BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2010), 160, 785–809

0007-1188/10
www.brjpharmacol.org

REVIEW

Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment

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Beyond direct synaptic communication, neurons are able to talk to each other without making synapses. They are able to send chemical messages by means of diffusion to target cells via the extracellular space, provided that the target neurons are equipped with high-affinity receptors. While synaptic transmission is responsible for the 'what' of brain function, the 'how' of brain function (mood, attention, level of arousal, general excitability, etc.) is mainly controlled non-synaptically using the extracellular space as communication channel. It is principally the 'how' that can be modulated by medicine. In this paper, we discuss different forms of non-synaptic transmission, localized spillover of synaptic transmitters, local presynaptic modulation and tonic influence of ambient transmitter levels on the activity of vast neuronal populations. We consider different aspects of non-synaptic transmission, such as synaptic–extrasynaptic receptor trafficking, neuron–glia communication and retrograde signalling. We review structural and functional aspects of non-synaptic transmission, including (i) anatomical arrangement of non-synaptic release sites, receptors and transporters, (ii) intravesicular, intra- and extracellular concentrations of neurotransmitters, as well as the spatiotemporal pattern of transmitter diffusion. We propose that an effective general strategy for efficient pharmacological intervention could include the identification of specific non-synaptic targets and the subsequent development of selective pharmacological tools to influence them.
British Journal of Pharmacology (2010) **160,** 785–809; doi:10.1111/j.1476-5381.2009.00624.x; published online 8 February 2010

Keywords: non-synaptic chemical transmission; extrasynaptic high-affinity receptors; extrasynaptic high-affinity transporters; extracellular space; tonic modulation; presynaptic modulation; receptor and transporter trafficking; targets of medicines

Abbreviations: 5-HT, serotonin; ACh, acetylcholine; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; AP, action potential; 2-AG, 2-arachidonoylglycerol; CB1, cannabinoid1; CNS, central nervous system; DA, dopamine; DAT, dopamine transporter; EAAT, excitatory amino acid transporter; GABA, γ-amino-butyric acid; Glu, glutamate; GlyR, glycine receptor; LTP, long-term potentiation; mAChR, muscarinic acetylcholine receptor; mGlu, metabotropic glutamate receptor; nAChR, nicotinic acetylcholine receptor; NA, noradrenaline; NET, noradrenaline transporter; NMDA, N-methyl-D-aspartate; SERT, serotonin transporter

Introduction

Our understanding of chemical transmission has been primarily influenced by the action of acetylcholine (ACh) at the neuromuscular junction, where ACh is released into the synaptic gap, diffuses 10–20 nm and interacts with intrasynaptic receptors of relatively low affinity. Over the years, functional, neurochemical and morphological evidence has shown that this assumption needs to be redefined, given that many neurotransmitters can diffuse out of the synaptic cleft to distant target receptors, and that the majority of transmitters may also be released from non-synaptic terminals.

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Received 17 July 2009; revised 31 October 2009; accepted 4 December 2009

The original observations that established the theory of non-synaptic transmission are both of functional (neurochemical) and morphological nature. During the 1950s and 1960s, the picture regarding neuronal communication appeared to be clear. Neurons were believed to communicate using chemicals to bridge the gaps between the pre- and postsynaptic sites of the synapse. Action potentials (APs) were understood to arrive at synaptic terminals in order to release transmitters and to transmit messages from one neuron to another, or between a neuron and its target cell. Based on the all-or-nothing nature of action potentials, the brain was thought to be a predominantly digital device of discrete computational units. This idea was held in general agreement until the end of the 1960s, when some novel discoveries broke the rule of synaptic transmission and challenged the dogma that the synapse is the exclusive site for the cell-to-cell information processing. Neurochemical evidence was obtained that noradrenaline (NA) released from

noradrenergic nerves presynaptically inhibits the release of ACh from the cholinergic interneurons in the Auerbach plexus and cerebral cortex (Vizi, 1968; Paton and Vizi, 1969; Vizi and Knoll, 1971). Light microscopic studies have shown a 'mismatch' between release sites and the locations of corresponding receptors (Herkenham, 1987; 1991). Electron microscopic ultrastructural studies have indicated that the majority of monoaminergic and cholinergic boutons in the CNS lack junctional complexes (Hökfelt, 1968; Descarries *et al.*, 1975; 1977).

These observations led to the suggestion (Vizi, 1974; 1980a,b; 1984) that in addition to synaptic chemical communication, there is another form of interaction among neurons. This interaction occurs when neurons release chemicals that diffuse far away in order to send messages without the need for close synaptic arrangements, thereby challenging the traditional view of synaptic transmission (Vizi, 1974; 1980a,b; 1984; Schmitt, 1984; Agnati *et al.*, 1986; 1995; Fuxe and Agnati, 1991; Bach-y-Rita, 1993; Zoli *et al.*, 1998; Descarries and Mechawar, 2000).

In this review, we aim to emphasize the fact that most CNS drugs primarily affect the non-synaptic system. In the periphery, several drugs are classic agonists and antagonists (such as adrenergic agonists and antagonists, cholinergic agonists, antimuscarinic agents, ganglionic blockers, and neuromuscular blockers); in the CNS, however, the typical drugs are modulators, and their targets are non-synaptic receptors, transporters, enzymes and ion channels. Non-synaptic receptors are more affected not only because they outnumber synaptic ones, but also because they are more sensitive to fine modulation of ambient concentrations of transmitters present in the extracellular space compared with receptors in synapses. In spite of these facts, many textbooks and some reviews (e.g. Krishnan and Nestler, 2008) still fail to discuss this form of interneuronal communication, indicating such ideas as 'increases in the amount of *synaptic* monoamines induced by antidepressants' have possible therapeutic effects.

This review attempts to summarize the evidence that has started to change and has already begun to shape our understanding of chemical transmission both between neurons and between neurons and non-neuronal target cells.

Neurochemical and morphological evidence for non-synaptic communication

Neurochemical evidence has demonstrated that there is a functional interaction between the noradrenergic and cholinergic axon terminals, such that NA released from noradrenergic neurons is able to inhibit the release of ACh (Vizi, 1968; 1972; 1974; 1979; 1980a,b; Paton and Vizi, 1969; Knoll and Vizi, 1970; 1971; Vizi and Knoll, 1971) despite the lack of synaptic contact between the two terminals (Silva *et al.*, 1971; Gordon-Weeks, 1982). Noradrenergic inhibition of ACh release can be viewed as presynaptic modulation, and, when compared with regulation at the postsynaptic receptor level, offers the physiologic advantage of economy in chemical transmission and the presynaptic inhibitory interaction. Removal of the noradrenergic control of cholinergic transmission by chemical lesion of the noradrenergic innervation or the administration of α_2-adrenoceptor antagonists resulted in a steady state increase of ACh release from the Auerbach plexus (Vizi, 1968; Paton and Vizi, 1969) and from isolated cerebral cortex slices (Vizi, 1974; 1980a). The results indicated that there was a tonic inhibition of ACh release by NA that was being continuously released into the fluid present in the extracellular space surrounding cholinergic release sites. Overall, it was suggested (Vizi, 1974) that '. . . there is morphological and histological evidence (Silva *et al.*, 1971) that the adrenergic terminals terminate not far from the cholinergic ones. This enables the released NA to reach the cholinergic nerve endings by diffusion and to act there via α-adrenoceptor'. A similar interaction was shown for dopamine (DA) and ACh release in the striatum (Vizi *et al.*, 1977), where DA inhibited the axonal stimulation-induced release of ACh via tonic activation of the D_2 receptor located on the axons of cholinergic cells. Inhibition of DA release or the removal of the nigrostriatal pathway resulted in a significant increase in ACh release, indicating the tonic influence on the release (Vizi *et al.*, 1977). This was the first evidence of the disinhibitory mechanism with no synaptic contacts among neurons. These observations validate a persistent tonic influence on neuronal activity at the presynaptic level (Vizi and Labos, 1991). Furthermore, they illustrate that even without synaptic connections, it is still possible to create functional interactions between two nerves that are equipped with different transmitter machineries, provided that the target neurons are equipped with receptors sensitive to the transmitter released from another neuron. Further evidence has shown that non-synaptic communication is a fundamental element of neuro-endocrine (Vizi *et al.*, 1992b; 1993) and neuro-immune (Vizi *et al.*, 1995; Elenkov *et al.*, 2000) interactions.

