Volume transmission in the CNS and its relevance for neuropsychopharmacology

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The terms 'wiring' and 'volume' transmission (WT and VT) have been introduced to provide a systematic categorization of intercellular communication in the brain. WT is one-to-one transmission and includes classical synapses, gap junctions and membrane juxtapositions, whereas VT is a one-to-many transmission and includes paracrine and endocrine-like transmissions in the brain extracellular space and cerebrospinal fluids. Any brain cell can participate in WT and VT and any kind of substance (e.g. ions, classical transmitters, peptides, neurosteroids) can be a signal in WT and VT. These concepts are relevant for the pharmacokinetics and actions of neuropsychoactive drugs. These drugs can be regarded as exogenous VT signals in that they diffuse in the cerebral extracellular space and are constrained there by the same factors that influence migration of endogenous VT signals. In addition, neuropsychoactive drugs can better mimic and more effectively interact with the relatively unconstrained VT-type transmissions than with the rigidly constrained WT mechanisms, such as synaptic transmission.

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In the past 20 years, a number of morphological and functional observations has led several authors[1] to suggest the existence of an alternative way, other than synaptic transmission, for interneuronal communication, which has usually been called non-synaptic or extrasynaptic transmission.

Two complementary modes for intercellular communication

These negative terms are, however, misleading as they overshadow the multiplicity of communication modes and cell types involved in the intercellular communication in the CNS. There has been, therefore, a need to formalize in a new way the intercellular communication in the CNS, bearing in mind that, on the one hand, central synaptic transmission might differ substantially from the classical paradigm of the neuromuscular junction and, on the other hand, that much evidence has accumulated on the existence in the CNS of many types of short- and long-distance transmissions of neuronal as well as non-neuronal origin besides synapses.

Therefore, 13 years ago, in an attempt to provide a positive and systematic definition of the categories of intercellular communication in the CNS, the terms wiring and volume transmission were introduced[2–4]. These terms are based on the ratio between the number of signal source structures (S, i.e. a part of the cell that releases the signal) and number of signal target structures (T, i.e. a part of the cell that recognizes and decodes the signal) in the transmission. For example, in the case of synaptic transmission, S would be the presynaptic terminal and T would be the postsynaptic density[4]. Thus, WT is a fast, one-to-one, point-to-point, 'private' intercellular communication ($S/T = 1$) via synapses, gap junctions and membrane juxtapositions, whereas VT is a slow, one-to-many, widespread, intercellular communication ($S/T < 1$) within the extracellular space (ECS) and cerebrospinal fluid (CSF). Other parameters, such as S/T distance, transmission delay, medium in which the transmission occurs (e.g. ECS vs. CSF), driving force for transmitter movements (e.g. diffusion versus convection), allow the known modes of intercellular communication to be classified further in a specific and comprehensive manner (see Table 1).

The distinction between WT and VT is based on general features of intercellular communication in the CNS, so that any cell type (e.g. neurones, astroglia, microglia) and any kind of substance (ions, amino acids, monoamines, peptides, gases, neurosteroids) can participate in WT and VT (see Table 2).

WT modes of communication

Within WT several types of communication can be distinguished. The chemical synapse is obviously the prototype for WT (see, however, the distinction between closed and open synapses, below). WT also occurs when informational molecules can pass directly from the source to the target cell as it occurs through gap junctions (electrical synapses)[5]. Astroglia, interconnected via gap junctions, act as an electrical syncytium, which allows *inter alia* spatial buffering of K^+ and long-range Ca^{2+} waves[6,7]. Ca^{2+} waves can affect the metabolism and excitability of the neurones embedded in the astrocyte network via Ca^{2+}-induced mobilization of glucose from glial glycogen stores, and secretion of neurotransmitters through reduction of free Ca^{2+} in synaptic clefts, respectively[5,6,8]. Overall, communication through gap junctions appears to be an integral component of the complex cell–cell interactions occurring in central neuronal–glial networks.

It is likely that other types of WT, besides chemical and electrical synapses, might exist. Some evidence indicates that plasma membrane juxtapositions constitute preferential sites for electrical interactions (ephapses) and, in general, for intercellular exchange of ions and transmitters between neuronal, as well as non-neuronal, cells[9–11].

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Table 1. Proposed classification of intercellular communication in the CNS

Transmission type	Source/target ratio	Source/target distance	Source/target delay
Wiring transmission			
Quasi-continuity			
Gap junction	1:1	2 to 3 nm	μs
Contiguity			
Closed synaptic transmission	1:1	20 to 50 nm	ms
Membrane juxtaposition	1:1	2 to 10 nm	ms
Volume transmission			
Diffusion-based			
Local ion currents	1:n; n>1/n>>1	100 nm to μm	ms to s
Paracrine transmission			
Open synaptic transmission	1:n; n>1/n>>1	100 nm to mm	ms to min
Nonsynaptic source[a]	1:n; n>1/n>>1	μm to mm	s to min
Para-axonal transmission	1:n; n>>1	mm	min
Convection-based			
Paravascular transmission	1:n; n>>1	mm to cm	min
Intracerebrospinal fluid transmission	1:n; n>>1	mm to cm	min

[a]Nonsynaptic sources can be neuronal (e.g. nonjunctional varicosities and reverse functioning of transmitter uptake mechanism) or non-neuronal (e.g. vesicular and nonvesicular release from glia and endothelial cells).

