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COMMENTARY

INTERCELLULAR COMMUNICATION IN THE BRAIN: WIRING VERSUS VOLUME TRANSMISSION*

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Abstract—During the past two decades several revisions of the concepts underlying interneuronal communication in the central nervous system have been advanced. We propose here to classify communicational phenomena between cells of the central neural tissue under two general frames: "wiring" and "volume" transmission. "Wiring" transmission is defined as intercellular communication occurring through a well-defined connecting structure. Thus, wiring transmission is characterized by the presence of physically identifiable communication channels within the neuronal and/or glial cell network. It includes synaptic transmission but also other types of intercellular communication through a connecting structure (e.g., gap junctions). "Volume" transmission is characterized by signal diffusion in a three-dimensional fashion within the brain extracellular fluid. Thus, multiple, structurally often not well characterized extracellular pathways connect intercommunicating cells. Volume transmission includes short- (but larger than synaptic cleft, i.e. about 20 nm) and long-distance diffusion of signals through the extracellular and cerebrospinal fluid. It must be underlined that the definitions of wiring and volume transmission focus on the modality of transmission and are neutral with respect to the source and target of the transmission, as well as type of informational substance transmitted. Therefore, any cell present in the neural tissue (neurons, astroglia, microglia, ependyma, tanycytes, etc.) can be a source or a target of wiring and volume transmission.

In this paper we discuss the basic definitions and some distinctive characteristics of the two types of transmission. In addition, we review the evidence for different types of intercellular communication besides synaptic transmission in the central nervous system during phylogeny, and in vertebrates in physiological and pathological conditions.

Key words: non-synaptic, extracellular fluid, neuropeptides, diffusion, central nervous system.

CONTENTS

Continued overleaf

*In memory of Professor Francesco Infantellina, Neurophysiologist in Bologna and Palermo.

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Abbreviations: CGRP, calcitonin gene-related peptide; CSF, cerebrospinal fluid; DA, dopamine; ECF, extracellular fluid; 5-HT, serotonin; LDCV, large dense-cored vesicle; NA, noradrenaline; PGD_2, prostaglandin D_2; SV, small vesicle; VT, volume transmission; WT, wiring transmission.

1. INTRODUCTION

The tension between the view that the brain works as a global system or as a collection of relatively independent elements has existed since the origin of modern neuroscience, when Golgi and Cajal had opposing views on the organization of neuronal networks (see e.g. Refs 40 and 71; see also the books by Shepherd[137] and Jacobson[91]).

Golgi supported the reticular theory of neuronal continuity, originally proposed by Kölliker[94] and von Gerlach,[67] while Cajal favoured the neuron theory of nerve cell contiguity, originally proposed by Waldeyer.[151] Golgi was very cautious about the controversy, as is evident from Golgi's letter reported by Luciani.[100] In fact, he pointed out that the techniques available at that time did not allow researchers to prove or to disprove any of the two theories, but from a functional standpoint he was inclined to surmise that, in executing several tasks, the CNS works as a global system (see also Ref. 72). This global mode of operation of the CNS is not always easily interpreted on the basis of a wired organization of the brain (see e.g. Ref. 20).

While from an anatomical standpoint the controversy has definitely been settled in favour of the neuron theory by electron microscopic studies, today Golgi's tenet has a solid ground from a functional standpoint. This is not so much in view of the discovery of membrane junctions that constitute a quasi-continuity between neurons,[47] but rather by the implications of volume transmission (VT) as a means of allowing a global mode of operation for the CNS. In fact, VT allows the setting of "entire provinces of the CNS" (intere provincie del Sistema Nervoso[72]) to the appropriate functional state so that they work as a unit, as Golgi pointed out at the beginning of this century.

2. DEVELOPMENT OF THE CONCEPT OF NEURONAL COMMUNICATION BESIDES SYNAPTIC TRANSMISSION

During the past two decades, several authors have proposed a revision of the concepts underlying interneuronal communication in the CNS.[4,8,9,19,23,43,48,76,84,112,131,148,149] All these proposals are quite different from each other, but they have as a common point of view the tenet that there exists another mode for interneuronal communication in the CNS besides synaptic transmission. The Guillemin,[76] Schmitt[131] and Agnati–Fuxe[4,8] proposals imply a wider type of communication than the ones suggested by Nicholson[112] and Vizi.[148] In fact, the original work of Nicholson dealt only with ion fluxes in the extracellular fluid of the brain and the possible functional consequences of these ionic currents (see e.g. Refs 112 and 113). Vizi's work, elegantly summarized in a book,[148] provided evidence in favour of the cross-talk between neighbouring axon terminals.

The name chosen to define the alternative way of intercellular communication already highlights an important difference among the proposals. Vizi,[148] Bach y Rita,[19] Descarries *et al.*[50] and Herkenham[84] have named the theory "non-synaptic transmission", while Schmitt[131] suggested the term "parasynaptic". Both these names (especially the former) stress the intention of the authors to deal exclusively with interneuronal communication, since "... it is possible to regard the synapse as the essential and defining property of the neuron".[138] Instead, we have suggested that neuron–glia and glia–glia communication should also be reconsidered in a new broader frame of studying intercellular communication in the CNS.[63] Furthermore, we think that a positive term (VT) should be employed rather than a negative term (non-synaptic transmission) as the latter term, on the one hand, does not give any hint as to what the alternative modes of communication might be, and on the other implies that any difference between other modes of transmission is negligible when compared to the difference between all of them and synaptic transmission.

On the basis of this and of some other considerations discussed in the Appendix, 10 years ago we suggested the terms wiring transmission and volume transmission as main conceptual categories for a systematization of intercellular communication in the CNS.[3,4,8,62]

3. DEFINITION OF WIRING AND VOLUME TRANSMISSION

Wiring transmission (WT) can be defined as intercellular communication occurring through a well-defined connecting structure. Thus, WT is characterized by the presence of physically identifiable communication channels within the neuronal and/or glial cell network.[8,63] Based on this structural definition, several functional features of WT can be derived. A structural link between two communicat-

ing cells determines a private, relatively permanent and safe 1:1 link.