Non-synaptic varicosities

Over half of the 100 billion neurons in the CNS release glutamate (Glu), and 30–40 billion release GABA (γ-aminobutyric acid) into the synaptic gap (van der Zeyden *et al.*, 2008). Furthermore, almost all neurons express receptors that are sensitive to Glu (van der Zeyden *et al.*, 2008). Glutamatergic and GABAergic neurons were thought to release their transmitters exclusively in synapses (Freund and Buzsaki, 1996). The exception to the rule has recently been discovered for GABAergic varicosities. Neurogliaform cells of the neocortex were found to release GABA from predominantly non-synaptic varicosities (Olah *et al.*, 2009). On the other hand, the overwhelming majority of monoaminergic neurons fail to make synaptic contacts (Descarries and Mechawar, 2000). A common feature of the monoaminergic system is that the cell bodies are located in small subcortical nuclei (for example, the locus coeruleus for noradrenergic, the substantia nigra for dopaminergic and the raphe nuclei for serotonergic neurons), but the varicose axon arborizations diffusely innervate large target areas, thereby creating vast neuronal assemblies (Figure 1). As revealed by concordant light microscopic fluorescence, radioautographic and immunocytochemical studies, preterminal monoaminergic axons branch profusely, and give rise to billions of varicose nerve endings without making synaptic contact (Table 1).

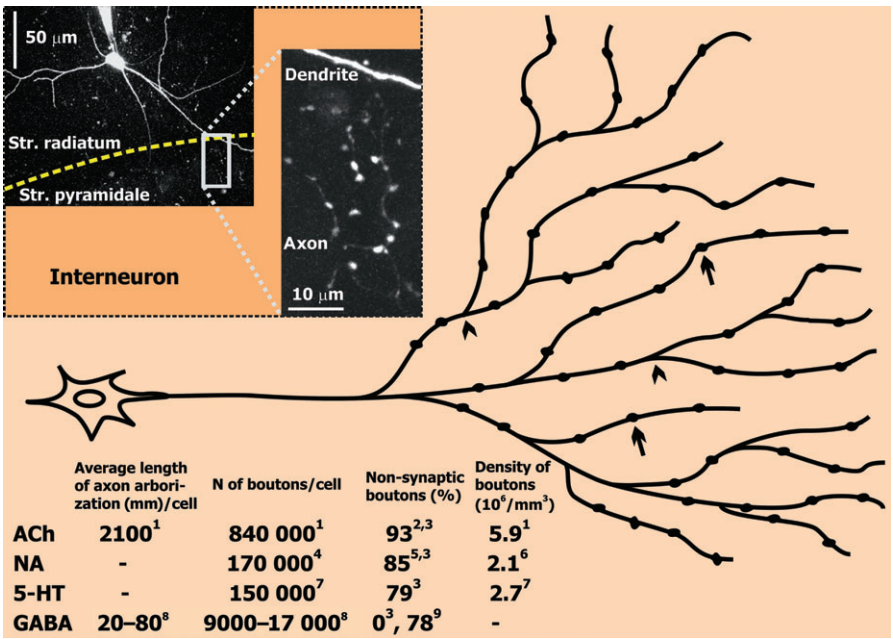

Figure 1 Scheme of the arborization of different neurons in the hippocampus. Arrows indicate varicosities, while the arrowheads show the bifurcation of branches. The average length of axonal arborization (mm) per cell body, number of boutons per cell body, percentage of non-synaptic boutons and density of boutons are given ($10^6 \cdot mm^{-3}$). [1]Aznavour *et al.* (2002), [2]Descarries *et al.* (1997), [3]Umbriaco *et al.* (1995), [4]Vizi and Kiss (1998), [5]Hökfelt (1968), [6]Oleskevich *et al.* (1989), [7]Descarries *et al.* (1990), [8]Gulyas *et al.* (1993), [9]Olah *et al.* (2009). The inset shows axonal varicosities of a GABAergic interneuron from the rat hippocampus (A. Barth, N. Hájos and E.S. Vizi, Institute of Experimental Medicine, unpublished data) using a two-photon laser-scanning microscope (for method see Rozsa *et al.* 2004 and Vizi *et al.*, 2004a). Scale bars are 10 and 50 μm, as indicated.

Table 1 Different neuronal projections in the brain without making synaptic contacts

Projections	*% of non-synaptic varicosities*	*Location*	*References*
Cholinergic	79–90	Somatosensory cortex	(Umbriaco *et al.*, 1994)
	86	Cerebral cortex	(Descarries *et al.*, 1997)
	93	Hippocampus (CA1)	
	91	Neostriatum	
	33	Human cerebral cortex	(Smiley *et al.*, 1997)
Noradrenergic	85	Hippocampus (CA1)	(Hökfelt, 1968; Umbriaco *et al.*, 1995)
	95	Cerebral cortex	(Descarries *et al.*, 1977)
	>60	Dorsal horn	(Ridet *et al.*, 1993)
	90	Frontal cortex	(Aoki *et al.*, 1998)
Dopaminergic	60–70	Neostriatum	(Descarries *et al.*, 1996; 2008; Wilson *et al.*, 1977)
		Median eminence and cerebral cortex	(Calas *et al.*, 1974; Descarries *et al.*, 1975; Tao-Cheng and Zhou, 1999)
	82	Raphe nucleus	(Kosofsky and Molliver, 1987)
Serotonergic	97	Somatosensory cortex	(DeFelipe and Jones, 1988)
	>60	Dorsal horn	(Ridet *et al.*, 1993)
	~50	Suprachiasmatic and supraoptic nuclei of hypothalamus	(Boulaich *et al.*, 1994)
	79	Hippocampus (CA1)	(Umbriaco *et al.*, 1995)
	90	Prefrontal cortex	(Smiley and Goldman-Rakic, 1996)
	>60	Spinal cord	(Alvarez *et al.*, 1998)
	80	Periaqueductal gray matter	(Lovick *et al.*, 2000)
Glutamatergic	0		(Freund and Gulyas, 1991)
Gabaergic	0	Hippocampus (CA1)	(Freund and Gulyas, 1991; Umbriaco *et al.*, 1995)
	78	Neurogliaform cells in the cortex	(Olah *et al.*, 2009)

Experiments are carried out in rats if otherwise not stated.

It has been calculated that each noradrenergic cell body that ipsilaterally innervates the CNS (700 per hemisphere in rats; Descarries and Saucier, 1972) has an axonal arborization, with an average of 170 000 varicosities within the hippocampus (Vizi and Kiss, 1998). With regard to cholinergic innervation of the hippocampus, the number is even higher: a single cholinergic cell (located in the median septum, or the diagonal band of Broca) has an axonal arborization that is

~2.1 m in total length within the ~56 mm^3 of the hippocampus, and is equipped with ~840 000 varicosities (Figure 1; Aznavour *et al.*, 2002). The majority of these varicosities exist mainly without synaptic contact (Descarries and Mechawar, 2000). Clearly, when an action potential is generated at the axon hillock, it will not cause release from all the ~840 000 varicosities. Furthermore, the cell body cannot dictate which of the specific varicosities should release transmitter. Rather, release probability is a subject of local modulation, and, as was shown for the innervation of the vas deferens, where ATP and NA are co-transmitters, low release probability is not due to failure of AP propagation but to a variable reliability of depolarization–secretion coupling (Jackson and Cunnane, 2001). This emphasizes the importance of local modulation of transmitter release; even though the AP propagates, whether it evokes release or not depends on the local environment of individual varicosities. It is also possible, however, that release is prevented by shutting down entire axonal branches (Debanne *et al.*, 1997; Kress and Mennerick, 2009). GABA-mediated increases in membrane conductance may be an important mechanism to control AP propagation to axonal branches (Segev, 1990; Verdier *et al.*, 2003).

By means of quantitative autoradiographic studies, the existence of non-synaptic communication in the CNS has been structurally shown. Monoaminergic (Hökfelt, 1968; Descarries *et al.*, 1975; Descarries *et al.*, 1977; Ridet *et al.*, 1993; Descarries *et al.*, 1996; Lovick *et al.*, 2000) and cholinergic axon terminals (Umbriaco *et al.*, 1995; Descarries and Mechawar, 2000) were found without synaptic contact, yet they are still equipped with releasing machinery. Only a small percentage (5–40%) of the varicosities formed synapses (Descarries *et al.*, 1975; Ridet *et al.*, 1993; Umbriaco *et al.*, 1995; Alvarez *et al.*, 1998; Lovick *et al.*, 2000). The overwhelming majority of non-synaptic boutons appear to have all the apparatuses that are associated with AP-dependent vesicular release and are able to release transmitters for long-distance diffusion. These findings indicate that the field of influence for a single neuron, which collects several hundreds or thousands of inputs, could be very large (Figure 1, Table 1).

It is interesting to note that all of the dopaminergic varicosities in the neostriatum or nucleus accumbens, which contain the vesicular transporter for Glu in addition to tyrosine hydroxylase and might therefore release Glu as a co-transmitter, make synaptic contacts (Descarries *et al.*, 2008). Other varicosities that contain only DA in their release machineries, however, fail to make synapses (Descarries *et al.*, 1996). The varicosities of serotonergic innervation that originate from the median raphe always form synapses with hippocampal interneurons (Freund and Buzsaki, 1996; Varga *et al.*, 2009); however, varicosities from the dorsal raphe do not make any synaptic contacts in the hippocampus (Kosofsky and Molliver, 1987). The experiments of Freund *et al.* (1985) may provide some explanation for this discrepancy. After reimplantation of substantia nigra into rats, in which the substantia nigra was previously removed, the nigro-striatal neurons, which otherwise do not synapse with other neurons (Wilson *et al.*, 1977) have made synapses 100% with cholinergic interneurons. It therefore appears very likely that during development, the time of innervation plays an important role in setting the synapse.