Table 2. Properties of wiring- and volume-transmission communication channels and circuits

Properties	Wiring transmission (WT)	Volume transmission (VT)
WT and VT communication channels		
Type of signal	Ions (e.g. Ca^{2+}) and neurotransmitters (e.g. amino acids)	Ions (e.g. K^+, Na^+), neurotransmitters (e.g. monoamines), neuropeptides, gases, neurosteroids, psychoactive drugs
Chemical signal concentration at receiver[a]	Usually high (μM to mM)	Usually low (nM)
Receiver affinity for chemical signal[a]	Usually low (high nM to μM)	Usually high (pM to low nM)
Transmission code[b]	Rate and temporal code	Rate code
Transmission delay	Low (ms)	High (s to min)
WT and VT circuits		
Cell composition	Usually only neurones or only astrocytes	Any cell type
Divergence[d]	Relatively low	Potentially high
Type of connectivity[e]	Preferentially serial	Preferentially parallel
Space filling[e]	High	Low
Time scale	ms to s	s to min
Biological effect	Typically phasic	Typically tonic

[a]Because the volume of even a small portion of the extracellular space is much larger than a synaptic cleft, the concentration of VT signals will be much lower than that of synaptic transmitters. Conversely, the affinity of receptors capable of recognizing VT signals must be, in principle, much higher than that of synaptic receptors[3,4].

[b]Rate code models propose that changes in the firing rate signal an event (a train of impulses) that in VT can be equated to the arrival of the VT signal at the receptor at suprathreshold concentration. The average rate of impulses in the train encodes the strength of the stimulus which in the VT can be equated to the VT-signal concentration at the target receptor level. Temporal codes propose that information is encoded by the precise occurrence of spikes over time. This constraint makes it unlikely that this type of code is used in VT.

[c]Although it is conceivable that most neural systems use both WT and VT, for sake of simplicity the main features of pure WT and VT circuits are shown here.

[d]Due to the 1:1 source/target ratio at WT communication channels, the number of targets that can be reached by a single cell (i.e. divergence) is relatively limited. On the contrary, VT signals released in the extracellular fluid by a single cell can in principle reach any cell in the brain. In view of this lower or higher divergence potential, WT circuits appear more suited for serial treatment of information, whereas VT circuits are better suited for parallel treatment of information.

[e]WT and VT circuits differ in terms of space filling and energy cost. In fact, essential components of WT, but not of VT, circuits are cellular processes (e.g. axons) that maintain electrical polarization and convey electrical signals in their membranes.

VT modes of communication

Several modalities of VT can be recognized in neuronal systems. One example of VT is the 'open' (or leaking) synapse, that is a morphologically recognizable synapse that allows its transmitter(s) to diffuse beyond the synaptic cleft (see below and Refs 1, 3, 4, 12) (Fig. 1a). Indeed, in some neuronal systems (especially peptidergic synapses), studies using electron microscopy have shown that the sites of transmitter release, where large dense-core vesicles rich in neuropeptides are preferentially concentrated, are not in strict contiguity with postsynaptic membranes (Fig. 1b). A second main source of neuronal VT signals are non-junctional varicosities where high densities of small vesicles exist in varicosities lacking synaptic specializations and located far from any recognizable postsynaptic density (Fig. 1c)[13–18]. In both cases, a spatial uncoupling between release sites for transmitters and their respective receptors (i.e. a 'transmitter-receptor mismatch') is to be expected. This is a common finding in morphological studies in the CNS (Refs 19–29). Other than VT signals deriving from vesicular release, neuronal (and glial) VT transmitters can be released by means of nonvesicular carrier-mediated mechanisms, i.e. by the reverse functioning of transmitter uptake carriers (Fig. 1d)[30,31].

In several cases, morphologically identified VT neuronal systems have been characterized from a functional standpoint (for example dopaminergic transmission in the retina[32], peptidergic and gaseous transmissions in the hippocampus[33,34] and peptidergic transmission in the rat spinal cord[35]; see also Refs 1, 3, 4, 12). A nice example of a well-characterized neuronal system working through VT mechanisms is represented by 5-HT-containing neurones in the lamprey spinal cord that control the swimming pattern[36]. In some cases, neuronal terminals or varicosities contact non-neuronal cells such as glial cells that contain G protein and ion-channel receptors[37], or capillaries[38] without morphologically identified synaptic specializations. A recently described case of neurone/capillary VT concerns the dense network of dopaminergic varicosities that are apposed to pericytes of cortical microvasculature and cause marked vasomotor responses[39]. Monoaminergic and peptidergic innervation of cerebral microvasculature could explain why changes in cerebral activity are associated with local alterations in blood flow in physiological and pathological (e.g. in schizophrenia and Parkinson's disease[40]) states[38].