It must be underlined that the features of WT mentioned above do not refer to the source cell–target cell link but rather to the link between the subcellular structure which releases the signal and that which recognizes the signal, i.e. in the case of synaptic transmission, the presynaptic terminal–postsynaptic density link. In this context, the 1:1 link seems appropriate for WT intercellular connecting structures. Instead, the 1:1 link requirement is not present in VT, where signals deriving from a single source structure can reach several target structures (see below).

Some functional features of WT, in particular the same 1:1 communication, can, however, be present in the absence of a well-defined physical structure connecting the source with the target cell. For instance, the work of Stjärne (see e.g. Ref. 140) shows that in certain conditions autonomic noradrenergic transmission is "functionally" a synapse without having the morphological features of a classical synapse. In fact, at low frequency of discharge ($\leqslant$ four pulses at 20 Hz) the released noradrenaline (NA), due to the highly active reuptake, can diffuse only to the $\alpha 2$ adrenoceptors of the muscle cell directly facing the varicosity (the so-called innervated "key" cell). At higher discharge frequency, NA can diffuse through extracellular fluid (ECF) to $\alpha 1$ adrenoceptors in the surrounds. Similar situations are likely to be present in the brain when varicosities devoid of postsynaptic densities are present. By extension of the WT concept as defined above, we propose to consider instances of functionally definite 1:1 intercellular communication as WT, even when the classical synapse is not demonstrated.

The definitions given above lead to the inclusion in WT of: (i) the classical synapse proposed by the "neuron doctrine" of Cajal (i.e. the presynaptic knob/synaptic cleft/postsynaptic density[137]); (ii) membrane junctions (especially important are the gap junctions);[21,47,111,138,152,155] (iii) close membrane juxtapositions, such as ephapses.[138] We also propose to broaden the class of WT communication to include functionally (although not morphologically) definite peripheral and central synapses.

VT is characterized by signal diffusion in a three-dimensional fashion within the brain ECF. Thus, multiple, structurally often not well characterized ECF pathways connect intercommunicating cells. Functionally, VT consists of an open, relatively unsafe, 1:n ($n \gg 1$) transmission.

This definition does not deny that some kind of organization of the cerebral microenvironment occurs. Some characteristics of the cerebral microenvironment have been summarized by the concepts of

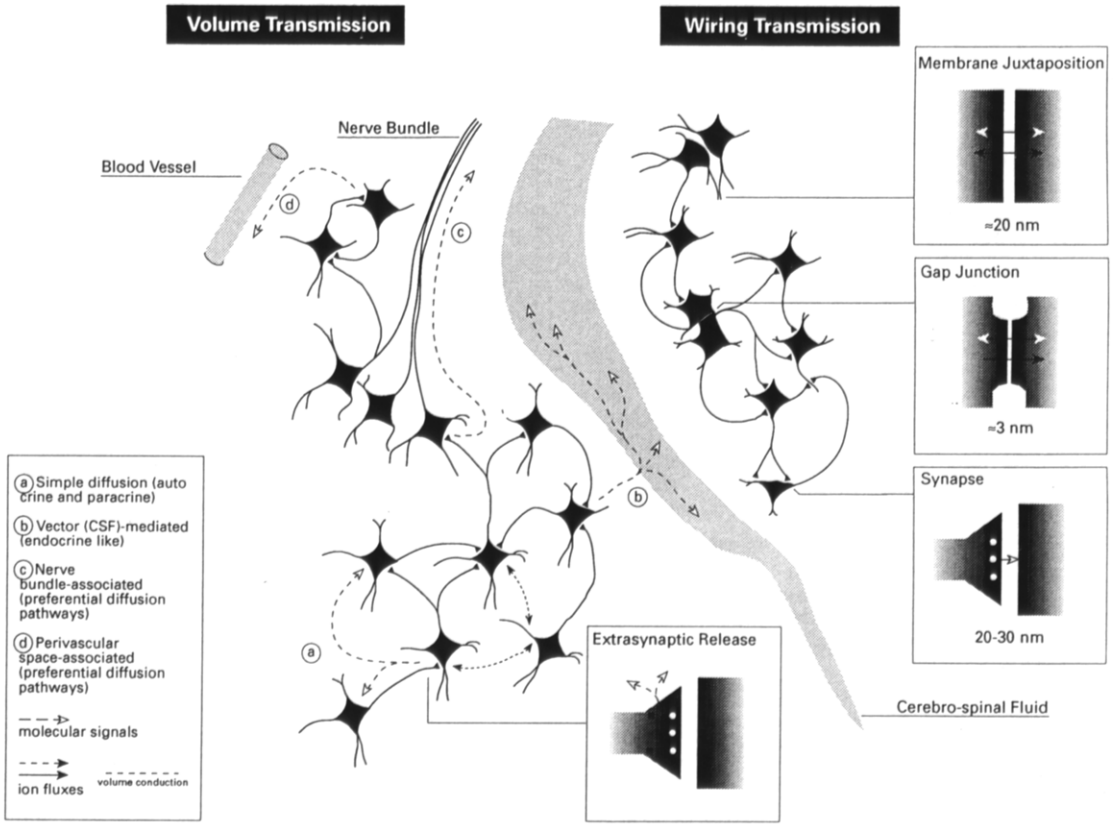

Fig. 1. Schematic representation of the principal types of intercellular communication in the CNS in the frame of wiring and volume transmission.

Table 1. Some differential features of wiring transmission and volume transmission

	Wiring transmission	Volume transmission
A. Coding		
1. Type of signals	chemical and electrical	chemical and electrical
2. Temporal coding for chemical signal	fast and slow components	mainly slow components
3. Concentration of chemical signal at receiver	usually high	usually low
4. Affinity of receiver for chemical signal	usually low	usually high
B. Structural features for chemical signals		
1. Site of chemical signal release/target link	1:1 link	1:n link ($n \gg 1$)
2. Space filling	high	very low
C. Functional features		
1. Speed	high	low
2. Safety	high	usually low
3. Energy cost	high	usually low

tortuosity and volume fraction[112,113,115] (see discussion below), which can vary in different regions and various physiopathological conditions. Diffusion in the ECF may thus occur via preferential pathways. Further examples of preferential pathways in the cerebral ECF have been characterized (see, for example, paravascular fluid circulation and nerve bundle-associated channels). A more detailed discussion of this subject is given below. Finally, in certain physiological conditions typical WT structures can also work via VT. For instance, a transmitter released at a synapse at high discharge frequency can overwhelm the reuptake and/or inactivation synaptic systems and diffuse in the neighbouring ECF.

