., 2007).

The second refers to the increasing evidence indicating that synaptic transmission is significantly complemented by other cell types forming the CCN (Agnati et al., 2014a,b). As illustrated above, elements of CCN communicate via two modes of connection, WT and VT, which are not mutually exclusive. From the connectomics point of view, it is also of interest that not only most of the WT communication channels (WT-connectomics), but also many VT ECS pathways (VT-connectomics) can be identified, mapped, characterized in terms of their neuroanatomical and biophysical features (Syková and Nicholson, 2008) and included in formal network models (Guidolin et al., 2007). It has to be pointed out that also VT connections could be highly regulated, for instance by controlling the perviousness of ECS pathways for chemical signals diffusion through a modulation of the extracellular matrix composition by neurons and glial cells (Bernardinelli et al., 2014; Marcoli et al., 2015).

Thus, a research effort in these directions could complement imaging connectomics and provide a more complete drawing of the connectome and of its plasticity in different functional conditions. These investigations could also make available a deeper insight into the relationship between the brain structure and function, since relevant implications for neurophysiology and neuropathology can be derived from this enlarged view on CNS organization and intercellular communication modes.

From the physiological point of view, if fully demonstrated to occur in the brain, recently discovered forms of WT (such as TNT) and VT (such as the roamer type of VT) could represent a new aspect of the extraordinary plasticity of the CNS. In this context, it is interesting to cite Smalheiser's proposal that exosomal transfer of proteins and RNA, especially from the postsynaptic dendrite to the presynaptic terminal, can play a role in synaptic plasticity (Smalheiser, 2007). In addition, investigations on the roamer type of VT has allowed us to introduce the new concept of 'transient changes of cell phenotypes', describing the transient acquisition of new signal release capabilities and/or new recognition/decoding apparatuses (e.g. receptors) by some cells of a CCN (Agnati et al., 2014a,b). The phenomenon of transient changes of cell phenotypes opens up the possibility of having a multiform use of the same CCN following some specific changes in some of its cell signalosomes. Exosomal secretion could also have pathological roles in the CNS, especially for conformational protein diseases, since a link has been suggested between these vesicles and prion disease pathogenesis (Vella et al., 2008). Furthermore, it has been shown that a minute fraction of amyloid- β peptides can be secreted from the cells in association with exosomes (Bellingham et al., 2012). Consistent with this finding, the presence of exosomal proteins has been observed in plaques from the brains of patients with Alzheimer's disease (AD) (Rajendran et al., 2006), supporting a potential role for exosomes in the pathogenesis of AD.

Acknowledgments: This work was supported by Grant 60A06-0515/15 from the University of Padova to DG.

Appendix

Just for illustrative purposes, an abstract model of a neuron potentially implementing both WT and VT modes of intercellular communication is briefly described here. It is based on the so-called neurotransmitter field theory, originally proposed by Greer (2007).

According to this approach, the physical quantity that considered a vehicle of information is the concentration of transmitters in the ECS and the neuron is seen as a processing element transforming an input (recognized) transmitter distribution into an output (released) transmitter distribution. In order to visualize (see Figure 5) how this computation can be performed on a neurotransmitter cloud, let us proceed through two steps:

1. We can imagine the dendrites of the neuron as a tree with its branches inside the cloud. The surface of the

tree is 'painted' with a shade of gray corresponding to its sensitivity to the neurotransmitter. When multiplied by the actual concentration of the neurotransmitter in the ECS and integrated over the dendritic tree surface, we shall obtain a first-order approximation of the neuron's response. More formally, if $h(x, y, z)$ ($x, y, z \in H = \text{ECS}$) represents the neurotransmitter cloud, $\mu(x, y, z)$ ($x, y, z \in \text{dendritic surface}$) is the sensitivity to the transmitter (e.g. the distribution of specific receptors) and σ is the usual sigmoidal activation function (Rumelhart et al., 1986), the neuron's response (α) will be

$$\alpha = \sigma \left(\int_H h(x, y, z) d\mu(x, y, z) \right)$$

This relationship can be further refined by introducing a 'dendritic-membrane transfer function' ($\chi_d(h)$)

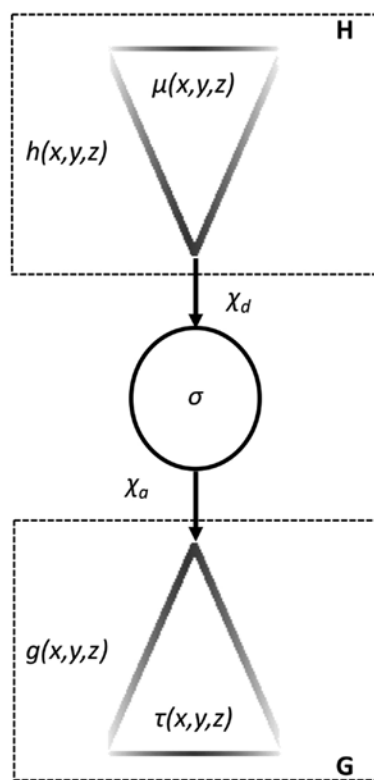


Figure 5: Schematic view of the abstract model of neuron.

The extracellular space (H) surrounding the dendritic tree hosts a cloud of neurotransmitter $[h(x, y, z)]$. The surface of the dendritic tree is represented with a gradient of gray levels to indicate its variable sensitivity $[\mu(x, y, z)]$ to the transmitter. χ_d, χ_a are the transfer functions (see the text) and σ is the activation function. The axonal tree is also represented with shades of gray to indicate the non-homogeneous spatial distribution of its release [described by the function $\tau(x, y, z)$]. It generates in the extracellular space (G) a cloud $g(x, y, z)$ of the released neurotransmitter.

accounting for the inherent nonlinear relationship between neurotransmitter concentration in the ECS and the gating of ion channels on the dendritic surface. Thus, we obtain

$$\alpha = \sigma \left(\int_H \chi_d(h(x, y, z)) d\mu(x, y, z) \right) \quad (1)$$

2. The neuron also has an axonal tree which releases the neurotransmitter into the extracellular space. Let $\tau(x, y, z)$ be the function that quantitatively describes the output of the neuron in terms of the spatial distribution of the chemical transmitter it generates. As a first-approximation, the product of the neuron's response α and the output function τ will provide the output transmitter cloud $g(x, y, z)$. However, to also take into account the intrinsic nonlinear response corresponding to the release of neurotransmitter by the axon terminals as a function of the neuron firing rate, an 'axonal-membrane transfer function' ($\chi_a(\alpha)$) will also be introduced. Thus, we can finally write

$$g(x, y, z) = \chi_a \left(\sigma \left(\int_H \chi_d(h(\xi, \eta, \zeta)) d\mu(\xi, \eta, \zeta) \right) \cdot \tau(x, y, z) \right) \quad (2)$$

where different symbols for the spatial coordinates were used in order to differentiate the input manifold from the output one.