Because there are no known specialized sites of intercellular communication for releasing chemical signals from non-neuronal cells, transmitters of glial, ependymal or endothelial origin are, by definition, VT signals[41]. This release could occur through vesicular or nonvesicular mechanisms (Fig. 1d,e).

A special source of VT signals are cells (neurones, glia or endothelial cells) that release gaseous transmitters (e.g. NO and carbon monoxide)[42] (Fig. 1f) and neurosteroids[43,44]. These VT signals are peculiar because they pass through biological membranes and find their target molecule inside the cells.

Increasing evidence points to the relevance of ions as intercellular signals[45–47]. On the one hand, extracellular K^+ accumulation, which is a result of impulse transmission and synaptic currents, is a powerful modulator of signal transmission, leading to glycogenolysis and increases in intracellular Ca^{2+} concentration, and serves as an important VT signal between neurones and glia[45,48]. On the other hand, K^+ accumulation during pathological events can dramatically impair neuronal and glial function[45] (see below).

H^+ is able to modulate membrane excitability, synaptic function and gap-junction conductance[49]. Notably, an allosteric site for H^+ has recently been identified on the extracellular surface loop of the NR1 subunit of NMDA receptors[50]. Similar actions have been proposed for extracellular Ca^{2+} as evidence has shown that receptors for Ca^{2+} are present in cell membranes of nerve terminals in several brain regions[51].

To work as an intercellular signal, a molecule must reach its target with a delay compatible with the task the two cells are performing. By simple diffusion, a small transmitter reaches postsynaptic receptors in a few microseconds[52], but takes more than 16 min to reach a target located 1 mm away.

These temporal constraints can be overcome if the signal is transported by convective forces together with the liquid in which it is dissolved (for example, hormones in the blood). Beginning with some seminal studies in the late 1960s (Ref. 53), much evidence has accumulated on convective fluid movements in the brain. Slow bulk flow from brain to cervical lymphatics[53] could be more relevant for the removal of compounds from brain tissue than for neurotransmission. Fast convective movements have been shown to occur in paravascular spaces of the brain and meninges[54,55]. Impulses for these movements are given, at least in part, by the pulsation of brain arterioles[54,55]. Because vasomotion of cerebral vessels mainly depends on local factors, the possibility that tissue activity influences convective movements is to be considered.

Cerebrospinal fluid might also represent an important vector for convection of VT signals, especially to peri- and paraventricular areas. Recent MRI studies indicate the existence of fluid movements from the CSF via the paravascular space and the ECS into the brain capillaries that might be of special importance for the convection of VT signals[54].

The synapse can switch between WT and VT

A wide spectrum of synaptic types is likely to exist in the CNS (Refs 4, 56–58). The focus of the present classification is on the distinction between the one-to-one (closed state) and one-to-many (open or leaking state[1]) synapses, which represent WT and VT, respectively[4] (see Table 1). Thus, the main feature of the VT-type synapse is the capability of permitting transmitter

diffusion outside the synaptic cleft at biologically relevant concentrations[4,56,57,59].

Recent research and modelling of glutamatergic synapses indicate that the concentration of glutamate required to open the low-affinity AMPA receptors can only be reached in the synaptic cleft (closed synaptic transmission). However, the concentration of glutamate that is reached upon diffusion outside the synaptic cleft (especially at high quantal contents) is sufficient to activate high-affinity NMDA receptors located in neighbouring (200–400 nm away) synapses (so called spill-over; short distance VT) (Fig. 1a)[4,56,60,61]. Therefore, spill-over of amino-acid transmitters from open synapses might constitute a cross-talk between closely associated synapses.

Extrasynaptic diffusion of the transmitter at functionally relevant concentration might be a common phenomenon in many types of central synapses. For example, voltammetric studies have shown that dopaminergic 'en passant' synapses of the nucleus accumbens and caudate-putamen favour extrasynaptic transmitter diffusion[57,59]. Accordingly, dopamine reuptake sites are located outside the synapses[62] and dopamine receptors are especially concentrated in extrasynaptic membranes of dendrites[24]. Recent findings showing that nigrostriatal dopamine-containing neurones co-store glutamate and form glutamatergic synapses, open up the possibility that dopamine–glutamate terminals make glutamatergic synapses with striatal neurones and release dopamine mainly from extrasynaptic sites[63]. Experiments simultaneously monitoring dopamine ECS levels and dopamine D1 receptor activation strongly suggested that dopamine diffusing outside the synaptic cleft or from nonsynaptic varicosities, or both, is responsible for the activation of dopaminoceptive neurones in rat striatum[59]. The features of some peptidergic synapses (e.g. preferential extrasynaptic location of releasing sites, absence of re-uptake mechanisms and preferential extrasynaptic location of high-affinity receptors[4]) indicate that they might preferentially function as open synapses (Fig. 1b).