The issue of safety of transmission in WT vs VT deserves further clarification. While changes in release or degradation rate of the signal modulate the efficacy of WT, they may make VT appear or disappear altogether. In fact, in VT a target cell may or may not be reached by the signal. In addition, establishment or loss of a synapse (or a gap junction, etc.) is a relatively long process, while the features of the ECF pathways can be easily modified by chemical changes in the extracellular milieu, as well as by changes in the geometry of cellular structures. In this sense, it seems appropriate to state that WT is safer and more permanent than VT.

The definitions given above lead to the inclusion in VT of short- (parasynaptic) and long-distance (paracrine) diffusion in the neural tissue of ions and chemicals. Moreover, we can recognize the endocrine-like and the nerve bundle-associated types of VT (Fig. 1). Endocrine-like VT is based on the use of cerebrospinal fluid (CSF) as a diffusion medium, allowing very far-reaching communication in the brain. The term is justified by the analogy with the peripheral endocrine system. In both cases, the signal, once released by the source cell, diffuses in the neighbouring ECF, enters a specialized fluid compartment (blood and the CSF, respectively) in which it is moved by convective forces, and finally diffuses into the ECF around the target cell. The nerve bundle-associated type of VT is a preferential pathway of signal convection in the CNS that occurs along the nerve bundles in small channels located outside the myelinated axons.[30,42,146]

It must be underlined that the definitions of WT and VT focus on the modality of transmission and are neutral with respect to the source and target of the transmission, as well as the type of informational substance transmitted. Therefore, any cell present in the neural tissue (neurons, astroglia, microglia, ependyma, tanycytes, etc.) can be a source or a target of VT and WT.

Some features of WT and VT are presented in Table 1. From these features, each transmission mode appears particularly suited for different tasks.

4. EVIDENCE FOR THE EXISTENCE OF VOLUME TRANSMISSION IN THE BRAIN

A substance must satisfy several criteria in order to be considered a neurotransmitter at a particular synaptic contact. Since several complex anatomical, neurophysiological and pharmacological techniques are necessary to prove these criteria, only very few transmitter candidates have been accepted as synaptic neurotransmitters.[35] Similarly, it may be useful to give a tentative list of criteria that should be fulfilled for a substance to be recognized as a VT signal. The substance: (i) is released by a cell (neuron, glial cell, etc.) of the nervous system in a regulated fashion; (ii) diffuses at relevant concentration in the cerebral ECF for a distance larger than the synaptic cleft (about 20 nm); (iii) is able to activate selective receptive molecules in a number of target cells; (iv) triggers a physiological response in the target cells.

In the following we will give some evidence for the existence of VT, along the lines indicated in the above-mentioned criteria. We will subsequently discuss some evidence for VT during phylogeny, and in vertebrates in physiological and pathological conditions.

4.1. *Release sites for volume transmission*

A strong piece of morphological evidence for VT-type chemical transmission is the demonstration of release sites for intercellular communication outside specialized junctions such as synapses. However, as

mentioned above, transmitters released from intrasynaptic stores may also diffuse in the cerebral ECF in certain physiopathological conditions. There is a wide body of literature on both large dense-cored (LDCV) and small (SV) vesicles concentrated outside classical synapses. In the last 20 years, the preferential location of LDCVs near, and their release from, non-synaptic membranes has been demonstrated in both invertebrates and vertebrates. Although LDCV fusion events are very rare in standard electron microscopic preparations of neural tissue, a large number of these events could be demonstrated thanks to techniques allowing immobilization of LDCVs during the fusion process.[37–39] Three types of release sites for LDCVs have been recognized:[69] synaptic, parasynaptic and non-synaptic sites. The three sites correspond to three domains in the nerve terminal. The synaptic domain of the terminal membrane is identified by the presence of synaptic thickenings. The parasynaptic domain corresponds to apparently undifferentiated areas of the terminal membrane lying adjacent to the postsynaptic cell. The non-synaptic domain corresponds to areas of the terminal membrane in contact with elements (e.g., glia) which have no conventional synaptic relationship with the terminal.[69] The proportion of these three types of sites is variable in different systems. It is interesting to note that in most cases extrasynaptic LDCV fusion events are much more frequent than synaptic events.

In the case of SVs, the standard view holds that a synaptic active zone is required for exocytosis.[46] However, several instances of varicosities containing monoaminergic or amino acidergic SVs and lacking synaptic specializations have been shown. In this case, it is presumable that the same, or similar, biochemical mechanisms are operating, although the molecules are not sufficiently concentrated, or regularly organized, to form an active zone recognizable at the electron microscope level. The most classical example is certainly the autonomic nervous system.[140] Some evidence for this phenomenon has also accumulated in the CNS (see discussion of some examples below).

In addition to exocytosis of LDCVs and SVs, other mechanisms may be active in releasing VT signals in the ECF. An interesting case is the non-vesicular carrier-mediated release of transmitters.[1,18] The molecular mechanism underlying this type of release is the reverse functioning of transmitter uptake carriers. These molecules can in fact take up specific transmitters from the ECF or release them into the ECF according to the transmembrane ion and voltage gradients.[1,18] Several instances in which this type of release mechanism may be physiologically relevant are summarized in Attwell *et al.*[18] A few features of reverse uptake are especially relevant to the present discussion. Contrary to the case of vesicular release, both neurons and glial cells can release transmitters through reverse uptake. Therefore, depolarization of glial cell membranes via activation of ionotropic receptors can release glial stores of a given transmitter and increase its concentration in the ECF. Overall, these findings provide a strong case for a regulated, non-vesicular release of VT transmitters by neurons and glial cells.