It was demonstrated (see Greer, 2007) that, when limited to the synaptic transmission (i.e. when the functions μ and τ are discrete), this model for neurons is computationally equivalent to the discrete classical models (Vogels et al., 2005) based on neuronal networks and synaptic weights.

This way to look at the neurotransmission, however, could quite easily accommodate features that can hardly be described in the conventional neuronal network-based models. In particular, the following extensions can likely be easily implemented:

- Different types of cells: the input-output map described by (2) can be applied with no formal changes to any other cell. Each cell type, of course, will have its own transfer functions, input and output surfaces.
- Different types of WT/VT: each of them can be described following the same strategy outlined before, i.e. by an input cloud $h(x, y, z)$, a sensitivity $\mu(x, y, z)$ and a release function $\tau(x, y, z)$.
- Changes in the structure of the ECS: the spatial variables (x, y, z) used in (1) and (2) refer to the ECS. Thus, changes in ECS, such as changes in the geometry of the ECS pathways or in the efficient diffusion coefficient (Syková and Nicholson, 2008), will lead to changes in the neurotransmitter clouds and in the system dynamics.
- Changes in cell phenotype: the acquisition of new recognition/decoding or release capabilities (occurring, for instance, as a consequence of the roamer type of VT) can also be easily implemented in the model. In fact, from a formal point of view, it corresponds to appropriate changes of the sensitivity $\mu(x, y, z)$ and/or release $\tau(x, y, z)$ functions.

References

- Adams, V.L., Goodman, R.L., Salm, A.K., Coolen, L.M., Karsch, F.J., and Lehman, M.N. (2006). Morphological plasticity in the neural circuitry responsible for seasonal breeding in the ewe. *Endocrinology* 147, 4843–4851.
- Agnati, L.F. and Fuxe, K. (1984). New concepts on the structure of the neuronal networks: the miniaturization and hierarchical organization of the central nervous system. (Hypothesis). *Biosci. Rep.* 4, 93–98.
- Agnati, L.F. and Fuxe, K. (2000). Volume transmission as a key feature of information handling in the central nervous system: possible new interpretative value of the Turing's B-type machine. *Progr. Brain Res.* 125, 3–19.
- Agnati, L.F., Fuxe, K., Zoli, M., Rondanini, C., and Ogren, S.O. (1982). New vistas on synaptic plasticity: the receptor mosaic hypothesis on the engram. *Med. Biol.* 60, 183–190.
- Agnati, L.F., Fuxe, K., Zoli, M., Ozini, I., Toffano, G., and Ferraguti, F. (1986). A correlation analysis of the regional distribution of central enkephalin and beta endorphin immunoreactive terminals and of opiate receptors in adult and old male rats. Evidence for the existence of two main types of communication in the central nervous system: the volume transmission and the wiring transmission. *Acta Physiol. Scand.* 128, 201–207.
- Agnati, L.F., Cortelli, P., Biagini, G., Bjelke, B., and Fuxe, K. (1994). Different classes of volume transmission signals exist in the central nervous system and are affected by metabolic signals, temperature gradients and pressure waves. *Neuroreport* 6, 9–12.
- Agnati, L.F., Fuxe, K., Nicholson, C., and Sykova, E., eds. (2000). Volume transmission revisited. *Progress in Brain Research* (Amsterdam: Elsevier).
- Agnati, L.F., Franzen, O., Ferré, S., Leo, G., Franco, R., and Fuxe, K. (2003). Possible role of intramembrane receptor-receptor interactions in memory and learning via formation of long-lived heteromeric complexes: focus on motor learning in the basal ganglia. *J. Neural Transm. Suppl.* 65, 1–28.
- Agnati, L.F., Genedani, S., Lenzi, P.L., Leo, G., Mora, F., Ferré, S., and Fuxe, K. (2005a). Energy gradients for the homeostatic control of brain ECF composition and for VT signal migration: introduction of the tide hypothesis. *J. Neural Transm.* 112, 45–63.
- Agnati, L.F., Tarakanov, A.O., Ferré, S., Fuxe, K., and Guidolin, D. (2005b). Receptor-receptor interactions, receptor mosaics, and basic principles of molecular network organization: possible implications for drug development. *J. Mol. Neurosci.* 26, 193–208.