Non-synaptic receptors

In addition to synaptic localization, there are receptors located non-synaptically. Their presence and capability of receiving chemical messages can be shown by morphological and functional methods (Table 2).

The current terminology for the location-based classification of receptors is inconsistent on several points. In this review, we do not intend to reform the terminology, and we will use the terms that seem the most logical and widely accepted. Still, we cannot avoid pointing out the inconsistencies. There are two major classification schemes based on both subcellular localization and function. One classification of receptors distinguishes synaptic and non-synaptic receptors. Non-synaptic receptors are sometimes referred to as extrasynaptic; however, we prefer to use the term extrasynaptic in a less inclusive manner, distinguishing synaptic, perisynaptic and extrasynaptic localizations (with the latter two groups both being non-synaptic). Perisynaptic receptors are located at the edges of synapses, while extrasynaptic receptors are located farther away. The perisynaptic–extrasynaptic boundary is rather subjective; thus, we propose to define it functionally. Perisynaptic receptors are those that are able to detect individual release events; therefore, the precise timing of digital information coding can be distinguished by these receptors. For extrasynaptic receptors, being far from the release sites, the precise temporal information is lost, and they can only follow the analogue signals, such as tonic changes in the ambient levels of the transmitter. The spatial limits of digital information exchange are termed the 'maximum effective radius' (Rice and Cragg, 2008), which is defined as the distance within which the release of one quantum of a transmitter reaches the EC50 concentration of the receptor. We propose that non-synaptic, somatodendritic receptors within the maximum effective radius should be termed perisynaptic. Unfortunately, we have no appropriate term for non-synaptic receptors located near non-synaptic release sites.

The other classification scheme is to distinguish presynaptic (axonal) and postsynaptic (somatodendritic) receptors. Presynaptic, axonal receptors are responsible for local modulation of depolarization-release coupling, including propagation of APs and modulation of release probability. Postsynaptic, somatodendritic receptors, on the other hand, modulate the integration of inputs, that is they decide whether or not inputs translate into APs. Unfortunately, we have no correct term for axonal and somatodendritic receptors in cases where there are no synapses; therefore, we must call these receptors 'presynaptic' and 'postsynaptic', respectively, despite the lack of morphologically distinguishable synapses. Presynaptic receptors can be further divided into autoreceptors (i.e. receptors that are sensitive to the neuron's own transmitter, which is termed as 'negative feedback' modulation) or heteroreceptors (i.e. receptors conveying the influence of other transmitters and neuromodulators).

Axonal receptors are sometimes divided into two functional categories, presynaptic and preterminal (Wonnacott, 1997). In this terminology, presynaptic receptors are only those

Table 2 Non-synaptic localization and site of action of different ionotropic and metabotropic receptors (subunits are indicated if needed)

Receptors	*Region in the brain*	*Site of action*	*References*
Ionotropic			
$GABA_A$	Cerebellum	Synaptic and non-synaptic expression in the somatodendritic compartment of granule cells; no expression in axons, nerve terminals or glial cells	(Somogyi *et al.*, 1989; Nusser *et al.*, 1995)
	Dorsal lateral geniculate nucleus	Synaptic and non-synaptic expression	(Soltesz *et al.*, 1990)
$GABA_A$ (δ)	Cerebellum	Exclusive extrasynaptic location in granule cells	(Nusser *et al.*, 1998)
$GABA_A$(α_4)	Hippocampus Dentate gyrus	Mainly perisynaptic; switch to synaptic in a model of ethanol withdrawal and dependence	(Liang *et al.*, 2006)
$GABA_A$(β_2)	Dentate gyrus	Extrasynaptic in granule cells	(Herd *et al.*, 2008)
$GABA_A$(β_3)	Dentate gyrus	Synaptic in granule cells	(Herd *et al.*, 2008)
AMPA	Hippocampus Dentate gyrus	Postsynaptic membrane specialization; in a lower number extrasynaptically on spines, dendrites, somata	(Baude *et al.*, 1995)
AMPA (GluR1)	Cerebellum	Synaptic localization; intracellular and glia cell labeling	(Baude *et al.*, 1994)
Kainate (GluR6/7)	Striatum	>70% intracellular; presynaptically both in the active zone (~20%) of asymmetric synapses and at non-synaptic sites (~80%); on spines in asymetric synapses (~40%) and at non-synaptic localization (~60%)	(Kieval *et al.*, 2001)
Kainate (KA2)	Striatum	>70% intracellular; presynaptically both in the active zone (~30%) of asymmetric synapses and at non-synaptic sites (~70%); on spines in asymetric synapses (~40%) and at non-synaptic localization (~60%)	(Kieval *et al.*, 2001)
NR2A	Hippocampus Cortex Thalamus	Mainly synaptic localization following postnatal development	(Kew *et al.*, 1998; Tovar and Westbrook, 1999; Liu *et al.*, 2004b; Thomas *et al.*, 2006)
NR2B	Hippocampus Cortex Thalamus	Mainly extrasynaptic localization following postnatal development (synaptic and extrasynaptic until postnatal development)	(Kew *et al.*, 1998; Tovar and Westbrook, 1999; Liu *et al.*, 2004b; Thomas *et al.*, 2006)
NR2C	Cerebellum (granule cell) Cortex (spiny stellate neuron)	Synaptic localization	(Rossi *et al.*, 2002; Binshtok *et al.*, 2006)
NR2D	Hippocampus Dorsal horn	Extrasynaptic localization	(Momiyama *et al.*, 1996; Momiyama, 2000; Lozovaya *et al.*, 2004; Harney *et al.*, 2008)
NR3A	Limited expression in rodent, but widespread expression in human		Eriksson *et al.*, 2007)
nACh (α_7)	Hippocampus	Presynaptic terminals in cultured neurons	(Fabian-Fine *et al.*, 2001)
	Ventral tegmental area	Glutamatergic terminals (perisynaptic)	(Jones and Wonnacott, 2004)
	Striatum	Nigro-striatal terminals	
	Cerebellum	Perisynaptic	(Caruncho *et al.*, 1997)
	Hippocampus	Soma	(Zarei *et al.*, 1999)
	Ventral tegmental area	Glutamatergic nerve terminals	(Jones *et al.*, 2001)
nACh	Chicken pretectum		(Ullian and Sargent, 1995)
Metabotropic (G-protein-coupled)			
α_{2A}	Prefrontal cortex	Preterminal axon	(Aoki *et al.*, 1998; Abbracchio *et al.*, 2009)
	Hippocampus	Axon terminal, glia	(Smiley *et al.*, 1994; Milner *et al.*, 1998)
D_1	Cerebral cortex Basal ganglia	Spiny dendrite, perikarya	(Smiley *et al.*, 1994; Yung *et al.*, 1995)
D_2	Midbrain Basal ganglia, subst. Nigra	Axon terminals, spines, dendrites (auto- and heteroreceptors)	(Sesack *et al.*, 1994; Yung *et al.*, 1995)
5-HT_{1A}	Substantia nigra	Cell body, dendrite	(Riad *et al.*, 2000)
5-HT_{1B}	Substia-nigra Globus pallidus	Preterminal axon	(Riad *et al.*, 2000)
$GABA_B$	Cerebellum	Purkinje cell Dendrites and spines	(Scanziani, 2000; Kulik *et al.*, 2006; Lujan and Shigemoto, 2006)
	Cortex	Pyramidal cell dendrite and spines	(Lopez-Bendito *et al.*, 2002)