4.2. *Diffusion pathways for volume transmission*

Cerebral ECF, and associated compartments such as CSF, constitute the medium for VT. A relevant objective of the research on VT is therefore the characterization of molecule diffusion in the brain extracellular microenvironment. These phenomena were first analysed by using monoamines (visualized by the Falck–Hillarp technique[32,127]) and radiotracers.[57,58] Subsequently, the diffusion characteristics of certain ions were studied using ion-selective microelectrodes.[114,116] Using these techniques, two main features of molecule diffusion in the extracellular microenvironment were determined: tortuosity and volume fraction. The tortuosity is the length of the diffusion path for a molecule in a given medium with respect to free medium. It is therefore a global index which takes into account, *inter alia*, the geometry of the boundaries limiting the microenvironment. The volume fraction represents the amount of volume in which an extracellular molecule can diffuse with respect to the overall volume of the brain region. Recently, using fluorophore-coupled dextran molecules of various molecular weights in combination with integrative optical imaging, the diffusion of high molecular weight compounds has been demonstrated in the brain extracellular microenvironment.[117] Thus, for the 40,000 and 70,000 mol. wt dextrans, significantly higher tortuosities were obtained compared with lower molecule weight dextrans. This observation does not seem to be relevant for brain neurotransmitters, since all of them are below this critical size, but it may be of importance for certain neurotrophic factors and for secreted proteins such as chromogranin, dopamine-β-hydroxylase and acetylcholinesterase.[16,41]

Also of relevance for VT is the evidence for paravascular fluid circulation in the mammalian CNS[123] provided by the rapid distribution in the brain of tracer proteins administered in the subarachnoid space. It has been suggested that there is movement of fluid and molecules from the paravascular network to the brain ECF. Interestingly, this movement is substantially increased by arteriolar pulsations. Therefore, paravascular fluid pathways of smooth muscle-containing vessels and the basal lamina of capillaries and venules can assure the movement of CSF and ECF within the CNS.[124]

Recent investigations involving magnetic resonance imaging and radionuclide tomography support the idea[74] that CSF circulation is directed by pulsatile flow and is made possible by expansion of intracranial arteries during cardiac systole. Furthermore, the majority of CSF transport into the blood stream is

direct and involves the paravascular and extracellular spaces of the brain. These findings are very relevant for VT in the brain, as they show the existence of convective fluid movements for the chemical signals present in the ECF of the brain.

Further evidence for long distance convection pathways along fibre bundles (a phenomenon originally described by Bondareff *et al.*[32] and Cserr and Ostrach[42]) has recently been obtained by using Texas Red-labelled dextran injections in the neostriatum.[30] Texas Red-labelled dextran, a marker for the extracellular space, is shown to be transported along myelinated fibre bundles extending throughout the neostriatum, reaching into the external capsule and the corpus callosum. Confocal laser microscopy indicated the presence of the marker in extracellular channels along the myelinated fibre bundles. This may represent a special system of extracellular fluid channels for long distance convection of neurotransmitters, as well as of neurotrophic molecules, which can use these pathways to reach distant targets.

4.3. *Receptors for volume transmission*

The existence of a distance larger than the synaptic cleft between release sites and receptors for a transmitter is to be expected for VT signals. In fact, morphological evidence has been obtained for transmitter release sites located far from their putative target receptors both at the light and electron microscopic levels.

At the regional level, morphological studies comparing the distribution of a given transmitter and its putative receptor population have shown that, in many instances, there is no obvious spatial correspondence between sites of transmitter storage and sites of receptor concentration.[4,84,85] This phenomenon has been called transmitter–receptor mismatch.[43,95] Some of these results can certainly be explained by technical factors (e.g., differences in the sensitivity of the techniques used). However, a transmitter–receptor mismatch could also be the morphological substrate for long distance VT signals.

Similar findings have been obtained in ultrastructural studies. Release sites not located close to their target are a common finding in central neuronal systems (e.g., a varicosity with LDCVs or, more rarely, SVs not facing a postsynaptic density; for reviews, see Refs 36, 69 and 143). More recent studies using anti-receptor antibodies have confirmed that many, and sometimes most, membrane receptors are not located in classical synapses. Extrasynaptic location was demonstrated for peptidergic,[44,77,99,121,139,144] monoaminergic[12,14,135] and glutamatergic[118] G-protein-linked receptors, but also for ion channel-gated receptors.[15,125] In addition, immunoreactivity for several types of ionotropic[22] and metabotropic[13,44] receptors was demonstrated in astrocytic processes, indicating that both types of receptors are present in astrocytes *in vivo*.

Even when technical artifacts are excluded, caution must be observed in interpreting instances of transmitter–receptor mismatches as demonstrations of VT. Other interpretations of the transmitter–receptor mismatch phenomenon have been proposed. For instance, a transmitter–receptor mismatch can be a sign of "superfluous" transmitters and/or non-functional receptors as well.[33]

Superfluous gene expression[56] has been proposed, *inter alia*, to explain the frequent failure of gene knockout experiments to demonstrate the expected phenotypes in mutated animals. This hypothesis holds that the expression of a non-functional protein in a certain tissue can continue precisely because it is non-functional. Of course, the protein will not be superfluous in the entire organism, otherwise its gene would become a pseudogene. In many instances, suppression of the expression of a protein in a given tissue would be less "economical" than its unrestrained expression, provided that the protein has no effect at that site.

The most straightforward solution for this puzzle is of course to demonstrate that a given type of VT is functional (see criteria summarized above). Relevant studies have been performed on a certain set of enzymes in several *Drosophila* species, demonstrating that their expression pattern is very variable across closely related species. However, high levels of each enzyme were present in the same locations for all the species. In these locations (called "primary" sites) the enzymes had obvious physiological significance. At the same time, mutation of genes expressed in highly variable sites ("secondary" sites) had no functional consequences, indicating that they were in fact superfluous. The distinction between primary and secondary sites may be used as a guideline for recognition of biologically relevant expression of a protein.