- Agnati, L.F., Leo, G., Genedani, S., Andreoli, N., Marcellino, D., Woods, A., Piron, L., Guidolin, D., and Fuxe, K. (2008). Structural plasticity in G-protein coupled receptors as demonstrated by the allosteric actions of homocysteine and computer-assisted analysis of disordered domains. *Brain Res. Rev.* 58, 459–474.
- Agnati, L.F., Baluška, F., Barlow, P.W., and Guidolin, D. (2009). ‘Mosaic’, ‘self-similarity logic’, and ‘biological attraction’ principles: three explanatory instruments in biology. *Commun. Integr. Biol.* 2, 552–563.
- Agnati, L.F., Guidolin, D., Guescini, M., Genedani, S., and Fuxe, K. (2010a). Understanding wiring and volume transmission. *Brain Res. Rev.* 64, 137–159.
- Agnati, L.F., Guidolin, D., Baluska, F., Leo, G., Barlow, P.W., Carone, C., and Genedani, S. (2010b). A new hypothesis of pathogenesis based on the divorce between mitochondria and their host cells: possible relevances for the Alzheimer’s disease. *Curr. Alzheimer Res.* 7, 307–322.
- Agnati, L.F., Guidolin, D., Vilardaga, J.P., Ciruela, F., and Fuxe, K. (2010c). On the expanding terminology in the GPCR field: the meaning of receptor mosaics and receptor heteromers. *J. Recept. Sig. Transduct. Res.* 30, 287–303.
- Agnati, L.F., Guidolin, D., Leo, G., Guescini, M., Pizzi, M., Stocchi, V., Spano, P.F., Ghidoni, R., Ciruela, F., Genedani, S., et al. (2011). Possible new targets for GPCR modulation: allosteric interactions, plasma membrane domains, intercellular transfer and epigenetic mechanisms. *J. Recept. Signal Transduct. Res.* 31, 315–331.
- Agnati, L.F., Guidolin, D., Maura, G., Marcoli, M., Leo, G., Carone, C., De Caro, R., Genedani, S., Borroto-Escuela, D.O., and Fuxe, K. (2014a). Information handling by the brain: proposal of a new “paradigm” involving the roamer type of volume transmission and the tunneling nanotube type of wiring transmission. *J. Neural Transm.* 121, 1431–1449.
- Agnati, L.F., Genedani, S., Spano, P.F., Guidolin, D., and Fuxe, K. (2014b). Volume Transmission and the Russian-doll Organization of Brain Cell Networks: Aspects of their Integrative Actions. *Neuronal Networks in Brain Function, CNS Disorders and Therapeutics*. C.L. Faingold, H. Blumenfeld, eds. (Amsterdam: Elsevier), pp. 103–119.
- Agnati, L.F., Guidolin, D., Cervetto, C., Borroto-Escuela, D.O., and Fuxe, K. (2016). Role of iso-receptors in receptor-receptor interactions with a focus on dopamine iso-receptor complexes. *Rev. Neurosci.* 27, 1–25.
- Akgün, E., Javed, M.I., Lunzer, M.M., Smeester, B.A., Beitz, A.J., and Portoghese, P.S. (2013). Ligands that interact with putative MOR-mGluR5 heteromer in mice with inflammatory pain produce potent atinociception. *Proc. Natl. Acad. Sci. USA* 110, 11595–11599.
- Anderson, M.L. (2010). Neural reuse: a fundamental organizational principle of the brain. *Behav. Brain Sci.* 33, 245–313.
- Anderson, M.L., Richardson, M.J., and Chemero, A. (2012). Eroding the boundary of cognition: implications of embodiment (1). *Top. Cogn. Sci.* 4, 717–730.
- Araque, A., Parpura, V., Sanzgiri, R.P., and Haydon, P.G. (1999). Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* 22, 208–215.
- Arvanitaki, A. (1942). Effects evoked in an axon by the activity of a contiguous one. *J. Neurophysiol.* 5, 89–108.
- Bassett, D.S., Wymbs, N.F., Porter, M.A., Mucha, P.J., Carlson, J.M., and Grafton, S.T. (2011). Dynamic reconfiguration of human brain networks during learning. *Proc. Natl. Acad. Sci. USA* 108, 7641–7646.
- Bellingham, S.A., Guo, B.B., Coleman, B.M., and Hill, A.F. (2012). Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? *Front. Physiol.* 3, 124.
- Bennett, E.L., Diamond, M.C., Krech, D., and Rosenzweig, M.R. (1964). Chemical and anatomical plasticity of brain. *Science* 146, 610–619.
- Bernardinelli, Y., Muller, D., and Nikonenko, I. (2014). Astrocyte-synapse structural plasticity. *Neural Plast.* 2014, 232105.
- Bhalla, U.S. and Iyengar, R. (1999). Emergent properties of networks of biological signaling pathways. *Science* 283, 381–387.
- Bjelke, B., and Fuxe, K. (1993). Intraventricular beta-endorphin accumulates in DARPP-32 immunoreactive tanycytes. *Neuroreport* 5, 265–268.
- Bloom, F., and Segal, D. (1980). Endorphins in the Cerebrospinal Fluid. *Neurobiology of Cerebrospinal Fluid*. J.H. Wood, ed. (New York: Plenum Press).
- Borroto-Escuela, D.O., Van Creanenbroek, K., Romero-Fernandez, W., Guidolin, D., Woods, A.S., Rivera, A., Haegeman, G., Agnati, L.F., Tarakanov, A.O., and Fuxe, K. (2011). Dopamine D2 and D4 receptor heteromerization and its allosteric receptor-receptor interactions. *Biochem. Biophys. Res. Commun.* 404, 928–934.
- Brezina, V. (2010). Beyond the wiring diagram: signalling through complex neuromodulator networks. *Phil. Trans. R. Soc. B* 365, 2363–2374.
- Bullmore, E.T. and Bassett, D.S. (2011). Brain graphs: graphical models of the human brain connectome. *Annu. Rev. Clin. Psychol.* 7, 113–140.
- Bullmore, E. and Sporns, O. (2012). The economy of brain network organization. *Nat. Rev. Neurosci.* 13, 336–349.
- Bunin, M.A. and Wightman, R.M. (1998). Quantitative evaluation of 5-hydroxytryptamine (serotonin) neuronal release and uptake: an investigation of extrasynaptic transmission. *J. Neurosci.* 18, 4854–4860.
- Bunin, M.A. and Wightman, R.M. (1999). Paracrine neurotransmission in the CNS: involvement of 5-HT. *Trends Neurosci.* 22, 377–382.
- Burbach, J.P. (1982). Neuropeptides and cerebrospinal fluid. *Ann. Clin. Biochem.* 19, 269–277.
- Bushong, E.A., Martone, M.E., and Ellisman, M.H. (2004). Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. *Int. J. Dev. Neurosci.* 22, 73–86.
- Calzolari, A., Raggi, C., Deaglio, S., Sposi, N.M., Stafnes, M., Fecchi, K., Parolini, I., Malavasi, F., Peschle, C., Sargiacomo, M., et al. (2006). TFR2 localizes in lipid raft domains and is released in exosomes to activate signal transduction along the MAPK pathway. *J. Cell Sci.* 119, 4486–4498.
- Cao, M., Huang, H., Peng, Y., Dong, Q., and He, Y. (2016). Toward developmental connectomics of the human brain. *Front. Neuroanat.* 10, 25.
- Carmignoto, G. (2000). Reciprocal communication systems between astrocytes and neurones. *Progr. Neurobiol.* 62, 561–581.
- Changeux, J.P. and Christopoulos, A. (2016). Allosteric modulation as a unifying mechanism for receptor function and modulation. *Cell* 166, 1084–1102.
- Chen, K.C. and Nicholson, C. (2000). Changes in brain cell shape create residual extracellular space volume and explain tortuosity behaviour during osmotic challenge. *Proc. Natl. Acad. Sci. USA* 97, 8306–8311.