As previously reviewed[4], each type of neurone has specific structural and functional arrangements which favour communication as a closed or open synapse. Structural factors can include glial ensheathment (which can be permanent in gliosis or transient in activity-induced swelling[5,64]), location of catabolic enzymes or reuptake sites, or both, whereas functional limitations can include amount of transmitter released, and single action potential versus bursts of action potentials. In general, the 'opening' of a synapse is accompanied by the progressive relaxation of the spatiotemporal constraints typical of the closed synapse.

It must be remembered that a one-to-one or one-to-many source:target ratio is a property of an individual form of transmission in the synapse. For example, a synapse containing several transmitters can work as an open synapse for one transmitter and as a closed synapse for another (e.g. ATP and noradrenaline in sympathetic synapses[65]). Further, a transmitter with several receptors can have closed transmission for one receptor species and open transmission for another (e.g. AMPA and NMDA receptors).

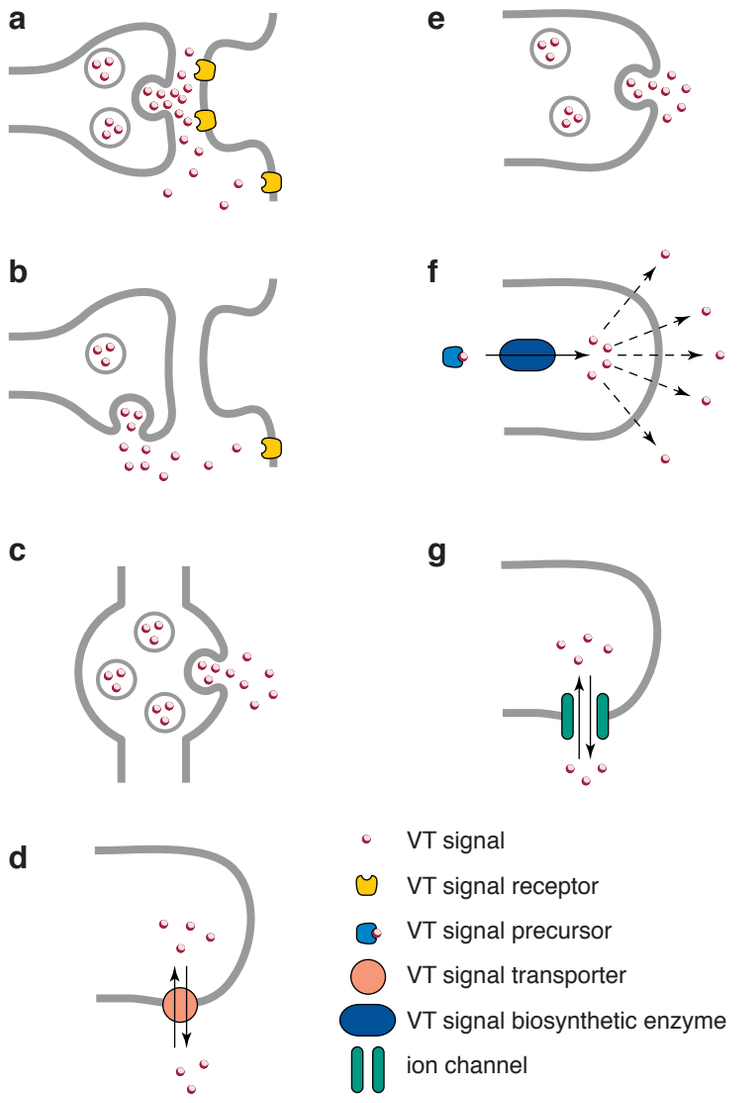

Fig. 1. Schematic representation of the main sources of volume transmission (VT) signals in the CNS. **a:** Open synaptic transmission (neuronal): intrasynaptic vesicular release followed by diffusion of the transmitter outside the synaptic cleft at effective concentration (synaptic spillover of, for example, amino acids). **b:** Open synaptic transmission (neuronal): extrasynaptic vesicular release. The transmitter is released directly into the extracellular fluid outside the synaptic cleft (nonsynaptic release of, for example, neuropeptides). **c:** Paracrine transmission (neuronal): vesicular release from nonjunctional varicosities (for example, diffuse catecholaminergic systems), i.e. varicosities lacking presynaptic specializations and postsynaptic densities. **d:** Paracrine transmission (neuronal or non-neuronal): reverse functioning of transmitter uptake carriers (for example, release of glutamate, D-serine and GABA from astroglia and glutamate and dopamine from neurones). **e:** Paracrine transmission (nonneuronal): Ca^{2+}-dependent and constitutive vesicular release from non-neuronal cells (e.g. endothelin release from endothelial cells). **f:** Paracrine transmission (neuronal and non-neuronal): release of gaseous transmitters (e.g. nitric oxide release from neurones and endothelial cells). **g:** Local ion currents (neuronal and non-neuronal): changes in the extracellular fluid concentration of ions (e.g. K^+, H^+ and Ca^{2+}) induced by the activity of transmitter or voltage gated ion channels located in neurones or glia.