A second issue which merits discussion concerns the features of receptors involved in VT. For the majority of chemical VT signals, the appropriate receptors must be located on the membrane. In this respect, gaseous transmitters (e.g., nitric oxide and carbon monoxide)[34,45,65,66] and neurosteroids[122] are exceptional in that these molecules can pass through biological membranes and find their target molecule inside the cell. In general, for both WT and VT there must be an equilibrium among extracellular concentration of the transmitter, volume of the structure in which the transmitter must diffuse to reach its target, and affinity of the active state of the receptor. Therefore, although some exceptions may be found, a distinctive characteristic of receptors for VT signals is their high (nanomolar to picomolar) affinity for the parent transmitter. A consideration of the difference (orders of magnitude) between the volume of a synaptic cleft and that of even a small portion of the extrasynaptic microenvironment makes obvious the requirement of high-affinity receptors for VT signals. Conversely, a high-affinity receptor will be easily saturated in a synapse.

As already mentioned, VT, especially in the case of long distance diffusion of signals, seems particularly suited for relatively slow global responses of neuronal and glial assemblies (see also below). Accordingly, receptors assuring long-lasting effects on the target cell, such as metabotropic receptors, seem appropriate for VT signals. In fact, metabotropic receptors activated by peptides, monoamines or amino acids are mainly located in membrane domains lacking synaptic specializations.[12,14,44,77,99,118,121,135,139,144]

In this context, it is interesting to mention the evidence concerning the internalization of neuropeptides after binding to their receptors. In several systems (e.g., neurotensin in the cholinergic basal forebrain system[11] and β-endorphin in periventricular areas[25]) the neuropeptide can bind to a receptor, cause a response in the target cell, and subsequently be internalized and transported towards the cell body.[11] It is tempting to speculate that through this mechanism the transmitter–receptor complex can modify the transcriptional activity of the target cell, thus inducing long-term modifications in its function.

5. VOLUME TRANSMISSION DURING PHYLOGENY

As suggested by the classical aphorism "*natura non facit saltus*", many examples of VT can be traced back to lower organisms. The basic machinery for regulated vesicular release of peptides and monoamines is present in cells ranging from protozoa to endocrine, neuroendocrine and neuronal cells in all multicellular organisms. In addition, as Schmitt[131] pointed out, during evolution neurons arose from cells that had axon-like elongations transporting and secreting molecules into the interstitial fluid of the organism in proximity of the target cells. Accordingly, there exist many examples of non-synaptic or parasynaptic release sites in invertebrates (for reviews see Refs 36, 37 and 69). This suggests that some type of VT exists in these organisms.

One interesting example of this kind of communication is found in neuroendocrine bag cells in the abdominal ganglia of the mollusc *Aplysia*.[107] Morphological analysis demonstrates that the axons of these bag cells only reach connective tissue surrounding the ganglia.[79] Upon activation peptides are released from these axons, diffuse into the ganglion and regulate the activity of the ganglionic neurons.

Arshavsky *et al.*[17] used the isolated pedal ganglia, which regulate locomotion, to show that molluscan neurons can also function in this manner. After disruption of all synaptic contacts, locomotor command neurons of the ganglion were still able (in approximately 25% of the cases) to regulate appropriately the other locomotor neurons of the network. Thus, at least in some systems, command neurons also operate via VT to organize networks, for example for locomotion.

Taken together, the VT mode of communication seems to be a common phenomenon in the invertebrate nervous system. Instead, as the CNS increases in complexity throughout phylogeny there is an increased demand for the more precise and rapid form of neuronal communication (i.e. synaptic transmission). This may be especially true for cortical and subcortical sensory and motor systems that govern detailed analysis of the environment and sophisticated motor skills.

6. SOME EXAMPLES OF VOLUME TRANSMISSION IN ADULT VERTEBRATE CENTRAL NERVOUS SYSTEM

6.1. *Dopamine in the retina*

Several lines of evidence indicate that amacrine and interplexiform dopamine (DA) cells of the vertebrate retina operate via VT. DA cell bodies are located in the inner nuclear layer and send terminals to the inner plexiform layer and, to a lesser extent, to the outer plexiform layer. The distribution of D_1 and especially D_2 receptors is much wider,[150] so that several instances of mismatch between DA release sites and DA receptors are present. It is worth noting (see above part on superfluous transmitters) that this pattern of distribution of DA terminals and receptors is remarkably preserved throughout phylogeny, suggesting that the components of DA transmission, as they are, play an important role in retinal physiology. Although some communication between DA cells and their targets is mediated through classical synapses (e.g., DA synapses in the outer plexiform layer), the D_2 receptors present in very high density in the inner and outer segments of the photoreceptors are located several tens of micrometres away from any DAergic terminal. In addition, in the inner plexiform layer, while nerve terminals have a laminar distribution, D_2 receptors have a homogeneous distribution, suggesting that most of them are located outside synaptic areas.

There is evidence that these DA receptors are biologically active.[52] They can regulate adenylate cyclase and play a role, *inter alia*, in the regulation of photoreceptor cells involving disc shedding and melatonin biosynthesis. The time scale of these effects (tens of seconds) also favours a transmission based on VT mechanisms. Accordingly, it has been shown in the eye of the clawed frog that vitreal DA concentration ranges between 100 and 1000 nM.[156] In addition, extracellular DA concentration varies in different physiological states, being higher in light-adapted than in dark-adapted eyes.

6.2. *Noradrenaline and serotonin in brain and spinal cord*

The available evidence indicates that widespread monoamine nerve terminal networks can operate via both VT and WT (see, however, Bloom[31] for the view that, contrary to the case of neuropeptides and steroids, there is no compelling evidence for VT in the case of monoamines). Both non-junctional and

junctional monoaminergic varicosities have been demonstrated within the cerebral cortex, the cerebellum, the locus coeruleus and the hippocampal formation.[48–50,109] It has previously been suggested by Descarries and colleagues that serotonin (5-HT) and NA can be released from those varicosities lacking synaptic junctions,[24,50] which would thus represent varicosities devoted mainly to VT. In addition, it seems possible that NA and 5-HT released from synaptic varicosities can leak from the synaptic cleft into the extracellular space. It is interesting to note that the prevalence of synaptic vs non-synaptic varicosities reflects the degree of divergence of the respective monoaminergic system.[50] For instance, in neocortex the more restricted (as far as regional and laminar patterns of distribution are concerned) DAergic system has a high incidence of synaptic varicosities; on the other hand, the NAergic, 5-HTergic and histaminergic, widely collateralized, projections to the entire neocortex have a high to very high incidence of non-synaptic varicosities.