- Ciranna, L. (2006). Serotonin as a modulator of glutamate- and GABA-mediated neurotransmission: implications in physiological functions and in pathology. *Curr. Neuropharmacol.* 4, 101–114.
- Cutsuridis, V., Wennekers, T., Graham, B.P., Vida, I., and Taylor, J.G. (2009). Microcircuits: their structure, dynamics and role for brain function. *Neural Netw.* 22, 1037–1038.
- DeFelipe, J., Alonso-Nanclares, L., and Arellano, J.I. (2002). Microstructure of the neocortex: comparative aspects. *J. Neurocytol.* 31, 299–316.
- Descarries, L. and Mechawar, N. (2000). Ultrastructural evidence for diffuse transmission by monoamine and acetylcholine neurons of the central nervous system. *Prog. Brain Res.* 125, 27–47.
- Descarries, L., Watkins, K.C., and Lapierre, Y. (1977). Noradrenergic axon terminals in the cerebral cortex of rat. III. Topometric ultrastructural analysis. *Brain Res.* 133, 197–222.
- Descarries, L., Bérubé-Carrière, N., Riad, M., Bo, G.D., Mendez, J.A., and Trudeau, L.E. (2008). Glutamate in dopamine neurons: synaptic versus diffuse transmission. *Brain Res. Rev.* 58, 290–302.
- De Wied, D. and Jolles, J. (1982). Neuropeptides derived from pro-opiomelanocortin: behavioral, physiological, and neurochemical effects. *Physiol. Rev.* 62, 976–1059.
- Diamond, J.S. (2001). Neuronal glutamate transporters limit activation of NMDA receptors by neurotransmitter spillover on CA1 pyramidal cells. *J. Neurosci.* 21, 8328–8338.
- Doly, S., Madeira, A., Fischer, J., Brisorgueil, M.J., Daval, G., Bernard, R., Vergé, D., and Conrath, M. (2004). The 5-HT_{2A} receptor is widely distributed in the rat spinal cord and mainly localized at the plasma membrane of postsynaptic neurons. *J. Comp. Neurol.* 472, 496–511.
- Eguiluz, V.M., Chialvo, D.R., Cecchi, G.A., Baliki, M., and Apkarian, A.V. (2005). Scale-free brain functional networks. *Phys. Rev. Lett.* 94, 018102.
- Eid, L. and Parent, M. (2016). Chemical anatomy of pallidal afferents in primates. *Brain Struct. Funct.* 221, 4291–4317.
- Färber, K. and Kettenmann, H. (2005). Physiology of microglial cells. *Brain Res. Rev.* 48, 133–143.
- Fellin, T. and Carmignoto, G. (2004). Neurone-to-astrocyte signaling in the brain represents a distinct multifunctional unit. *J. Physiol.* 559, 3–15.
- Février, B. and Raposo, G. (2004). Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr. Opin. Cell Biol.* 16, 415–421.
- Flechsigs, P. (1901). Developmental (myelogenetic) localization of the cerebral cortex in the human subject. *Lancet* 158, 1027–1030.
- Floresco, S.B. (2007). Dopaminergic regulation of limbic-striatal interplay. *J. Psychiatry Neurosci.* 32, 400–411.
- Floresco, S.B., West, A.R., Ash, B., Moore, H., and Grace, A.A. (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat. Neurosci.* 6, 968–973.
- Friston, K.J. (2011). Functional and effective connectivity: a review. *Brain Connectivity* 1, 13–36.
- Fuxe, K. and Agnati, L.F., eds. (1991). Volume Transmission in the Brain, Novel Mechanisms for Neural Transmission, Vol. 1 (New York: Raven Press).
- Fuxe, K., Hökfelt, T., Eneroth, P., Gustafsson, J.A., and Skett, P. (1977). Prolactin-like immunoreactivity: localization in nerve terminals of rat hypothalamus. *Science* 196, 899–900.
- Fuxe, K., Agnati, L.F., Benfenati, F., Celani, M., Zini, I., Zoli, M., and Mutt, V. (1983). Evidence for the existence of receptor-receptor interactions in the central nervous system. Studies on the regulation of monoamine receptors by neuropeptides. *J. Neural Transm.* S18, 165–179.
- Fuxe, K., Jansson, A., Diaz-Cabiale, Z., Andersson, A., Tinner, B., Finnman, U.B., Misane, I., Razani, H., Wang, F.H., Agnati, L.F., et al. (1998). Galanin modulates 5-hydroxytryptamine functions. Focus on galanin and galanin fragment/5-hydroxytryptamine_{1A} receptor interactions in the brain. *Ann. N.Y. Acad. Sci.* 863, 274–290.
- Fuxe, K., Dahlström, A., Höistad, M., Marcellino, D., Jansson, A., Rivera, A., Diaz-Cabiale, Z., Jacobsen, K., Tinner-Staines, B., Hagman, B., et al. (2007). From the Golgi-Cajal mapping to the transmitter-based characterization of the neuronal networks leading to two modes of brain communication: wiring and volume transmission. *Brain Res. Rev.* 55, 17–54.
- Fuxe, K., Dahlström, A., Jonsson, G., Marcellino, D., Guescini, M., Dam, M., Manger, P., and Agnati, L.F. (2010). The discovery of central monoamine neurons gave volume transmission to the wired brain. *Prog. Neurobiol.* 90, 82–100.
- Fuxe, K., Borroto-Escuela, D.O., Tarakanov, A., Romero Fernandez, W., Manger, P., Rivera, A., van Craenenbroeck, K., Skierska, K., Diaz-Cabiale, Z., Filip, M., et al. (2013). Understanding the balance and integration of volume and synaptic transmission. Relevance for psychiatry. *Neurol. Psych. Brain Res.* 19, 141–158.
- Gainetdinov, R.R., Premont, R.T., Bohn, L.M., Lefkowitz, R.J., and Caron, M.G. (2004). Desensitization of G protein-coupled receptors and neural functions. *Annu. Rev. Neurosci.* 27, 107–144.
- Gally, J.A., Montague, P.R., Reeke, G.N., and Edelman, G.M. (1990). The NO hypothesis: possible effects of a short-lived, rapidly diffusible signal in the development and function of the nervous system. *Proc. Natl. Acad. Sci. USA* 87, 3547–3551.
- Garthwaite, J. (2016). From synaptically localized to volume transmission by nitric oxide. *J. Physiol.* 594, 9–18.
- Geffen, L.B., Jessell, T.M., Cuellar, A.C., and Iversen, L.L. (1976). Release of dopamine from dendrites in rat substantia nigra. *Nature* 260, 258–260.
- Gerdes, H.H. and Carvalho, R.N. (2008). Intercellular transfer mediated by tunneling nanotubes. *Curr. Opin. Cell Biol.* 20, 470–475.
- Glasser, M.F., Coalson, T.S., Robinson, E.C., Hacker, C.D., Harwell, J., Yacoub, E., Uğurbil, K., Andersson, J., Beckmann, C.F., Jenkinson, M., et al. (2016). A multi-modal parcellation of human cerebral cortex. *Nature* 536, 171–178.
- Glaume, C. (2010). Astroglial wiring is adding complexity to neurological networking. *Front. Neuroenerg.* 2, 129.
- Golding, N.L., Staff, N.P., and Spruston, N. (2002). Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* 418, 326–331.
- Golgi, C. (1914). La moderna evoluzione delle dottrine e delle conoscenze sulla vita, XLVII (1). Rendiconti Regio Istituto Lombardo, Milano, Italy.
- Gong, G., He, Y., Concha, L., Lebel, C., Gross, D.W., Evans, A.C., and Beaulieu, C., et al. (2009). Mapping anatomical connectivity patterns of human cerebral cortex using in vivo diffusion tensor imaging tractography. *Cereb. Cortex* 19, 524–536.
- Goto, Y., Otani, S., and Grace, A.A. (2007). The yin and yang of dopamine release: a new perspective. *Neuropharmacology* 53, 583–587.
- Greer, D.S. (2007). Neurotransmitter fields. ICANN'07 – Proceedings of the 17th International Conference on Artificial Neu-