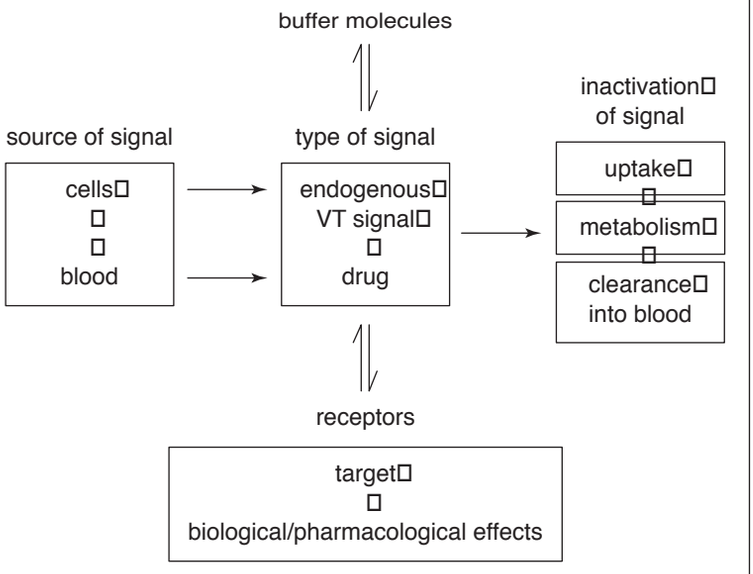

Fig. 2. Schematic representation of the similarities between endogenous volume transmission (VT) signals and neuropsychoactive drugs (i.e. exogenously administered VT signals) in the brain. Although most endogenous VT signals are hydrophilic and therefore confined to the extracellular space (see, however, the case of nitric oxide and neurosteroids as examples of lipophilic endogenous VT signals), most neuropsychoactive drugs are lipophilic and can enter brain cells. In this latter case, the volume of diffusion is the entire brain space and buffer molecules as well as metabolic mechanisms, can be present in both the extracellular and intracellular spaces.

Neuropsychoactive drugs as VT signals

Neuropsychoactive drugs can be considered to work mechanistically as though they were VT signals. First, these drugs come from systemic blood and diffuse into the brain as if they were exogenous VT signals (Fig. 2). Second, drugs can more easily affect the relatively unconstrained VT rather than the severely spatiotemporally constrained WT. This discussion will therefore be divided into two main topics: (1) what are the features of molecular movement in the brain and how does this phenomenon influence the action of endogenous and exogenous VT signals? and (2) how do drugs affect VT and WT?

Migration of molecules in the brain

In vivo and *ex vivo* studies have allowed the crucial parameters that describe the behaviour of molecules in the brain to be defined[66]. The volume fraction (volume of molecule diffusion/total tissue volume) is the proportion of the tissue in which a given substance can diffuse. It typically amounts to 0.2 for molecules confined to the extracellular compartment but is higher for lipophilic molecules such as gaseous transmitters and neurosteroids, which can enter the brain cells. The brain tortuosity (λ) is the ratio between the diffusion in free medium (D) and the apparent diffusion coefficient (ADC) of a given molecule in the brain. This is expressed in the formula: $\lambda = (D/ADC)^{0.5}$. It has been shown that the migration of molecules in the brain is dependent on their size (tortuosity varies from around 1.5 for small molecules to around 2.5 for large molecules like albumin[66]) and shape (even polymers with Mr of about 1 000 000 can diffuse in the ECS if they have an elongated shape[67]). The limited available evidence does not support an influence of molecular charge (differences between the ADC of anions and cations) on diffusion in brain ECS (Ref. 68). However, extracellular matrix (ECM) proteoglycans, particularly hyaluronic acid (which has highly negatively charged branches) can slow down the diffusion of anions.

Diffusion parameters are region-specific, heterogeneous within a given region and vary during development, ageing and with pathological states (see below). In addition, the migration of substances is in some regions isotropic, e.g. the cortex, whereas in others it is anisotropic (i.e. there are different ADCs along the three cartesian axes)[66], being, for instance, favoured along the direction of myelinated axon bundles (Fig. 3). We have called this type of transmission para-axonal transmission (Table 1). Anisotropy has been demonstrated in the cerebellum, the hippocampus and in the myelinated white matter of the spinal cord and corpus callosum[47,69–71].