In other regions such as the mesencephalic central gray and the dorsal horn of the spinal cord, morphological evidence favours a predominant VT mode for 5-HT and NA innervation. The prevalence of synaptic vs non-synaptic varicosities is different in the dorsal and ventral spinal cord.[126] In fact, in the former region non-synaptic varicosities predominate, while the opposite is true in the latter region. The distribution of 5-HT receptors confirms the prevalence of VT in the dorsal horn and of WT in the ventral horn of the spinal cord, as low-affinity 5-HT_2 receptors predominate in the ventral horn and high-affinity 5-HT_1 receptors are prevalent in the dorsal horn.[101]

An interesting feature of monoaminergic diffuse systems is that non-synaptic varicosities are often in proximity to astrocytic processes.[105,126] The astroglia are known to contain high-affinity G-protein-coupled receptors for monoamines (for reviews see Refs 78, 89 and 136). The activation of these receptors can lead to, for example, changes in cell shape,[110] regulation of energy metabolism,[89,141] and stimulation of astroglial transmitter release and uptake.[78,141] Thus, monoamines can indirectly influence neuronal function via informational, metabolic and trophic signals originating in the astroglia.

Monoamines are known to exert widespread effects on neuronal function throughout the brain, and thus to play a role in mass sustained functions, such as the sleep–wake cycle, mood tone and nociception/antinociception. These slow long-term modulations of the neuronal activity of entire networks suggest VT as the main mode of intercellular communication.

6.3. *Neuropeptide systems*

Several features of peptidergic transmission make neuropeptides prototypic VT signals. In varicosities of peptidergic nerve terminals, neuropeptides are stored in LDCVs mostly located in parasynaptic or non-synaptic positions,[69,103,143] from which they can easily reach the extracellular space. In addition, neuropeptides, unlike classical transmitters, are not taken up again into the neuron via specific reuptake mechanisms. Instead, they diffuse in the ECF and undergo cleavage, by the action of proteases and neuropeptidases, leading to the formation of inactive or active fragments. A slow clearance of the neuropeptide from the ECF may also occur at the target receptor level through internalization of the neuropeptide–receptor complex (see above). Thus, neuropeptides or their fragments can persist in the ECF for a long time. Finally, neuropeptide receptors are typically G-protein-linked receptors with very high affinity. The majority of the neuropeptide systems are also characterized by the presence of clear-cut transmitter–receptor mismatches. This is true, for example, for the neuropeptide Y neuronal system and the opiate peptide system.[4,64,84,159]

A well characterized example of a neuropeptide working as a VT signal in the brain is represented by dynorphin in hippocampal mossy fibres.[154] Dynorphin, which is co-stored with glutamate in mossy fibres, can cause a slow-onset and long-lasting (30–60 min) inhibition of mossy fibre synaptic responses by decreasing glutamate release. This effect is also physiologically present at distant synapses (so called heterosynaptic depression). Indeed, tetanization of one mossy fibre pathway is able to induce a long-lasting depression of the activity in a second unstimulated pathway. Accordingly, the long duration of dynorphin action is not due to intracellular effects in target cells (as dynorphin action is immediately blocked by administration of an opiate antagonist) but rather to permanence of dynorphin in the ECF. In conclusion, dynorphin works as a VT signal, slowly diffusing in the ECF to inhibit glutamate synapses located at a distance.

It is interesting to consider co-existence of multiple neuropeptides in one nerve terminal in the frame of VT. It is now accepted that many LDCVs contain several neuropeptides.[8,87,88] In addition, LDCVs can contain monoamines, although co-existing classical neurotransmitters are also stored in SVs present in the same nerve terminals.[103] In most neurons, LDCVs and SVs are present in the same nerve terminal but have different intracellular locations (LDCVs are mainly concentrated close to non-synaptic membranes) and are differently regulated (in particular, LDCVs are mainly released after high-frequency electrical stimulation[103]).

The fact that co-stored neuropeptides can be VT signals is an intriguing explanation of co-existence itself. In fact, if we suppose that neuropeptides can diffuse in the ECF and reach multiple targets, the existence of multiple transmitters in the same neurons acquires its full relevance. Inside a synapse, a transmitter or a transmitter plus a modulator would be sufficient to assure information transfer. Instead, an

extended number of transmitters markedly increases information transfer through VT: in fact, each VT signal can reach a specific set of targets and trigger a specific response.

The metabolic fate of neuropeptides in the ECF is particularly crucial for VT. As already mentioned, peptides once released are not taken up by cell processes. Their metabolism is instead regulated by the presence of proteolytic enzymes present in the ECF.[134,142] Interestingly, proteolytic cleavage does not always lead to inactive fragments, but rather to active fragments with a spectrum of activity different from that of the parent peptide.[51] The collection of active fragments plus the parent peptide, diffusing in the ECF to reach multiple targets, may induce a complex response in the tissue, which has been called a "syndromic response".[2,6] This cascade of fragments and their effects constitute a kind of "chemical network" in the cerebral microenvironment parallel to the cellular networks.[5,6,51,83,106]

Important evidence for this view was discussed in a paper by Duggan and co-workers,[54] in which they demonstrated the release, spread and persistence of neurokinin A in the dorsal horn after continuous noxious stimulation. The peptide was detected by antibody microprobes. The noxious stimulation caused a diffusion of immunoreactive neurokinin A throughout the dorsal horn and even in the white matter of the spinal cord, while the immunoreactive substance P was restricted to the substantia gelatinosa. Such findings may help explain long-term changes in the excitability of spinal cord neurons after activation of unmyelinated primary afferents of the dorsal roots,[108] and present compelling experimental evidence for the existence of VT in nerve terminal networks of the dorsal horn under such experimental conditions.