- ral Networks. J. Marques de Sá, L.A. Alexandre, W. Duch, D. Mandic, eds. (Berlin-Heidelberg: Springer-Verlag), pp. 19–28.
- Grillner, S. and Graybiel, A.M., eds. (2004). Report of the 93rd Dahlem Workshop on ‘Microcircuits: The Interface between Neurons and Global Brain Function’ (Berlin: Germany).
- Griszi, F. and Chiriva-Internati, M. (2005). The complexity of anatomical systems. *Theor. Biol. Med. Model* 2, 26.
- Guescini, M., Leo, G., Genedani, S., Carone, C., Pederzoli, F., Ciruela, F., Guidolin, D., Stocchi, V., Mantuano, M., Borroto-Escuela, D.O., et al. (2012). Microvesicle and tunneling nanotube mediated intercellular transfer of g-protein coupled receptors in cell cultures. *Exp. Cell Res.* 318, 603–613.
- Guidolin, D., Fuxe, K., Neri, G., Nussdorfer, G.G., and Agnati, L.F. (2007). On the role of receptor-receptor interactions and volume transmission in learning and memory. *Brain Res. Rev.* 55, 119–133.
- Guidolin, D., Albertin, G., Guescini, M., Fuxe, K., and Agnati, L.F. (2011). Central nervous system and computation. *Q. Rev. Biol.* 86, 265–285.
- Guidolin, D., Agnati, L.F., Marcoli, M., Borroto-Escuela, D.O., and Fuxe, K. (2015). G protein-coupled receptor type A heteromers as an emerging therapeutic target. *Expert Opin. Ther. Targets* 19, 265–283.
- Guidolin, D., Tortorella, C., De Caro, R., and Agnati, L.F. (2016). Does a self-similarity logic shape the organization of the nervous system? The Fractal Geometry of the Brain (Series in Computational Neuroscience). A. Di Ieva, ed. (New York: Springer). doi: 0.1007/978-1-4939-3995-4_9.
- Guillemin, R. (1978). Peptides in the brain: the new endocrinology of the neuron. *Science* 202, 390–402.
- Hagmann, P. (2005). From diffusion MRI to brain connectomics. PhD Thesis, Ecole Polytechnique Fédérale de Lausanne, Lausanne.
- Hagmann, P., Kurant, M., Gigandet, X., Thiran, P., Wedeen, V.J., Meuli, R., and Thiran, J.P. (2007). Mapping human whole-brain structural networks with diffusion MRI. *PLoS One* 2, e597.
- He, Y., and Evans, A. (2010). Graph theoretical modeling of brain connectivity. *Curr. Opin. Neurol.* 23, 341–350.
- Herrick-Davis, K., Grinde, E., Cowan, A., and Mazurkiewicz, J.E. (2013). Fluorescence correlation spectroscopy analysis of serotonin, adrenergic, muscarinic, and dopamine receptor dimerization: the oligomer number puzzle. *Mol. Pharmacol.* 84, 630–642.
- Hilgetag, C.C., Burns, G.A.P.C., O’Neill, M.A., Scannell, J.W., and Young, M.P. (2000). Anatomical connectivity defines the organization of clusters of cortical areas in the macaque monkey and the cat. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 355, 91–110.
- Hirasawa, H., Contini, M., and Raviola, E. (2015). Extrasynaptic release of GABA and dopamine by retinal dopaminergic neurons. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 370, 20140186.
- Hirase, H., Qian, L., Barth, P., and Buzsáki, G. (2004). Calcium dynamics of cortical astrocytic networks *in vivo*. *PLoS Biol.* 2, E96.
- Hokfelt, T., Kellerth, J.O., Nilsson, G., and Pernow, B. (1975). Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons. *Brain Res.* 100, 235–252.
- Holtmaat, A. and Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Rev. Neurosci.* 10, 647–658.
- Hopcroft, J.E. and Ullman, J.D. (1979). Introduction to Automata Theory, Languages and Computation (Boston: Addison-Wesley).
- Hrabetová, S., Hrabec, J., and Nicholson, C. (2003). Dead-space microdomains hinder extracellular diffusion in rat neocortex during ischemia. *J. Neurosci.* 23, 8351–8359.
- Jacob, F. (1970). La logique du vivant. Une histoire de l’hérédité. (Paris: Gallimard).
- Jansson, A. (2000). Long distance signalling in volume transmission. Focus on clearance mechanisms. *Prog. Brain Res.* 125, 399–413.
- Jansson, A., Maze, I.T., Andbjør, B., Rosen, L., Guidolin, D., Zoli, M., Syková, E., Agnati, L.F., and Fuxe, K. (1999). Effects of nitric oxide inhibition on the spread of biotinylated dextran and on extracellular space parameters in the neostriatum of the male rat. *Neuroscience* 91, 69–80.
- Jansson, A., Descarries, L., Cornea-Hebert, V., Riad, M., Verge, D., Bancila, M., Agnati, L.F., and Fuxe, K. (2002). Transmitter receptor mismatches in central dopamine serotonin and neuropeptide systems. The Neuronal Environment, Brain Homeostasis in Health and Disease. W. Walz and N.J. Totowa, eds. (New York: Humana Press), pp. 83–107.
- Jennings, K.A. (2013). A comparison of the subsecond dynamics of neurotransmission of dopamine and serotonin. *ACS Chem. Neurosci.* 4, 704–714.
- Kenakin, T., Agnati, L.F., Caron, M., Fredholm, B., Guidolin, D., Kobilka, B., Lefkowitz, R.W., Lohse, M., Woods, A., and Fuxe, K. (2010). International workshop at the Nobel Forum, Karolinska Institutet on G protein-coupled receptors: finding the words to describe monomers, oligomers, and their molecular mechanisms and defining their meaning. Can a consensus be reached? *J. Recept. Signal Transduct. Res.* 30, 284–286.
- Kerchner, G.A. and Nicoll, R.A. (2008). Silent synapses and the emergence of a postsynaptic mechanism for LTP. *Nat. Rev. Neurosci.* 9, 813–825.
- Kim, J.W., Wieckowski, E., Taylor, D.D., Reichert, T.E., Watkins, S., and Whiteside, T.L. (2005). Fas ligand-positive membranous vesicles isolated from sera of patients with oral cancer induce apoptosis of activated T lymphocytes. *Clin. Cancer Res.* 11, 1010–1020.
- Kullmann, D.M., Erdemli, G., and Asztely, F. (1996). LTP of AMPA and NMDA receptor-mediated signals: evidence for presynaptic expression and extrasynaptic glutamate spill-over. *Neuron* 17, 461–474.
- Lakkaraju, A. and Rodriguez-Boulán, E. (2008). Itinerant exosomes: emerging roles in cell and tissue polarity. *Trends Cell Biol.* 18, 199–209.
- Lanciego, J.L. and Wouterlood, F.G. (2011). A half century of experimental neuroanatomical tracing. *J. Chem. Neuroanat.* 42, 157–183.
- Le Bihan, D. and Johansen-Berg, H. (2011). Diffusion MRI at 25: exploring brain tissue structure and function. *NeuroImage* 61, 324–341.
- Le Boudec, J.Y. and Thiran, P. (2001). Network Calculus: A Theory of Deterministic Queuing Systems for the Internet (Lecture Notes in Computer Science) (Berlin Heidelberg: Springer).
- Lee, Y., El Andaloussi, S., and Wood, M.J. (2012). Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. *Hum. Mol. Genet.* 21, R125–R134.