Much work is still needed to determine what cellular components and molecular mechanisms contribute to the dynamics of molecular migration in ECS and how far various transmitters can actually travel in the brain. For example, current techniques cannot discriminate between true diffusion and microconvection (i.e. convection occurring in the local space around cell processes), and therefore both types of physical processes could contribute to determining the ADC in brain tissue. It is thought that microconvection occurs as a consequence of astroglial uptake and release of ions, transmitters and water. It occurs in a pulsatile manner and could cause parallel pulsatile changes in the local ECS volume[47,72,73]. Pulsatility leading to microconvection might also arise from the contraction of pericytes located in the basal lamina of brain capillaries[38,39].

Molecules in the ECM might have a major role in controlling ECS diffusion parameters and could change under pathophysiological conditions[74–78]. Glycosaminoglycan chains of proteoglycans can form gels of varying pore size and charge density, which can be remodelled by matrix metalloproteases under physiological and pathological conditions[79]. In addition, it has been shown that ECM binds some molecules, including calcium, as well as diffusible proteins, such as certain types of growth factors[80]. Furthermore, tortuosity and anisotropy decrease during ageing, concomitantly with the loss of ECM molecules (fibronectin and chondroitin sulphate proteoglycan)[81]. Available evidence is, however, circumstantial and firm experimental evidence for the role of ECM in VT is still lacking.

In general, the dynamics of substance migration in the ECS is altered according to the presence of molecules either in the ECM or on membranes facing the ECS, which are able to bind molecules and release them back into the ECS. In this context, classical concepts such as

that of acceptor versus receptor or silent versus effective binding site can be revised. Acceptors or silent binding sites are molecules that bind a transmitter or a drug without triggering a biological effect. These molecules might work as buffers regulating the diffusion of their ligands in the brain ECS. In most cases it is difficult to evaluate their impact on the physiological or pharmacological action of neuroactive molecules. However, such buffering sites could protect their ligands from fast degradation and assure permanence of biologically relevant concentrations in the ECS for a prolonged period, thus contributing to the persistence of their effects in a particular local brain network. At the same time, their potential long-distance diffusion will become reduced so that distant high affinity receptors can no longer be reached and activated.

Distinctive features of drug diffusion in the brain ECS

When considering neuropsychoactive drugs, i.e. exogenously administered VT signals, some specific factors must be considered. Contrary to endogenous VT signals, which generally derive from definite sources, systemically administered drugs have access to the entire brain. This access is almost simultaneous, although a certain heterogeneity results from regional differences in blood flow and blood–brain-barrier permeability[82–87]. Once in the brain, drug diffusion will meet the same constraints as endogenous VT (Fig. 2). Region-specific factors acting on VT signals could markedly influence the fate of drugs migrating in the brain. For example, region-specific uptake or degradation mechanisms (e.g. local concentration of monoamine oxidases or peptidases[88,89]) might accelerate drug clearance in the ECS. On the other hand, region-specific ECS buffer molecules might protect drugs and prolong their permanence in the ECS. Some putative buffer molecules for endogenous ligands have been identified. An example is the recently discovered 100 kDa neurotensin receptor, which is a non-G-protein-coupled receptor and identical to gp95/Sortilin, that might act as a buffering protein for extracellular neurotensin peptides[90]. Another example is the soluble endogenous interleukin 1 (IL-1) receptor antagonist (25 kDa) and the type II IL-1 receptor (60 kDa), which is a decoy receptor without signalling that can be membrane-bound or soluble[91]. Future work will probably identify various families of such proteins that can alter the diffusion of transmitters as well as drugs by altering their clearance or degradation, or both, and leading to changes in the activation of the target cells. Overall, region-specific features of brain ECS could help to explain regional heterogeneities of drug effects in the brain.

Because most psychoactive drugs are lipophilic, their migration in the brain is not limited to the ECS. Their behaviour is similar to that of lipophilic endogeneous VT signals such as gases[92,93] and neurosteroids[43,44]. Therefore, apart from ECS characteristics, diffusion features of the intracellular milieu (such as existence of intracellular buffering molecules) might be important determinants of the migration of drugs in the brain.

The nonphysiological source of drugs has important consequences for their effects. As previously reviewed[3], rich receptor populations are, in many cases, located at sites distant from sources of endogenous transmitters (so-called 'transmitter-receptor mismatch'). The presence of mismatch receptors is, by definition, a feature of VT (Ref. 94). However, some functional mismatch receptor populations might never be reached by endogenous transmitters, being therefore 'superfluous'[3,95]. This issue is of primary importance for the physiology of brain transmission as well as for the action of neuropsychoactive drugs. In fact, all functional and accessible receptors (physiologically active or superfluous) constitute the target of a systemic drug.

These concepts could contribute to the understanding of the strong reinforcing actions of dopamine-releasing or dopamine-uptake blocking drugs, or both, such as d-amphetamine and cocaine. By strongly increasing VT for dopamine in reward networks, these drug treatments could lead to the activation of many dopamine receptors that normally are rarely reached by relevant extracellular concentrations of dopamine[96]. Many classical and especially novel antidepressants such as fluoxetine are powerful blockers of the 5-HT transporter[97] and lead to increases in ECS 5-HT levels and diffusion[98]. For example, the prolonged potentiation of 5-HT VT by antidepressants would tonically activate large numbers of extrasynaptic 5-HT receptors in limbic regions.