Subsequently, the same group was able to demonstrate that the calcitonin gene-related peptide (CGRP) can cause an intraspinal spreading of substance P following peripheral activation.[129] After microinjections of CGRP into the dorsal horn, nerve stimulation caused diffusion of immunoreactive substance P from the spinal cord surface into the ventral horn, a pattern very similar to that found after application of peptidase inhibitors.[55] These results indicate that CGRP (which is co-stored in many substance P-containing sensory neurons) can reduce the degradation of substance P via inhibition of endopeptidases, thus increasing VT for substance P. The mixture and relative content of co-stored peptides may therefore be crucial for their VT-dependent effects.

These results indicate that the nociceptive afferents to the substantia gelatinosa of the spinal cord operate not only via their release of synaptic transmitter, namely glutamate,[53,128] mediating fast synaptic transmission, but also via release of various neuropeptides[96,128] which via VT can lead to long-term effects on nociception. In addition, the neuropeptides can have different metabolic patterns, some being more suited for short-distance diffusion and others for long-distance diffusion.

VT is also likely involved in other phenomena related to nociception. Electrical stimulation-induced analgesia from the mesencephalic central gray appears to involve the activation of antinociceptive systems from the reticular formation of the brainstem innervating the outer layers of the dorsal horn. These systems may use both monoamines and various neuropeptides, such as opioid peptides, for mediating the long-lasting effects observed after a relatively short electrical stimulation. The involvement of VT could explain both the latency and the very long duration of action of the stimulation-induced analgesia.[7]

6.4. *Classical transmitter systems*

Although classical neurotransmitters, such as glutamate, GABA and acetylcholine, are considered as synaptic transmitters operating via receptors coupled to ion channels within the synaptic cleft, several lines of evidence indicate that they may also work as VT signals.

It has long been known that glial cells participate in the metabolic cycle of glutamate and GABA. Astrocytes (and neurons) can both uptake and release, via a reverse uptake mechanism (see above), several amino acid transmitters and modulators.[1,18] Electrophysiological studies both *in vivo* and *in vitro*[93] show that astrocytes bear membrane receptors for various transmitters, including ionotropic amino acid transmitters. Finally, recent ultrastructural studies demonstrate that ionotropic receptors are located on astrocyte membranes outside classical synapses.[22] We have, therefore, all the elements for VT chemical circuits between neurons and/or neurons and astrocytes using amino acid transmitters as signals. These mechanisms may be relevant in both physiological (e.g., horizontal cell communication with cones in the retina, and modulation of *N*-methyl-D-aspartate transmission by reverse uptake of glycine and/or D-serine from astroglia[18,130]) and pathological conditions (e.g., potentiation of excitotoxicity by reverse uptake of glutamate from astroglia during ischaemia[18]).

In addition, classical transmitters can be released extrasynaptically via a vesicular system or leak out from the synaptic cleft to activate high-affinity G-protein-coupled receptors for glutamate (metabotropic receptors), GABA ($GABA_B$ receptors) and acetylcholine (muscarinic receptors). For instance, several papers have shown a large prevalence of non-synaptic release sites in cholinergic systems (see, for example, cholinergic varicosities in nerve terminals of rat parietal cortex[145]).

6.5. *Nitric oxide and carbon monoxide*

A novel mechanism for neuronal transmission and especially for VT is provided by nitric oxide.[34,65,66] Activation of nitric oxide synthase occurs upon

activation of glutamate receptors, leading to depolarization and increases in the intracellular calcium levels.[66] The nitric oxide represents a short-action rapidly diffusible signal for VT. In fact, nitric oxide is a gas which can easily cross cell membranes and link the activities of neurons in a restricted brain volume by activation of guanylate cyclase.[34,65] Nitric oxide is the prototype of a new class of transmitters, which also includes carbon monoxide.[45] This class of gaseous transmitters is relatively unique among VT signals in that it can activate cytosolic enzymes and does not operate via membrane receptors.[34]

6.6. *Diffusible signals of non-neuronal/non-glial origin*

As mentioned in the section on VT and WT definitions, any kind of cell in neural tissue can be a source or target for communication via WT and VT. An intriguing example of VT stemming from non-neuronal/non-glial cells is sleep–wake cycle regulation by prostaglandins. Prostaglandins D_2 (PGD_2) and E_2 are the most powerful regulators of the sleep–wake cycle known.[81] PGD_2 promotes sleep by acting in the preoptic area, while prostaglandin E_2 promotes wakefulness via an action in the posterior hypothalamus.[80] Recent papers show that PGD synthase is found almost exclusively in the leptomeninges, the choroidal cells and, to a lesser extent, in brain oligodendrocytes.[147] Consistent with this pattern of expression, it has recently been demonstrated that β-trace, a major constituent of CSF, is homologous if not identical to PGD synthase.[86] Accordingly, a number of studies have shown that CSF contains sleep-promoting substances.[90] These data suggest that PGD_2 or PGD synthase itself can diffuse in CSF and then in brain ECF to reach its targets in specific brain regions.

6.7. *Global regulatory signals*

It has recently been proposed that overall tuning of the WT and VT of entire networks can be achieved by the action of "global regulatory signals".[9] The proposal holds that carbon dioxide, hydrogen ion concentration, temperature gradients and pressure waves can affect the efficacy of both WT and VT in entire brain regions.

Carbon dioxide is another gas of interest for communication in the brain. It not only plays an important role in pH control in the CNS, but it can also lead to the formation of α-carbamates from L-amino acids present in the ECF of the CNS.[104] Some of the carbamates have been characterized as powerful agonists of *N*-methyl-D-aspartate receptors.[104,119] Thus, carbon dioxide and α-carbamates represent metabolic signals which can affect both WT and VT and participate in the regulation of the sensitivity of receptors for WT and VT. These metabolic signals may be of particular importance for the maintenance of the spontaneous activity in CNS networks, a phenomenon which may be essential for a safe (noise-resistant) and high-capacity transmission of information along neuronal networks. In fact, "basic roles of spontaneous activity are to keep the synaptic apparatus trimmed for use, preventing disuse, and to serve as a background for inhibition".[73]