- Lendvai, B. and Vizi, E.S. (2008). Nonsynaptic chemical transmission through nicotinic acetylcholine receptors. *Physiol. Rev.* 88, 333–349.
- Lichtman, J.W. and Sanes, J.R. (2008). Ome sweet ome: what can the genome tell us about the connectome? *Curr. Opin. Neurobiol.* 18, 346–353.
- Ljungdahl, A., Hökfelt, T., and Nilsson, G. (1978). Distribution of substance P-like immunoreactivity in the central nervous system of the rat. I. Cell bodies and nerve terminals. *Neuroscience* 3, 861–943.
- Lorente de Nö, R. (1938). Architectonics and structure of the cerebral cortex. *Physiology of the Nervous System*. J.F. Fulton, ed. (New York: Oxford University Press), pp. 291–330.
- MacMillan, S.J., Mark, M.A., and Duggan, A.W. (1998). The release of β -endorphin and the neuropeptide-receptor mismatch in the brain. *Brain Res.* 794, 127–136.
- Marcoli, M., Agnati, L.F., Benedetti, F., Genedani, S., Guidolin, D., Ferraro, L., Maura, G., and Fuxe, K. (2015). On the role of the extracellular space on the holistic behavior of the brain. *Rev. Neurosci.* 26, 489–506.
- Marullo, S. and Bouvier, M. (2007). Resonance energy transfer approaches in molecular pharmacology and beyond. *Trends Pharmacol. Sci.* 28, 362–365.
- McEwen, B.S. (2010). Stress, sex, and neural adaptation to a changing environment: mechanisms of neural remodeling. *Ann. N.Y. Acad. Sci.* 1204, 38–59.
- Meunier, D., Lambiotte, R., and Bullmore, E.T. (2010). Modular and hierarchically modular organization of brain networks. *Front. Neurosci.* 4, 200.
- Mori, S. and van Zijl, P.C. (2002). Fibertracking: principles and strategies – a technical review. *NMR Biomed.* 15, 468–480.
- Mountcastle, V.B. (1998). *Perceptual Neuroscience: The Cerebral Cortex* (Boston: Harvard University Press).
- Mundorf, M.L., Joseph, J.D., Austin, C.M., Caron, M.G., and Wightman, R.M. (2001). Catecholamine release and uptake in the mouse prefrontal cortex. *J. Neurochem.* 79, 130–142.
- Nagya, J.I., Dudekb, E.F., and Rashb, J.E. (2004). Update on connexins and gap junctions in neurons and glia in the mammalian nervous system. *Brain Res. Rev.* 47, 191–215.
- Nelson, S.M., Cohen, A.L., Power, J.D., Wig, G.S., Miezín, F.M., Wheeler, M.E., Velanova, K., Donaldson, D.I., Phillips, J.S., Schlaggar, B.L., et al. (2010). A parcellation scheme for human left lateral parietal cortex. *Neuron* 67, 156–170.
- Nicholson, C. (1979). Brain cell microenvironment as a communication channel. *The Neurosciences: Fourth Study Program*. F.O. Schmitt, F.G. Worden, eds. (Cambridge, MA: MIT Press), pp. 457–476.
- Nicholson, C. (2001). Diffusion and related transport mechanisms in brain tissue. *Rep. Prog. Phys.* 64, 815–884.
- Nicholson, C. and Phillips, J.M. (1981). Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. *J. Physiol. (Lond.)* 321, 225–257.
- Onfelt, B., Nedvetzki, S., Benninger, R.K., Purbhoo, M.A., Sowinski, S., Hume A.N., Seabra, M.C., Neil, M.A., French, P.M., and Davis, D.M. (2006). Structurally distinct membrane nanotubes between human macrophages support long-distance vesicular traffic or surfing of bacteria. *J. Immunol.* 177, 8476–8483.
- Oviedo-Orta, E. and Evans, W.H. (2004). Gap junctions and connexin mediated communication in the immune system. *Biochim. Biophys. Acta* 1662, 102–112.
- Ovsepian, S.V., O’Leary, V.B., and Zaborszky, L. (2016). Cholinergic mechanisms in the cerebral cortex: beyond synaptic transmission. *Neuroscientist* 22, 238–251.
- Pasti, L., Volterra, A., Pozzan, T., and Carmignoto, G. (1997). Intracellular calcium oscillations in astrocytes: a highly plastic, bidirectional form of communication between neurons and astrocytes in situ. *J. Neurosci.* 17, 7817–7830.
- Patlak, C.S., Hospod, F.E., Trowbridge, S.D., and Newman, G.C. (1998). Diffusion of radiotracers in normal and ischemic brain slices. *J. Cereb. Blood Flow Metab.* 18, 776–802.
- Pereira, A. and Furlan, F.A. (2010). Astrocytes and human cognition: modeling information integration and modulation of neuronal activity. *Progr. Neurobiol.* 92, 405–420.
- Picard, N.A. and Zanardi, C.A. (2015). Brain motion and volume transmission: keeping the interstice flowing. *Med. Hypotheses* 85, 41–44.
- Plested, A.J.R. (2016). Structural mechanisms of activation and desensitization in neurotransmitter-gated ion channels. *Nat. Struct. Mol. Biol.* 23, 494–502.
- Quah, B.J., Barlow, V.P., McPhun, V., Matthaei, K.I., Hulett, M.D., and Parish, C.R. (2008). Bystander B cells rapidly acquire antigen receptors from activated B cells by membrane transfer. *Proc. Natl. Acad. Sci. USA* 105, 4259–4264.
- Rajendran, L., Honsho, M., Zahn, T.R., Keller, P., Geiger, K.D., Verkade, P., and Simons, K. (2006). Alzheimer’s disease beta-amyloid peptides are released in association with exosomes. *Proc. Natl. Acad. Sci. USA* 103, 11172–11177.
- Rakic, P. (2008). Confusing cortical columns. *Proc. Natl. Acad. Sci. USA* 105, 12099–12100.
- Rash, J.E., Dillman, R.K., Bilhartz, B.L., Duffy, H.S., Whalen, L.R., and Yasumura, T. (1996). Mixed synapses discovered and mapped throughout mammalian spinal cord. *Proc. Natl. Acad. Sci. USA* 93, 4235–4239.
- Reichenbach, A., Derouiche, A., and Kirchhoff, F. (2010). Morphology and dynamics of perisynaptic glia. *Brain Res. Rev.* 63, 11–25.
- Rice, M.E. and Cragg, S.J. (2008). Dopamine spillover after quantal release: rethinking dopamine transmission in the nigrostriatal pathway. *Brain Res. Rev.* 58, 303–313.
- Rice, M.E. and Patel, J.C. (2015). Somatodendritic dopamine release: recent mechanistic insights. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 370, 20140185.
- Ridet, I. and Privat, A. (2000). Volume transmission. *Trends Neurosci.* 23, 58–59.
- Rivera, A., Agnati, L.F., Horvath, T.L., Valderrama, J.J., de La Calle, A., and Fuxe, K. (2006). Uncoupling protein 2/3 immunoreactivity and the ascending dopaminergic and noradrenergic neuronal systems. Relevance for volume transmission. *Neuroscience* 137, 1447–1461.
- Robertson, J.M. (2002). The astrocentric hypothesis: proposed role of astrocytes in consciousness and memory formation. *J. Physiol. (Paris)* 96, 251–255.
- Rouach, N., Koulakoff, A., Abudara, V., Willecke, K., and Glaume, C. (2008). Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* 322, 1551–1555.
- Rózsa, M., Baka, J., Bordé, S., Rózsa, B., Katona, G., and Tamás, G. (2015). Unitary GABAergic volume transmission from individual interneurons to astrocytes in the cerebral cortex. *Brain Struct. Funct.* doi: 10.1007/s00429-015-1166-9.
- Rumelhart, D., Hinton, G., and McClelland, J. (1986). A general framework for parallel distributed processing. *Parallel*