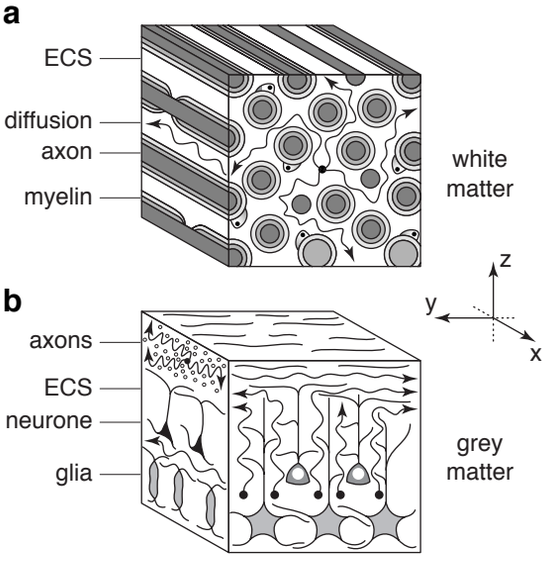

Fig. 3. Schematic representation of substance diffusion in the extracellular space (ECS). **a** (white matter): Anisotropic diffusion in myelinated white matter. Movement of substances is faster along the axons than in the directions perpendicular to them. **b** (grey matter): Anisotropic diffusion is determined by the geometry of the ECS as well as by the size, number and shape of neurones, glial cells and their processes.

VT as target of neuropsychoactive drugs

When considering the pharmacology of central neurotransmission, it is possible to distinguish between drugs interfering with transmitter release, transmitter diffusion between source and target, and transmitter action on target cells. Although drugs acting on release or target cell action can, in principle, influence either WT or VT (but see below), drugs acting on transmitter diffusion mainly interfere with VT. In a schematic way (Fig. 2), substance migration in the brain depends on the following characteristics:

(1) general features of the local ECS (volume fraction, tortuosity, inhomogeneity, anisotropy);

(2) transmitter degradation or conversion into biologically active fragments (e.g. peptidic fragments[99]), for example, by monoamine oxidase A (mainly neuronal), monoamine oxidase B (mainly glial[100]) and specific or nonspecific peptidases[101,102];

(3) transmitter uptake by the parent or other cell types, e.g. involving dopamine and 5-HT transporters[62,103];

(4) transmitter clearance via the brain–blood barrier, e.g. regulated by NO (Refs 86, 104, 105);

(5) presence in the ECS or in cell membranes facing the ECS (and/or inside the cells, in case of lipophilic VT signals) of molecules able to buffer the transmitter (acceptors or silent binding sites, or both)[90,91].

By definition, mechanisms (1), (4) and (5) are relevant for VT but not for WT. In most cases mechanisms (2) and (3) also affect VT rather than WT. In fact, termination of transmitter action in closed synapses is mainly assured by diffusion of the transmitter outside the cleft, as is the case of glutamate[56,60]. Intrasynaptic degrading enzymes might, however, have an important role in some closed synapses, for example, acetylcholinesterase in the neuromuscular junction. In the brain, non-junctional cholinergic varicosities are predominant in many regions and acetylcholine might therefore function mainly as a VT signal. Acetylcholinesterase, which, in the brain, is present in cholinergic as well as noncholinergic systems, might regulate the local spread of acetylcholine from nonjunctional varicosities[15]. Much evidence has accumulated that monoamine reuptake sites are located on nerve terminals outside synapses, whereas amino acid reuptake sites are mainly located on glial cells (see above). Reuptake blockers are therefore powerful regulators of transmitter levels in the ECS rather than in the synaptic cleft, and they control VT. For example, reuptake block does not influence dopamine concentration at the synapse[57]. Moreover, in the absence of uptake mechanisms, the main regulators of peptide transmitter extracellular concentrations are degrading or converting enzymes which are located in the ECS or in nonsynaptic cell membranes including membranes from target cells and glial cells[99,101,102]. Peptidase inhibitors will therefore substantially increase peptidergic VT.

Some synapses can switch from closed to open state and vice versa, whereas other synapses, such as the peptidergic synapses, might always be in an open state, as the large peptide storing granular vesicles are not concentrated close to the synaptic cleft[4]. A primary physiological mechanism responsible for synapse opening is increased release of the transmitter via bursts of action potentials[57,60,106]. Drugs acting on transmitter release, such as blockers of autoreceptors at cell bodies and terminals, could mimic this physiological mechanism. Therefore, besides drugs acting on WT or VT, or both, drugs such as monoamine uptake blockers, which modulate the switch between WT and VT, should be considered.