Table 2 *Continued*

Receptors	*Region in the brain*	*Site of action*	*References*
CB1	Hippocampus	Preterminal axon of cholecystokinin-containing gabaerg interneuron	(Katona *et al.*, 1999)
A_1	Striatum Hippocampus Widely distributed	Pre- and postsynaptic	(Wu and Saggau, 1997; Ciruela *et al.*, 2006)
A_{2A}	Striatum Hippocampus Cerebral cortex Widely distributed	Pre- and postsynaptic	(Johansson and Fredholm, 1995; Cunha *et al.*, 1996; Rosin *et al.*, 1998; Ciruela *et al.*, 2006)
mGlu1	Hippocampus Cerebellum Neocortex	Concentrated in a perisynaptic annulus of postsynaptic membrane; extrasynaptically at spines, dendrites and somata	(Baude *et al.*, 1993; Martin *et al.*, 1992; Lujan *et al.*, 1996; 1997)
mGlu2	Hippocampus Cerebellum	Extrasynaptically along axons and axon terminals	(Lujan *et al.*, 1997; Shigemoto *et al.*, 1997; Ohishi *et al.*, 1998)
mGlu3	Hippocampus Cerebellum Striatum Thalamus	Extrasynaptically along axons and axon terminals; associated with asymmetrical synapses both in the postsynaptic specialization and in a perisynaptic position, glial processes	(Shigemoto *et al.*, 1997; Tamaru *et al.*, 2001)
mGlu4	Striatum Cerebellum Hippocampus Thalamus	Presynaptic active zone, postsynaptic density, spines, dendrites and somata	(Bradley *et al.*, 1996; Shigemoto *et al.*, 1997; Corti *et al.*, 2002)
mGlu5	Hippocampus Cerebellum Striatum	Concentrated in a perisynaptic annulus of postsynaptic membrane; extrasynaptically at spines, dendrite and somata	(Lujan *et al.*, 1996; 1997; Uchigashima *et al.*, 2007)
mGlu6	Retina	Somata, Located on dendrites of on-bipolar cells far from the release site (400–800 nm)	(Nakajima *et al.*, 1993; Nomura *et al.*, 1994; Vardi *et al.*, 2000)
mGlu7	Hippocampus Dorsal horn	Presynaptic active zone	(Ohishi *et al.*, 1995; Shigemoto *et al.*, 1997)
mGlu8	Hippocampus	Presynaptic active zone	(Shigemoto *et al.*, 1997)
M1	Striatum Cerebral cortex	Extrasynaptically at somata, spines and dendrites; postsynaptic densities of asymmetric and symmetric synapses	(Mrzljak *et al.*, 1993; Hersch *et al.*, 1994; Alcantara *et al.*, 2001; Narushima *et al.*, 2007; Uchigashima *et al.*, 2007)
M2	Striatum Cerebral cortex Nucleus basalis magnocellularis	Extrasynaptically at somata, dendrites; postsynaptic densities of asymmetric and symmetric synapses; presynaptic terminals of asymmetric and symmetric synapses; non-synaptic varicosities and perikarya of cholinergic neurons	(Mrzljak *et al.*, 1993; Hersch *et al.*, 1994; Bernard *et al.*, 1998; Rouse *et al.*, 2000; Decossas *et al.*, 2003)
M3	Striatum	Postsynaptic densities of asymmetric synapses; presynaptic terminals of asymmetric synapses	(Hersch *et al.*, 1994)
M4	Striatum	Extrasynaptically at somata, spines and dendrites; postsynaptic densities of asymmetric synapses; presynaptic terminals of asymmetric synapses; non-synaptic sites of cholinergic perikarya	(Hersch *et al.*, 1994; Bernard *et al.*, 1999)
M5			(Yamada *et al.*, 2003)

located in close proximity to the synapse (possibly even within the synaptic cleft), and their activation results in transmitter release even in the absence of functional axonal sodium channels. Thus, the release is tetrodotoxin (TTX) insensitive. Release evoked by preterminal receptors, on the other hand, requires axonal sodium channels to amplify and conduct the signal; therefore, it is TTX sensitive. This classification, however, can only be used for facilitatory axonal receptors, that is when receptor activation evokes transmitter release. Furthermore, the term presynaptic is more often used in a more general way, including receptors with different axonal locations.

In summary, we can conclude that, thus far, there is no logical terminology for non-synaptic receptors. We have no better option but to call certain groups 'perisynaptic' and 'presynaptic' even when they are connected not to morphologically observable synapses but to non-synaptic release sites. This inconsistency originates from the fact that, until recently, non-synaptic communication was peripheral in scientific thinking, and synaptic communication was used as a synonym of interneuronal communication. Thinking influences terminology, and terminology might have also influenced thinking.

Cell surface exchange of receptors between synaptic and non-synaptic sites (receptor trafficking)

At the neuromuscular junction the nicotinic acetylcholine receptors (nAChRs) are trapped at the synapse on the postsynaptic site, and are not localized extrasynaptically. Upon

innervation of skeletal muscle, nAChRs congregate in the junctional end-plate area. When the motor nerve has been severed, the nAChRs start to move away from the synapse and the receptors become distributed across the whole surface of the striated muscle (Axelsson and Thesleff, 1959).

A key mechanism regulating synaptic efficiency in the CNS is receptor trafficking composed of the lateral (between synaptic and non-synaptic compartments) and vertical (mobility between intracellular stores and cell surface by endocytosis and exocytosis) mobility of receptors described for many receptor types (Lau and Zukin, 2007; Groc *et al.*, 2009; Jaskolski and Henley, 2009). Formerly, vertical mobility has been considered the mechanism that promotes rapid changes in receptor number at postsynaptic density, however, recently, a complementary hypothesis has emerged. According to this, the mobility of receptors in/out of synapses is composed of two phases: (i) lateral diffusion of receptors in/out of the synapse, (ii) exocytosis/endocytosis of receptors in/out of the non-synaptic compartment (Triller and Choquet, 2005; Yudowski *et al.*, 2007). The finding of endocytic zones on spines lateral from synapse (Blanpied *et al.*, 2002) reveal that lateral diffusion of receptors between synapse and non-synaptic regions may be the part of the process of receptor recycling (Lau and Zukin, 2007).

Examination of Glu, GABA and glycine receptor trafficking is a prominent field of neuroscience. Glu receptor (NMDA, AMPA) trafficking into and out of synapses is influenced by neuronal activity (Lau and Zukin, 2007; Frischknecht *et al.*, 2009), and is an important mechanism to change the number of functional receptors in the synapse during plasticity (Choquet and Triller, 2003). The synaptic scaffolding proteins (e.g. PSD-95, PSD-93, stargazin) are involved in the stabilization of NMDA and AMPA receptors at the synapse (Schnell *et al.*, 2002; Lau and Zukin, 2007; Santos *et al.*, 2009). Synaptic $GABA_A$ and glycine receptors are also directly recruited from their non-synaptic counterparts and maintained in the synapse by direct binding to the multifunctional protein gephyrin (Meier *et al.*, 2001; Thomas *et al.*, 2005; Jacob *et al.*, 2008). This highly dynamic and regulated exchange between synaptic and non-synaptic membrane (Triller and Choquet, 2003) influences the number of non-synaptic receptors, thereby it may also affect synaptic plasticity and the effectiveness of medicines.

G-protein-coupled receptors (Table 2)

Peptidergic receptors. Neuropeptides are typical non-synaptic transmitters, which are released extrasynaptically (Torrealba and Carrasco, 2004; Wotjak *et al.*, 2008). Because of the large number of neuropeptides, in this paper, we are unable to give a detailed discussion of peptidergic transmission.

Adrenoceptors. Noradrenergic neurotransmission is mostly non-synaptic in the CNS (Figure 1, Table 1). Of the nine subtypes of adrenoceptors (α_{1A}, $_B$ and $_D$, α_{2A}, $_B$ and $_C$, β_1, β_2 and β_3), α_2 receptors are the most important in presynaptic modulation (Gilsbach and Hein, 2008). An electron microscopic immunocytochemical study has shown that α_{2A}-adrenoceptors are located mostly on extrasynaptic areas, including axons, dendrites and astrocytic membranes in the prefrontal cortex (Aoki *et al.*, 1998). β-adrenoceptors expressed on noradrenergic axons (van Zwieten and Timmermans, 1983) and on immune cells (Elenkov *et al.*, 2000) have been shown to be localized extrasynaptically.

Dopamine receptors. Morphological evidence for the non-synaptic role of DA in the CNS includes the sparsity of dopaminergic synapses (Table 1) in the striatum and substantia nigra (Wilson *et al.*, 1977), as well as the predominance of extrasynaptic DA receptors (Sesack *et al.*, 1994; Yung *et al.*, 1995; Table 2).

Serotonin (5-HT, 5-hydroxytryptamine) receptors. Serotonergic transmission in the CNS is predominantly non-synaptic (Figure 1, Table 1). Using immunogold labelling, extrasynaptic localization was shown for the $5\text{-}HT1_A$ and $5\text{-}HT1_B$ receptors. Both receptors are negatively coupled to adenylate cyclase. While $5\text{-}HT1_A$ immunoreactivity was found on mostly non-synaptic locations of cell bodies and dendrites, $5\text{-}HT1_B$ receptors were preferentially associated with unmyelinated preterminal axons, but were not found on axon terminals (Riad *et al.*, 2000). These receptors can be both inhibitory auto- and heteroreceptors.

GABAB receptors. $GABA_B$ receptors are largely located non-synaptically (Scanziani, 2000; Kulik *et al.*, 2006), and are also abundant in axons (Kulik *et al.*, 2003; Vigot *et al.*, 2006). On the postsynaptic Purkinje cells of the cerebellum, $GABA_{B1}$ and $GABA_{B2}$ receptors are localized to the extrasynaptic and perisynaptic plasma membrane (Lujan and Shigemoto, 2006).

Metabotropic glutamate receptors (mGlu). The mGlu receptors consist of eight subtypes classified into three subgroups. Group I receptors (mGlu1 and 5) couple to $G_{q/11}$ protein and induce LTD (Bellone *et al.*, 2008), group II (mGlu2 and 3) and group III receptors (mGlu4, 6, 7 and 8) inhibit cAMP formation through G_i protein (Bellone *et al.*, 2008) and suppress transmitter release (Scanziani *et al.*, 1995; Takahashi *et al.*, 1996; Giustizieri *et al.*, 2005; see Figure 2 and Table 2 for localization).