- Distributed Processing, Explorations in the Microstructure of Cognition. Vol. 1: Foundations. D. Rumelhart, J. McClelland, PDP Research group, eds. (Cambridge, MA: The MIT Press).
- Rustum, A., Saffrich, R., Markovic, I., Walther, P., and Gerdes, H.-H. (2004). Nanotubular highways for intercellular organelle transport. *Science* 303, 1007–1010.
- Savtchenko, L.P. and Rusakov, D.A. (2007). The optimal height of the synaptic cleft. *Proc. Natl. Acad. Sci. USA* 104, 1823–1828.
- Schipke, C.G., Haas, B., and Kettenmann, H. (2008). Astrocytes discriminate and selectively respond to the activity of a subpopulation of neurons within the barrel cortex. *Cereb. Cortex* 18, 2450–2459.
- Schmahmann, J.D. and Pandya, D.N. (2007). Cerebral white matter – historical evolution of facts and notions concerning the organization of the fiber pathways of the brain. *J. Hist. Neurosci.* 16, 237–267.
- Schmitt, F.O. (1984). Molecular regulators of brain function. A new view. *Neuroscience* 13, 991–1001.
- Shepherd, G.M. (1979). *The Synaptic Organization of the Brain* (New York: Oxford University Press).
- Simons, M. and Raposo, G. (2009). Exosomes – vesicular carriers for intercellular communication. *Curr. Opin. Cell Biol.* 21, 575–581.
- Skieterska, K., Duchou, J., Lintermans, B., and Van Craenenbroeck, K. (2013). Detection of G protein-coupled receptor (GPCR) dimerization by coimmunoprecipitation. *Methods Cell Biol.* 117, 323–340.
- Smalheiser, N.R. (2007). Exosomal transfer of proteins and RNAs at synapses in the nervous system. *Biol. Direct.* 2, 35.
- Smith, J.S., and Rajagopal, S. (2016). The β -arrestin: multifunctional regulators of G protein-coupled receptors. *J. Biol. Chem.* 291, 8969–8977.
- Sowinski, S., Jolly, C., Berninghausen, O., Purbhoo, M.A., Chauveau, A., Köhler, K., Oddos, S., Eissmann, P., Brodsky, F.M., Hopkins, C., et al. (2008). Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission. *Nat. Cell Biol.* 10, 211–219.
- Sporns, O. (2012). *Discovering the Human Connectome* (Cambridge, MA: MIT Press).
- Sporns, O. (2013). The human connectome: origins and challenges. *NeuroImage* 80, 53–61.
- Sporns, O. and Betzel, R.F. (2016). Modular brain networks. *Annu. Rev. Psychol.* 67, 613–640.
- Sporns, O. and Zwi, J.D. (2004). The small world of the cerebral cortex. *Neuroinformatics* 2, 145–162.
- Sporns, O., Tononi, G., and Kötter, R. (2005). The human connectome: a structural description of the human brain. *PLoS Comput. Biol.* 1, 245–251.
- Stam, C.J. (2010). Characterization of anatomical and functional connectivity in the brain: a complex networks perspective. *Int. J. Psychophysiol.* 77, 186–194.
- Stam, C.J. and Reijneveld, J.C. (2007). Graph theoretical analysis of complex networks in the brain. *Nonlinear Biomed. Phys.* 1, 3.
- Steinert, J.R., Kopp-Scheinflug, C., Baker, C., Challiss, R.A.J., Mistry, R., Hausteint, M.D., Griffin, S.J., Tong, H., Graham, B.P., and Forsythe, I.D. (2008). Nitric oxide is a volume transmitter regulating postsynaptic excitability at a glutamatergic synapse. *Neuron* 60, 642–656.
- Steno, N. (1965). *Lecture on the Anatomy of the Brain* (Copenhagen: Nordisk Forlag Brusck).
- Stevens, B. (2008). Neuron-astrocyte signaling in the development and plasticity of neural circuits. *Neurosignals* 16, 278–288.
- Sun, M.K. and Alkon, D.L. (2002). Carbonic anhydrase gating of attention: memory therapy and enhancement. *Trends Pharmacol. Sci.* 23, 83–89.
- Syková, E. and Chvátal, A. (2000). Glial cells and volume transmission in the CNS. *Neurochem. Int.* 36, 397–409.