Another type of neuropsychoactive drug that could preferentially influence VT is the transmitter precursor. In fact, although precursors will feed both WT and VT, drug effects will more easily mimic VT mechanisms than the spatially constrained WT mechanisms. This is likely the case of the antiparkinsonian drug L-dopa[107]. L-Dopa is transformed into dopamine in L-dopa decarboxylase (DDC)-positive cells in the brain, which comprise dopamine-containing neurones but also other catecholaminergic neurones and cells that contain DDC but not the other catecholamine biosynthetic enzymes, such as 5-HT neurones and pericytes[108]. Furthermore, in parkinsonian patients dopaminergic neurones are markedly decreased and clearly insufficient to assure WT to their striatal targets. Therefore, the therapeutic efficacy of L-dopa treatment will depend on the migration of dopamine released by dopaminergic and non-dopaminergic cells in striatal ECS to reach the denervated supersensitive dopamine receptors more than on the potentiation of the few surviving dopaminergic synapses. Indeed, as discussed below, experimental evidence points to the fact that a potentiation of VT is an endogenous compensatory mechanism for the loss of dopaminergic neurones.

VT in brain pathology

Several pathological conditions cause and, in turn, can be aggravated by alterations in VT. Several cellular and molecular events related to pathological states involve abnormal release of VT signals and long-term changes in ionic composition and physico-chemical parameters of the ECS (Ref. 47). These include lack of energy reserves, excessive release of transmitters and other neuroactive substances, cell loss or proliferation, glial swelling and loss of ionic homeostasis.

During hypoxia and terminal anoxia, there is both an increased release of transmitters and ions (such as glutamate and K^+) and profound changes in ECS. The mechanism for glutamate release is probably the reversal of the glial uptake for glutamate during anoxia[31]. The ECS volume fraction in rat cortex or spinal cord *in vivo* markedly decreases[109–111], whereas tortuosity increases, possibly due to molecular crowding and swelling of glial processes[47]. These changes could, therefore, decrease the diffusion of molecules and aggravate the accumulation of ions, excitotoxic transmitters and neurotoxic metabolites, thus contributing to ischaemic damage. Indeed,

changes in diffusion parameters might also affect the access of therapeutic agents to the cellular elements in lesioned tissue. Reductions of ECS volume fraction could play a role in epilepsy but can also improve metabolic pumping rate through Cl^--induced increases in membrane stability[112], which suggests that, under certain conditions, such changes in volume fraction can improve post-traumatic recovery.

In chronic and often gliotic lesions, increased volume fraction and tortuosity might contribute to functional deficits. For example, in both X-irradiated cerebral cortex and spinal cord during experimental auto-immune encephalomyelitis, marked increases in ECS volume fraction and tortuosity were observed[113,114]. The observed long-term increase in tortuosity, seemingly related to astrogliosis, suggests that diffusion is substantially hindered even long after mild irradiation. These changes could contribute to impaired signal transmission by diluting VT signals (ions and neuroactive substances) as well as by hindering their diffusion.

Overall, many pathological processes in the CNS are accompanied by alterations in ECM, astrogliosis, demyelination and loss of neuronal cells or processes, all of which can affect the ADC of neuroactive substances. Although much work remains to be done, the roles of some tissue factors in determining ADC have started to emerge. In particular, the presence of astrogliosis is well correlated with persistent increases in tortuosity that is independent from changes in ECS volume fraction. This evidence has led to the hypothesis that glial cell processes or ECM molecules, or both, produced by proliferating and hypertrophied astrocytes can be main diffusion barriers in the CNS (Ref. 47). Nevertheless, new findings indicate that under certain conditions reactive astrocytes can help axonal outgrowth provided they express appropriate recognition molecules[115].

Alterations in VT in brain pathological states might also represent compensatory changes aimed at recovering an impaired transmission. Experiments in animal models of Parkinson's disease strongly suggest that a switch from short-distance to long-distance VT in dopaminergic striatal transmission represents a major compensatory mechanism to delay the onset of the motor symptoms produced by the ongoing degeneration of the ascending dopamine-containing neurones[3,94,116]. It has also been suggested that such switches towards long-distance VT improve rehabilitation after CNS damage[117]. Moreover, the compensatory efficacy of VT mechanisms is supported by experiments on CNS grafts. For example, the studies on grafted dopamine neurones show that diffusion of dopamine and not only regeneration or sprouting of dopaminergic nerve terminals, or both, underlie the therapeutic actions of such transplants, the diffusing dopamine reaching high affinity supersensitive dopamine receptors within the striatum[118]. Similarly, transplantation experiments indicate that B3 5-HT rhombencephalic embryonic cells transplanted into the transected spinal cord selectively innervate the dorsal horn in a nonsynaptic way. This VT signalling by 5-HT can contribute to post-traumatic rehabilitation[119].

In conclusion, marked changes in VT occur during CNS diseases and might aggravate tissue alterations or represent an attempt to compensate for the ongoing pathological modifications. It is, in any case, important for the researcher and the physician to consider the existence of these changes in order to understand the pathogenesis of CNS diseases and choose an optimal pharmacological strategy.

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