Muscarinic ACh receptors (mAChRs). Cholinergic neurotransmission is mainly non-synaptic in the CNS (Figure 1, Table 1). The five mAChR subtypes are referred to as M_1–M_5. The M_1, M_3, M_5 receptors couple to PLC through $G_{q/11}$ activation, while M_2 and M_4 inhibit adenylyl cyclase via G_i protein (Caulfield and Birdsall, 1998). Species- (Plummer *et al.*, 1999), age- (Decossas *et al.*, 2005), region- and cell-specific differences (Rouse and Levey, 1996; Nathanson, 2008) in the subcellular distribution of various subtypes of mAChRs and caveats in the immunocytochemical techniques (Nathanson, 2008) obscure the ability to distinguish between receptor subtypes (for detailed localization data, see Table 2).

P2Y receptors. P2Y receptors have two distinct subgroups. The first contains $P2Y_{1,2,4,6}$ and $_{11}$, which couple to $G_{q/11}$ protein, and the second constitutes $P2Y_{12,13,14}$, and inhibits adenylyl cyclase through G_i protein activation (Abbracchio *et al.*, 2009). P2Y receptors have been shown to be present at presynaptic, postsynaptic and extrasynaptic sites in the

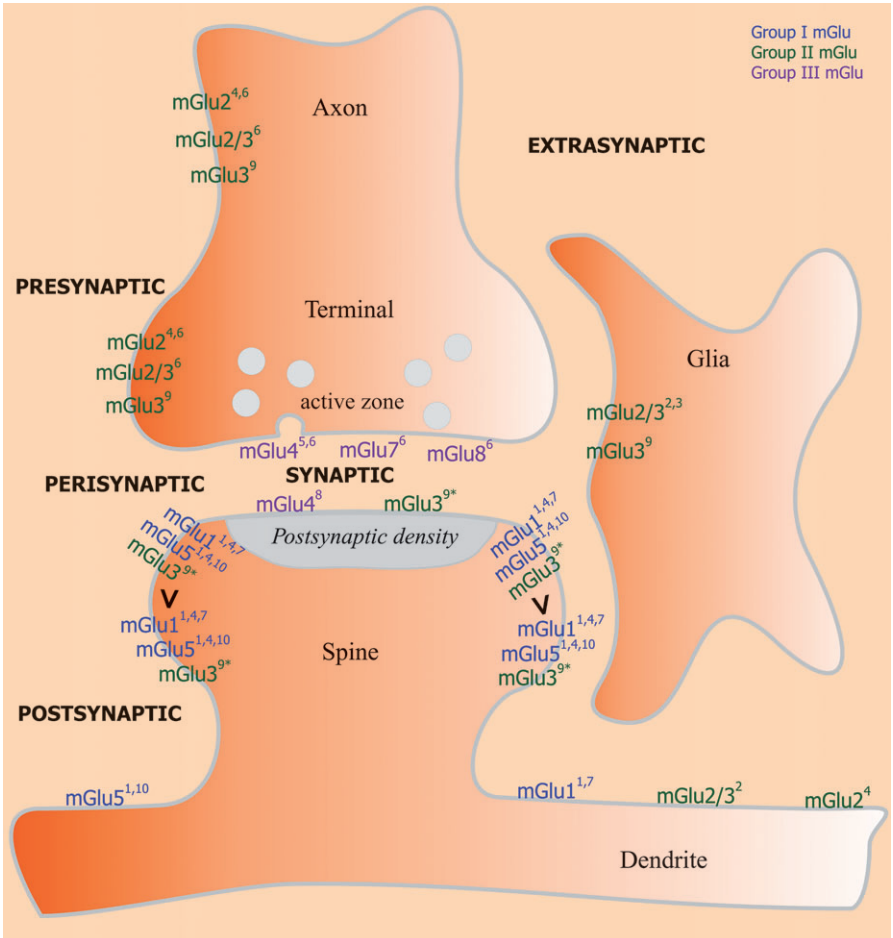

Figure 2 Subcellular localization of mGlu receptors at synaptic and non-synaptic (perisynaptic or extrasynaptic) sites. Group I mGlu receptors are concentrated at the postsynaptic membrane in a perisynaptic annulus, but are also present extrasynaptically. Group II mGlu receptors are located extrasynaptically along axons and axon terminals, while group III mGlu receptors are expressed in the presynaptic active zone. [1]Lujan *et al.* (1996), [2]Ohishi *et al.* (1994), [3]Petralia *et al.* (1996), [4]Lujan *et al.* (1997), [5]Corti *et al.* (2002), [6]Shigemoto *et al.* (1997), [7]Baude *et al.* (1993), [8]Bradley *et al.* (1996), [9]Tamaru *et al.* (2001), [10]Uchigashima *et al.* (2007). *mGlu3 can be found in a postsynaptic localization in the hippocampus and striatum but not in the ventrobasal nuclear complex of the thalamus.

hippocampus and in glial cells underlying the non-synaptic action of ATP (Fields and Stevens, 2000; Rodrigues *et al.*, 2005; Hussl and Boehm, 2006; Sperlagh *et al.*, 2007a). P2Y receptors also have a prominent role in the sensory pathways (Housley *et al.*, 2009). Many immunocytochemical data, however, should be handled with caution due to the lack of verification of the specificity (Sperlagh *et al.*, 2007a).

Adenosine receptors. The extracellular nucleotides are degraded by ectoenzymes to adenosine (Zimmermann, 2008), which in turn acts on postsynaptic metabotropic receptors ($A_{1,\ 2A,\ 2B,\ 3}$) without any postsynaptic morphological arrangements. The high-affinity A_1 and A_{2A} receptors (affinities in μM: A_1, ~0.07; A_{2A}, ~0.15; A_{2B}, ~5.1; A_3, ~6.5) are primarily responsible for the effects of adenosine in the brain, because basal extracellular adenosine concentrations (0.01–0.5 μM) are sufficient to activate them tonically (Latini *et al.*, 1999; Dunwiddie and Masino, 2001). In addition to their postsynaptic location, A_1 (Johansson and Fredholm, 1995; Wu and Saggau, 1997) and A_{2A} (Rosin *et al.*, 1998) receptors have been described presynaptically (Table 2; Ciruela *et al.*, 2006). The first evidence that adenosine and nucleotides are able to inhibit AP-induced transmitter release from cholinergic terminals was shown in the cerebral cortex and the Auerbach plexus (Vizi and Knoll, 1976). Presynaptic A_1 receptor activation reduces transmitter release mainly through the inhibition of Ca^{2+} channels and activation of K^+ conductances (Milusheva *et al.*, 1990; Yawo and Chuhma, 1993; Haas and Selbach, 2000), while A_{2A} receptors enhance neurotransmitter release (Popoli *et al.*, 1995; Nishizaki *et al.*, 2002; Cunha, 2008). Adenosine influences immunity via activation of A_{2B} receptor (Hasko *et al.*, 2009). A_{2A} receptors are also found in glial cells (Hettinger *et al.*, 2001).

Cannabinoid CB1 receptors. Major physiological effects of cannabinoids (Freund and Katona, 2007) can be linked to the regulation of neurotransmitter release via presynaptic CB1 receptors (Katona *et al.*, 1999; Freund *et al.*, 2003; Kofalvi *et al.*, 2003; Kofalvi *et al.*, 2005; Schoffelmeer *et al.*, 2006; Katona and Freund, 2008). Immunogold staining methods have shown that CB1 receptors are localized extrasynaptically in the preterminal axons (Katona *et al.*, 1999),

and also in the perisynaptic annulus on the presynaptic terminals, but not in the active zone (Nyiri *et al.*, 2005). Some endocannabinoids (e.g. 2-arachidonoylglycerol, 2-AG) are released from the postsynaptic membrane in response to the activation of primary transmitters (e.g. Glu and 5-HT; Katona *et al.*, 2006; Balazsa *et al.*, 2008). They stimulate extrasynaptic and perisynaptic CB1 receptors and inhibit transmitter release (Wilson and Nicoll, 2002; Lau and Schloss, 2008; Lau *et al.*, 2009) as a function of postsynaptic activity (Katona *et al.*, 2006). Other endocannabinoids such as anandamide, on the other hand, may have a presynaptic origin, since N-acylphosphatidylethanolamine-hydrolysing phospholipase D, which is a recently identified synthesizing enzyme of this bioactive lipid (Okamoto *et al.*, 2007), is localized to the presynaptic site (Nyilas *et al.*, 2008). The presynaptic release of anandamide, however, has not yet been shown.

It is very likely that these non-synaptically located CB1 receptors are the possible targets of endogenous cannabinoids and cannabis, which is used as a recreational drug. The question arises as to whether or not endogenous cannabinoids can tonically activate these CB1 receptors. Since the CB1 antagonist (SR141716A) failed to affect GABA release (Schlicker *et al.*, 1997), it seems likely that there is no tonic influence on GABAergic inhibitory transmission. In contrast, it is very likely that, when taken as a recreational drug of abuse, cannabis can tonically inhibit GABA release, thereby removing the inhibition of DA release by GABA (Ferraro *et al.*, 1996) in the nucleus accumbens. As a consequence of this disinhibitory action of cannabis, the release of DA increases and could then positively influence the reward system (Sperlagh *et al.*, 2009).