- Syková, E. and Nicholson, C. (2008). Diffusion in brain extracellular space. *Physiol. Rev.* 88, 1277–1340.
- Syková, E., Mazel, T., Vargová, L., Vorísek, I., and Prokopová-Kubinová, S. (2000). Extracellular space diffusion and pathological states. *Prog. Brain Res.* 125, 155–178.
- Szapiro, G. and Barbour, B. (2007). Multiple climbing fibers signal to molecular layer interneurons exclusively via glutamate spillover. *Nat. Neurosci.* 10, 735–742.
- Tang, A.-H., Chen, H., Li, T.P., Metzbow, S.R., MacGillavry, H.D., and Blanpied, T.A. (2016). A trans-synaptic nanocolumn aligns neurotransmitter release to receptors. *Nature* 536, 210–214.
- Theodosis, D.T., Poulain, D.A., and Oliet, S.H.R. (2008). Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. *Physiol. Rev.* 88, 983–1008.
- Ungerstedt, U., Butcher, L.L., Butcher, S.G., Andén, N.E., and Fuxe, K. (1969). Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat. *Brain Res.* 14, 461–471.
- Van den Heuvel, M.P. and Sporns, O. (2013). Network hubs in the human brain. *Trends Cogn. Sci.* 17, 683–696.
- Van Essen, D.C., Drury, H.A., Joshi, S., and Miller, M.I. (1998). Functional and structural mapping of human cerebral cortex: solutions are in the surfaces. *Proc. Natl. Acad. USA* 95, 788–795.
- Van Essen, D.C., Ugurbil, K., Auerbach, E., Barch, D., Behrens, T.E.J., Bucholz, R., Chang, A., Chen, L., Corbetta, M., Curtiss, S.W., et al. (2012). The Human Connectome Project: a data acquisition perspective. *NeuroImage* 62, 2222–2231.
- van Niel, G., Porto-Carreiro, I., Simoes, S., and Raposo, G. (2006). Exosomes: a common pathway for a specialized function. *J. Biochem.* 140, 13–21.
- Vella, L.J., Sharples, R.A., Nisbet, R.M., Cappai, R., and Hill, A.F. (2008). The role of exosomes in the processing of proteins associated with neurodegenerative diseases. *Eur. Biophys. J.* 37, 323–332.
- Vizi, E.S. (1980a). Modulation of cortical release of acetylcholine by noradrenaline released from nerves arising from the rat locus coeruleus. *Neuroscience* 5, 2139–2144.
- Vizi, E.S. (1980b). Non-synaptic modulation of transmitter release: pharmacological implication. *Trends Pharmacol. Sci.* 1, 172–175.
- Vizi, E.S. (1984). *Non-Synaptic Interactions between Neurons: Modulation of Neurochemical Transmission* (Chichester: John Wiley and Sons).
- Vizi, E.S. (2000). Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the CNS. *Pharmacol. Rev.* 52, 63–89.
- Vizi, E.S., Fekete, A., Karoly, R., and Mike, A. (2010). Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment. *Br. J. Pharmacol.* 160, 785–809.
- Vogels, T.P., Rajan, K., and Abbott, L.F. (2005). Neural network dynamics. *Annual Rev. Neurosci.* 28, 357–376.
- Volterra, A., and Meldolesi, J. (2005). Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci.* 6, 626–640.
- Watts, D., and Strogatz, S. (1998). Collective dynamics of small world networks. *Nature* 393, 440–442.

- Wig, G.S., Schlaggar, B.L., and Petersen, S.E. (2011). Concepts and principles in the analysis of brain networks. *Ann. N.Y. Acad. Sci.* 1224, 126–146.
- Wreggett, K.A., and Wells, J.W. (1995). Cooperativity manifest in the binding properties of purified cardiac muscarinic receptors. *J. Biol. Chem.* 270, 22488–22499.
- Yano, S., Brown, E.M., and Chattopadhyay, N. (2004). Calcium-sensing receptor in the brain. *Cell Calcium* 35, 257–264.
- Zalesky, A., Fornito, A., Harding, I.H., Cocchi, L., Yucel, M., et al. (2010). Whole-brain anatomical networks: does the choice of nodes matter? *NeuroImage* 50, 970–983.