7. VOLUME TRANSMISSION IN BRAIN PATHOLOGICAL STATES

VT in pathology has been especially studied in models of Parkinson's disease, i.e. in DA-denervated striatum. In physiological conditions, the major mode of DA communication within the neostriatum seems to be synaptic transmission.[92] Ultrastructural studies have demonstrated DAergic small-symmetric synapses located on the dendritic spines of medium spiny striatal neurons representing 59% of all dopaminergic synapses.[59,75] Synaptic release sites in DAergic axons correspond to boutons *en passant*, usually located in the thin intervaricose portions of the axons. Only rarely do DAergic varicosities contain a synapse. Although no direct evidence is available, it is possible that DA released by the varicosities acts via a VT mode of action. In agreement with this hypothesis is the recent evidence that a large proportion of D_1 and D_2 receptors in the striatum is located in non-synaptic membranes.[157] This may be the substrate of the non-synaptic action of DA on striatal cholinergic interneurons (for review, see Ref 149). It has also been possible to determine that striatal ECF levels are sufficiently high to activate high-affinity DA receptors.[62] However, in physiological conditions the VT modality of transmission seems to be relatively atypical for striatal DA.[92]

Recent studies in a rat model of hemiparkinsonism (i.e. unilateral DA denervation induced by intranigral injection of 6-hydroxydopamine indicate that the role of VT may be potentiated after lesion.[29] After one month of recovery, systemic administration of D-amphetamine (a catecholamine-releasing agent) could cause DA-like actions (e.g. increase in c-*fos* immunoreactivity and inhibition of striatal neuronal firing) in both innervated and denervated striata. However, the effects were observed with a latency of about 5 min in the intact striatum and about 30 min in the denervated striatum. The inhibition of firing disappeared after depletion of the catecholamine stores.[29] These results can be explained on the basis that supersensitive DA receptors can react to very low concentration of DA diffusing via the CSF from the unlesioned side and/or via the ECF from the few surviving DA terminals of the lesioned side.

This interpretation is also supported by experiments performed in cats whose striatal DA system has been depleted by 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) treatment. Six weeks after MPTP treatment, DA innervation recovers in the ventromedial, but not in the dorsolateral, part of the striatum. KCl-induced release of DA in ventromedial striatum leads to the appearance, with a delay,

of increased DA levels in the denervated dorsolateral part, while direct KCl infusion in the dorsolateral striatum is almost ineffective.[132] These experiments indicate that DA can diffuse for a long distance (millimetres) in the partially denervated striatum.

Experiments on intrastriatal adenohypophyseal transplants in DA innervated and denervated striata also support the notion of long-distance diffusion of DA in the striatum. Adenohypophyseal transplants have been shown to survive in the rat neostriatum and to secrete prolactin into the surrounding ECF.[26–28] DA denervation induced by 6-hydroxydopamine injection in the substantia nigra markedly increased the zone of the striatum where prolactin-like immunoreactivity could be detected. These results indicate that endogenous extracellular DA concentrations within the non-denervated neostriatum are sufficiently high to allow DA to reach the transplant. High-affinity D_2 receptors on prolactin cells are then activated, leading to a reduction in the release of prolactin.

These results, together with many others,[61,158] indicate that compensatory enhancement of short- and long-distance VT in remaining DA nerve terminal networks in Parkinson's disease can help explain the slow progression of the symptoms of this disease. It therefore seems likely that the therapeutic action of L-DOPA is related to its decarboxylation to DA in remaining DA nerve terminal networks, followed by release and diffusion in the ECF pathways to reach supersensitive DA receptors. Treatments aimed at increasing DA ECF levels, such as blockade of DA uptake and inhibition of DA catabolism by monoamine oxidase, may also lead to an enhancement of VT and thus to therapeutic improvements in Parkinson's disease.[82,120]

Finally, although some synaptic contacts are established between grafted DA neurons and the host,[60] it seems likely that the mechanism underlying the therapeutic actions of adrenal medulla and embryonic midbrain transplants into the striatum of parkinsonian patients consists of the diffusion of DA from the catecholamine-containing cells and fibres of such transplants.[97,98] Bilateral therapeutic actions of unilateral transplants may also be explained by diffusion of chemicals into the ECF and CSF, and their action as VT signals.

In general, it must be emphasized that, in contrast to a therapy based on WT potentiation, which must preserve the spatiotemporal code of communication, a therapy based on VT potentiation can also use drugs directly acting on target cell receptors (such as postsynaptic agonists[10]). Therefore, the therapeutic actions of DA receptor agonists such as bromocriptine in Parkinson's disease probably reflect restoration of a VT-type communication, with improvement of movement and cognition, which are tonically controlled.[133] However, such treatments are not expected to improve the electrophysiological responses to reward-associated stimuli in DA nerve cells which are fast and require synaptic transmission (see also Ref. 10).

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APPENDIX

The terms WT and VT are derived from the very early definition given by Volta (Alessandro Volta, 1745–1827, the inventor of the voltaic battery) of the conduction phenomenon. In 1793, Volta discovered the existence of two types of conductors for electric current: the "metal conductors" (class I conductors, according to his original definition) made by metal wires, and the "wet conductors" (class II conductors, according to his original definition) made by solutions.[68]

Therefore, the term WT has been derived from Volta's concept of the class I conductor and by the meaning of the word "wire", which also indicates "the telegram system, i.e. to send a message by wire" (see *Webster's New Collegiate Dictionary*[153]). The wire is, in our opinion, a good analogy not only for classical synaptic transmission, but also for other types of fast intercellular communication (e.g., gap junctions and membrane juxtapositions) by which information flows in constrained channels in the CNS represented by cell structures.

The term VT has been derived from Volta's concept of the class II conductor and by the name of a theory that describes the capability of the ECF to work as this type of conductor, namely the "volume conduction theory" (see, e.g., Ref. 102). This theory describes the flow of ionic currents generated by nerve cells through ECF under various conditions of cellular activity.

It is interesting to note that Golgi already suggested this type of interneuronal communication in 1891[70] by pointing out that " ... the structural contact or fusion between two nerve fibres is not a necessary condition to have functional relationships among different neurons ... since studies on electricity show that electrical currents can link two conductors [*class I conductors*] not in direct contact [*but instead connected by a class II conductor, such as the ECF*]" (the italics are ours).