Ionotropic receptors (Table 2)

The majority of synapses within the CNS use mainly ionotropic receptors (receptor ion channels, transmitter-gated channels), because these receptors are equipped for fast (submillisecond) activation, making them able of transmitting information with minimal delay. Nevertheless, it has been discovered, that they are also abundant extrasynaptically (see Table 2).

Glutamate receptors. In spite of the predominantly synaptic nature of glutamatergic transmission (Table 1), different types of non-synaptic communication have been proven in various parts of the CNS (Vizi and Mike, 2006). Glutamate that is released into the synaptic gap can 'escape' from the synapse (Asztely *et al.*, 1997; Kullmann, 2000), which enables it to reach extrasynaptic receptors (Pankratov and Krishtal, 2003). This phenomenon is referred to as 'spillover' (Asztely *et al.*, 1997; Rusakov *et al.*, 1999), which could appear at extreme activity of the synapse (Drew *et al.*, 2008).

Ionotropic glutamate receptors are classified into three major groups, AMPA, kainate and NMDA receptors. All three types can be found at extrasynaptic locations (Table 2); however, this does not prove that they are functional extrasynaptically. Extrasynaptic AMPA receptors on neurons (mostly of the same subtype as synaptic receptors – Lu *et al.*, 2009) may only serve as spare receptors: a reservoir for synaptic plasticity. On the other hand, NMDA and kainate receptors show subtype-specific subcellular localization, and different subtypes have been shown to be involved in synaptic and non-synaptic functions.

NMDA receptors have a tetrameric structure that is composed of two NR1 subunits and at least one type of NR2 subunit (A, B, C or D). The NR2A or NR2B subunits (Figure 3)

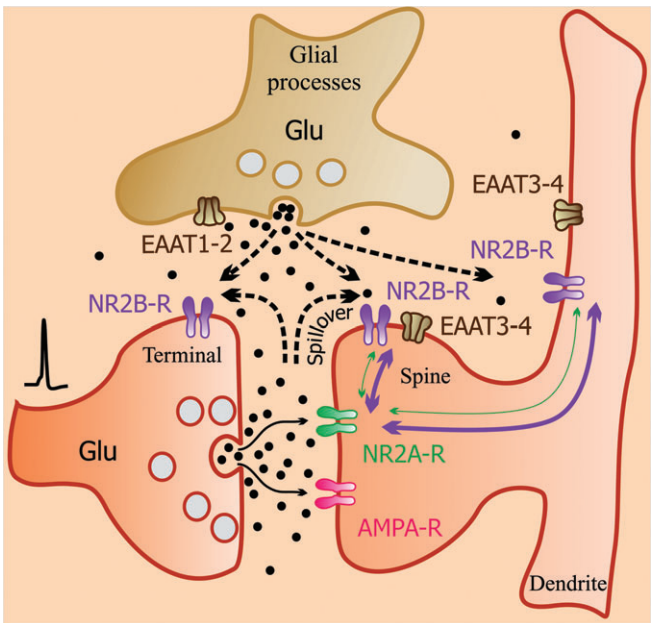

Figure 3 Relevance of NMDA receptor localization composed of NR1/NR2A or NR1/NR2B assemblies. It was proposed that NR1/NR2A receptors, which have a lower affinity for glutamate and glycine than NR1/NR2B receptors (Yamakura and Shimoji, 1999), reside in the synapse, while NR1/NR2B receptors are located at extrasynaptic sites. However, it has been shown that this separation evolves only during postnatal development when the NR1/NR2B receptors exchange for NR1/NR2A receptors in the synapse (Tovar and Westbrook, 1999). This dynamic view of NMDA receptor subtypes was completed by the observation that NMDA receptors can move laterally between synaptic and extrasynaptic sites within the plasma membrane (Tovar and Westbrook, 2002; Triller and Choquet, 2005). Groc *et al.* have shown that NR1/NR2A assemblies diffuse laterally more than two order of magnitude slower than NR1/NR2B receptors (Groc *et al.*, 2009; Groc *et al.*, 2006; see thick purple and thin green arrows representing the mobility of NR1/NR2A and NR1/NR2B assemblies, respectively). The different subcellular locations of NR1/NR2A and NR1/NR2B assemblies determine the source of glutamate being able to activate these receptors. While the NR1/NR2A assemblies are activated by glutamate released in the synapse, the origin of glutamate activating NR1/NR2B assemblies can be: (i) the spillover of synaptic glutamate following high frequency stimulation (Huang, 1998; Vizi and Mike, 2006), (ii) the reversal of glutamate transporters (EAAT1-4) mainly in neurons (Rossi *et al.*, 2007), (iii) the vesicular glutamate release from glial processes in the tripartite synapses (Haydon and Carmignoto, 2006; Perea *et al.*, 2009). The latter is the basis of glia-neuron interaction and can affect both the presynaptic and postsynaptic NR1/NR2B assemblies and either enhances synaptic strength or mediates slow inward currents, respectively (Fellin *et al.*, 2004; Jourdain *et al.*, 2007). Functions of synaptic NR1/NR2A and extrasynaptic NR1/NR2B receptors are opposing to some extent (Haydon and Carmignoto, 2006): (i) NR1/NR2A promote, while NR1/NR2B inhibit AMPA receptor trafficking (Kim *et al.*, 2005), (ii) NR1/NR2A induces the neuroprotective CREB (cAMP response element binding protein) pathway, while NR1/NR2B shuts it down and promotes neuronal death (Hardingham *et al.*, 2002), (iii) NR1/NR2A induces long-term potentiation, while NR1/NR2B seems to be relevant in long-term depression (Liu *et al.*, 2004a; Massey *et al.*, 2004).

are predominant in humans (Wenzel *et al*., 1997; Cull-Candy *et al*., 2001). It has been observed that NR2A subunit-containing NMDA receptors are predominantly located synaptically, whereas NR2B subunits are primarily found in extrasynaptic locations (Stocca and Vicini, 1998; Mohrmann *et al*., 2000; Townsend *et al*., 2003), although this segregation is not an absolute rule (Thomas *et al*., 2006; Gardoni *et al*., 2009). A recent development has shown that NR2B-containing subtype of Glu receptors is also part of the postsynaptic compartment in the synapse, and is involved in the physiological induction of long-term potentiation (LTP; Gardoni *et al*., 2009). Nevertheless, accumulating evidence indicates that the synaptic NR2A receptors mediate synaptic plasticity and cell survival signals, whereas extrasynaptic NR2B receptors are responsible for excitotoxicity (Hardingham and Bading, 2003; Vanhoutte and Bading, 2003; Zhou and Baudry, 2006; Liu *et al*., 2007). It has also been shown that NMDA receptors evoke the production of peroxynitrite (product of superoxide and the diffusible nitric oxide interaction), the highly toxic reactive oxygen species, in regions vulnerable to ischemia but not in regions relatively resistant to ischemic injury (Fekete *et al*., 2008).

As for kainate receptors, although they mediate synaptic transmission by postsynaptic receptors, their predominant role appears to be presynaptic modulation of glutamate and GABA release (Jaskolski *et al*., 2005; Lerma, 2006; Coussen, 2009). They are located presynaptically near the release site and in the somatodendritic compartment both within the postsynaptic density (e.g. in the hippocampal mossy fiber synapse) and extrasynaptically (Jaskolski *et al*., 2005; Table 2).

Non-synaptic AMPA and kainate receptors are commonly present in glial cells (astrocytes, oligodendrocytes and microglia; Matute *et al*., 2001). Different subtypes of NMDA receptors, including NR3A, but not NR3B, have also been shown in the myelinating processes of oligodendrocytes, where the small intracellular space could lead to a large rise in glutamate concentration and mediate excitotoxic injury (Karadottir *et al*., 2005; Salter and Fern, 2005).

GABAA receptors. It is generally accepted that the main inhibitory transmitter, GABA is predominantly synaptic (Figure 1, Table 1), and functions as an agonist with high potency but low efficacy. The first evidence that $GABA_A$ receptors could be localized extrasynaptically was found by Somogyi *et al*. (1989) using an immunogold technique in cerebellar granule cells. This observation was a milestone in neuroscience, because it provided the first convincing morphological evidence that ionotropic receptors are also expressed extrasynaptically. In the same laboratory, it was shown that although the density of $GABA_A$ receptors is higher within the synapses, with regard to the overall number, extrasynaptically localized receptors outnumber those that are present at the synapse (Nusser *et al*., 1995). The presence of the δ subunit was shown to be a characteristic of $GABA_A$ receptors that are localized extrasynaptically (Nusser *et al*., 1998) or perisynaptically (Huang and Pickel, 2002). Herd *et al*. (2008) demonstrated that tonic currents are predominantly mediated by β_2 containing $GABA_A$ receptors, while synaptic currents are mediated by $GABA_A$ receptors that contain the β_3 subunit. These findings indicate that non-synaptic GABA receptors are different from those that are present at the synapse. While the millimolar concentration of GABA in the synapse (Mody *et al*., 1994; Jones and Westbrook, 1996) results in a phasic and transient inhibition of the postsynaptic cell, GABA escaping from the synapse can result in a persistent or tonic activation of non-synaptic $GABA_A$ receptors (Semyanov *et al*., 2004; Farrant and Nusser, 2005). Subsequent studies have indicated that GABA-mediated tonic conductance exists in many different nerve cells of distinct brain areas (Farrant and Nusser, 2005), such as the cerebellum, hippocampus, dentate gyrus, cortex, thalamus and brainstem (Glykys and Mody, 2007). In fact, GABA-mediated tonic conductance on dendrites and cell bodies has also been found under *in vivo* conditions (Chadderton *et al*., 2004).

Simulation studies suggest that a decrease in membrane resistance at the opening of $GABA_A$ receptor channels efficiently prevents propagation of APs into presynaptic terminals (Segev, 1990). Verdier *et al*. (2003) recently reported that $GABA_A$ receptors could affect AP propagation in branches of afferents in brainstem slices. The δ subunit-containing $GABA_A$ receptors that include either α4 or α6 subunits characteristically have a high affinity and a low efficacy for GABA (Saxena and Macdonald, 1996; Brown *et al*., 2002), and low degree of desensitization to agonists that are continuously present in the extracellular space (Wohlfarth *et al*., 2002; Bianchi and Macdonald, 2003; Table 2). Their EC_{50} is in the tens of nM range, which is well within the concentrations that are present in the extracellular space (Nyitrai *et al*., 2006).

A novel form of GABAergic inhibition has recently been described (Tamas *et al*., 2003; Szabadics *et al*., 2007). A class of cortical GABAergic interneurons, the neurogliaform cells produce slow ionotropic responses (IPSCs lasting for tens of milliseconds), with properties that are intermediate between phasic and tonic inhibitory signalling. Most (78%) axonal varicosities of this cell type lack synapses, but non-synaptic release from its unusually dense axonal arborization can provide an effective widespread inhibition in the local environment (Olah *et al*., 2009). Slow ionotropic responses have proven to be particularly sensitive to the modulation of reuptake, as well as to certain types of benzodiazepines.

5-HT3 receptors. $5\text{-}HT_3$ receptors have been shown to be present on axons and presynaptic terminals, and in large dense-core vesicles, however, they do not associate with the synaptic differentiation of terminals (van Hooft and Vijverberg, 2000; Miquel *et al*., 2002; Huang *et al*., 2004). Immunoreactive terminals containing abundant dense-core vesicles often lack recognizable synapses, but those containing few dense core vesicles form asymmetric or symmetric synapses (Huang *et al*., 2004). $5\text{-}HT_3$ receptors are present on spines and distal portion of dendrites, mainly within the hippocampus. $5\text{-}HT_3$ receptors are concentrated at the postsynaptic differentiation, but can also be found non-synaptically on spines and dendrites, occasionally in a perisynaptic location (Miquel *et al*., 2002; Huang *et al*., 2004), and are also localized on glial processes (Huang *et al*., 2004).

Nicotinic receptors. There are at least 12 different functional pentamer nACh receptors (Lendvai and Vizi, 2008), which can be found in extrasynaptic locations on different neurons

in the hippocampus (Hill *et al.*, 1993; Ullian and Sargent, 1995; Fabian-Fine *et al.*, 2001; Jones and Wonnacott, 2004). It was shown in the ventral tegmental area (Jones and Wonnacott, 2004) with both pre- and post-embedding electron microscopy that 27% of a7-nACh receptors are extrasynaptic on the glutamatergic axons, 61% are perisynaptic and only 11% are expressed at the active zone, opposed to the postsynaptic density. Functionally, it was shown that tonic activation of extrasynaptic α7-nAChRs by cholinergic agonists – depending on timing – either activates or depresses back-propagating action potentials (Rozsa *et al.*, 2004; 2008; Lendvai and Vizi, 2008) of CA1 stratum radiatum interneurons. A stimulation paradigm that normally failed to produce LTP in the Schaffer collaterals was able to boost LTP production when sufficient depolarization was evoked by α7-nAChR stimulation and was timed to coincide with the somatic stimulation. Similarly, the activation of non-synaptic nAChRs resulted in a potentiation of synaptic glutamatergic inputs (Vizi *et al.*, 2004a). Szabo *et al.* (2008) showed that the activation of non-synaptic nAChRs results in Ca^{2+} transients in pyramidal spines and induces firing of APs. These findings indicate that ACh or its metabolite choline, which itself is an endogenous agonist of the α7 subtype of nAChRs, are able to produce 'postsynaptic' signalling following diffusion far away from their release sites. It seems likely that non-synaptic nAChR activation through ionotropic channels is a potential alternative for metabotropic muscarinic modulation of hippocampal plasticity, which exhibits a rather slow action (Buzsaki, 2002).

Glycine receptors (GlyRs). The subunits (α(1–4) and β) of pentameric GlyRs form heteromeric or α homomeric receptors. α subunits are responsible for agonist binding (Lynch, 2004), while the β subunit binds the anchoring protein gephyrin with high affinity (Kirsch *et al.*, 1993; Meyer *et al.*, 1995), which immobilizes postsynaptic GlyRs at the subsynaptic cytoskeleton. Homomeric α GlyRs, which do not contain the β subunit, are expected to freely diffuse in the plasma membrane, thus representing extrasynaptic GlyRs.

P2X receptors. Adenosine triphosphate (ATP) is a transmitter released from both axon terminals along with another transmitter (Buzsaki, 2002; Zimmermann, 2008; Abbracchio *et al.*, 2009) and from the postsynaptic sites in response to receptor stimulation by the primary transmitter (Vizi *et al.*, 1992a; Sperlagh *et al.*, 2002). Glia is also a major source of extracellular ATP (Cunha, 2008). ATP can act on postsynaptic sites, where it is released from termed 'cascade' transmission (Vizi *et al.*, 1992a) or on presynaptic sites. Ionotropic receptors of ATP are called P2X receptors, and seven subunits ($P2X_{1,2,3,4,5,6,7}$) can form homo- or heterotrimeric ion channels (Burnstock, 2008b). P2X receptors are involved in fast synaptic transmission (North and Verkhratsky, 2006), where ATP is co-released with glutamate, and P2X receptors have been shown to be located both within glutamatergic synapses and perisynaptically (Rubio and Soto, 2001). P2X receptors mediating non-synaptic, especially neuro-glial interactions are even more widespread (Abbracchio and Ceruti, 2006). Purinergic receptors are also involved in the modulation of transmitter release (Sperlagh *et al.*, 2007a). P2X receptors typically facilitate release of other transmitters, including GABA (Sperlagh *et al.*, 2002) and NA (Papp *et al.*, 2004), as well as the carrier-mediated release of Glu from the hippocampus.

Non-synaptic transporters

As in the case of the discovery of non-synaptic localization of different receptors, the observation that several transporters are also localized extrasynaptically (Huang *et al.*, 2004; Torres and Amara, 2007; Daws, 2009) has also helped to change our view. There are two ion-coupled co-transporter families that are responsible for the transport of transmitters: the Na^+/Cl^--dependent carriers for monoamines (NA, dopamine and serotonin), glycine and GABA, and the Na^+-dependent transporters for glutamate and aspartate (Torres and Amara, 2007). The role of these transporters is to remove transmitters that have been released into the synaptic gap or are present in the extracellular space in millimolar to nanomolar concentrations and to return them to nerve terminals. This would prevent postsynaptic receptors from becoming desensitized and help the synapses recover so they can relay transmissions again at a high frequency.

It is generally accepted that transporters are equally capable of operating in either the forward or reverse direction, and that they play a critical role in setting the ambient concentrations of different transmitters and the measure of tonic inhibition (Richerson and Wu, 2003). When the transporters operate in the forward direction, they clear neurotransmitters from the synaptic cleft, which recycles the transmitters after release. When they operate in the reverse direction, they release transmitters that are present in the cytoplasm in a $[Ca^{2+}]_o$-independent manner (Table 3). This type of release is not the subject of presynaptic inhibition (Vizi *et al.*, 1996), and ensures a permanent and continuous release of transmitters into the extrasynaptic space.

A further characteristic of transporters that are expressed on non-synaptic varicosities is their indiscriminate nature. Monoamine transporters are members of the same structural family with a high degree of homology (Amara and Kuhar, 1993) and are able to take up other transmitters besides their own (Devoto *et al.*, 2001; Moron *et al.*, 2002; Vizi *et al.*, 2004b) from the extracellular space. Monoaminergic varicosities are also able to release different transmitters in response to depolarization. This is also the case with noradrenergic varicosities, which are able to both take up and release DA (Devoto *et al.*, 2001), as well as the case with serotonergic terminals, which are able to take up and release NA (Vizi *et al.*, 2004b). This effect is independent of their machinery, which synthesizes their transmitters. It means that there is a

Table 3 Origin of transmitters released in response to neuronal firing and reversal of transporter (for details see the text)

	Transmitter release due to	
	Axonal firing	*Reversed transporter function*
Origin of transmitter	Vesicle	Cytoplasm
$[Ca^{2+}]_o$-dependence	Yes	No
Presynaptic modulation	Yes	No
Lowering temperature	Not sensitive	Sensitive

Table 4 Concentrations of transmitters in the vesicle, cytosol, synapse and extracellular space

Concentration of transmitters	*Vesicle*	*Cytosole*	*Synapse*	*Extracellular space*
Acetylcholine[1]	~100 mM	n.d.	~3 mM	0.01–3 μM
Noradrenaline[2]	~200 mM	n.d.	~1 mM	14–78 nM
Dopamine[3]	300–350 mM	n.d.	n.d.	14 nM–0.7 μM
GABA[4]	~100 mM	1–7 mM	~1 mM	0.2–0.8 μM
Glutamate[5]	~100 mM	2–10 mM	~1 mM	0.03–3 μM
Serotonin[6]	~90 mM	n.d.	~6 mM	1–100 nM

Our calculation based on:
[1]Descarries *et al.* (1997) and Zhang *et al.* (1996).
[2]Abercrombie *et al.* (1988) and Tso *et al.* (2004).
[3]Chen and Budygin (2007), Cragg *et al.* (2001), Jaffe *et al.* (1998), Nirenberg *et al.* (1997) and Pothos *et al.* (1998).
[4]Jones and Westbrook (1995), Lerma *et al.* (1986), Mody *et al.* (1994) and Otsuka *et al.* (1971).
[5]Barbour *et al.* (1994), Cavelier *et al.* (2005), Clements *et al.* (1992), Nedergaard et al. (2002) and van der Zeyden *et al.* (2008).
[6]Barnes et al. (1992); Bunin and Wightman (1998) and Crespi et al. (1988).
n.d., no data available.

functional overlap between different monoaminergic transporters. In the synapse, this phenomenon either does not happen or is negligible, because the concentration of the primary transmitter is about 1000 times higher in the synapse than the concentration of another transmitter diffusing from the extrasynaptic space (see Table 4).

Transporters are able to generate current in response to substrates through a channel-based mechanism (Bringmann *et al.*, 2009). This was strongly supported by electrophysiological evidence for the dopamine transporter (DAT) (Ingram *et al.*, 2002; Carvelli *et al.*, 2004) and for the Glu transporter (Veruki *et al.*, 2006). It was shown that transporter-associated anion current produced by Glu can hyperpolarize the presynaptic terminal and thus reduce transmitter release (Veruki *et al.*, 2006). In contrast, substrates of DAT, such as DA and amphetamine, activate anion conductances and increase the firing rate of dopaminergic neurons (Ingram *et al.*, 2002). It appears likely that the explanation for these observations is that the Cl^- reversal potential might vary in different neurons.

Transporter trafficking to and from the plasma membrane (Melikian, 2004) could be a response to chronic stress, which would result in a change in neuronal function (Torres *et al.*, 2003; Miner *et al.*, 2006; Robinson, 2006; Torres and Amara, 2007). The finding that chronic stress results in increased plasma membrane expression of the NA transporter (NET) in the prefrontal cortex suggests that there is an activity-dependent change in the subcellular localization of NET (Miner *et al.*, 2006), which is otherwise 85–90% localized in the cytoplasm. Agonists for the serotonin transporter (SERT; Zhu *et al.*, 2004) and NET (Zhou *et al.*, 1998) increase the delivery of transporter molecules to the plasma membrane. These studies illustrate that, even during axonal firing, the transmitter release and uptake machinery of the neuronal network is able to adapt to the new circumstances. Transporter molecules could traffic between intracellular pools and the cell surface (Miner *et al.*, 2003). It remains to be discovered whether or not these translocation changes of transporters occur extrasynaptically or in synapses. More than likely, both scenarios are involved.

NET. The NET is present on noradrenergic fibres at non-synaptic sites (Schroeter *et al.*, 2000; Moron *et al.*, 2002; Miner *et al.*, 2003; Liprando *et al.*, 2004; Daws, 2009) and serves as a target for multiple antidepressants and psychostimulants. Immunogold labelling of the NET was more frequently observed in the cytoplasm than on the plasma membrane (Miner *et al.*, 2003). The NET is also able to take up DA from the extracellular space (Moron *et al.*, 2002). The inhibition of the NET therefore results in an increase in the extracellular levels of both NA and DA.

Dopamine transporter. Ultrastructural analysis of DAT immunolabeling revealed that the DAT is preferentially localized non-synaptically (Smiley *et al.*, 1994; Nirenberg *et al.*, 1996; 1997) rather than to the synaptic part of the presynaptic membrane. This was supported by voltammetric investigations that showed the re-uptake of DA at a distance from the original release sites (Garris and Wightman, 1994; Garris *et al.*, 1994). Many current pharmacological or recreational psychotropic drugs, including psychostimulants, antidepressants and neurotoxins, exert their actions, at least in part, via an interaction with the DAT (Sotnikova *et al.*, 2006). Studies in DAT-KO mice have confirmed this hypothesis, showing a total lack of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) neurotoxicity in these mice (Gainetdinov *et al.*, 1997; Bezard *et al.*, 1999). The transporter is also of great importance for the toxic action of drugs of abuse (such as methamphetamine and ecstasy), which exert their actions through mobilization of DA from intracellular stores to the extracellular space via DAT-mediated outward transport (Fleckenstein *et al.*, 1997; Fumagalli *et al.*, 1998).

SERT. The SERT is mainly localized extrasynaptically on the plasma membrane (Zhou *et al.* 1998; Tao-Cheng and Zhou, 1999) of intervaricosity segments of the axons (Tao-Cheng and Zhou, 1999; Huang and Pickel, 2002; Huang *et al.*, 2004) shown by immunogold immunocytochemistry (Tao-Cheng and Zhou, 1999). This distribution is in contrast to what has been observed in the soma and dendrites, where the labelling is prevalent in the cytoplasm. Neurochemical evidence showed that the SERT is capable of taking up NA from the extracellular space and releasing it in response to axonal stimulation (Vizi *et al.*, 2004b). The non-synaptic SERT, but not those in the synaptic gap, is the main target of the selective transporter inhibitors, as well as many drugs of abuse, such as cocaine, ecstasy and methamphetamine.

Glutamate transporter (excitatory amino acid transporter, EAAT). Five EAATs have been identified in the brain. EAAT1 (GLAST) and EAAT2 (GLT-1) are predominantly expressed in astrocytes, while EAAT3 (EAAC1) and EAAT4 are localized in neurons. EAAT5 is expressed primarily in the retina (Amara and Fontana, 2002). The majority of Glu uptake occurs in glia rather than in neurons (Yi and Hazell, 2006). The EAAT3 transporter is expressed both in the synaptic cleft and non-synaptically (Conti *et al.*, 1998). Electrophysiological studies have shown that inhibition of Glu uptake leads to Glu spill-over (Diamond, 2002). While the activity of Glu transporters minimally affects synaptic currents (Rusakov and Kullmann, 1998), EAATs represent a major mechanism for removing extrasynaptie Glu in order to ensure a high signal-to-noise ratio and to enable a high frequency of synaptic transmission (Beart and O'Shea, 2007). Although an important role of the EAATs is to maintain the extracellular Glu concentration below neurotoxic levels, the $[Ca^{2+}]_o$-independent (Sperlagh *et al.*, 2007b) reversal of Glu transporter activity after ischemic injury, when the Na^+/K^+ electrochemical gradient collapses, seems to be involved in the accumulation of extracellular Glu concentrations.

GABA transporters. Inhibition of the GABA transporter results in an excessive accumulation of GABA in the synaptic gap, which is followed by a spillover of GABA (Isaacson *et al.*, 1993). In addition to the vesicular release, GABA can be released also by the reversal of the GABA transporter, which can be induced by pathological or therapeutic conditions, such as high intracellular sodium concentration, which occurs during ischemia, depolarization due to the accumulation of extracellular potassium during seizure activity, or in the presence of the anticonvulsants gabapentin or vigabatrin (Wu *et al.*, 2001; 2007; Richerson and Wu, 2003). The non-synaptic GABA transporter-1 regulates tonic inhibition by controlling the ambient, non-synaptic, extracellular GABA concentrations. The increase in tonic GABAergic inhibition that resulted from the activation of non-synaptic, high-affinity $GABA_A$ receptors, which do not desensitize, is related to the size of the reversal of the GABA transporter. It is supposed that this mechanism may operate physiologically as well, and can serve as a delicate sensor of intracellular sodium and extracellular GABA concentration (Wu *et al.*, 2007). Release of GABA by reverse operation of GABA transporters have been shown to be evoked by increased glutamate uptake. Increased tonic conductance by GABA receptor activation thus provides a compensatory mechanism for glutamatergic overexcitation (Heja *et al.*, 2009).

Choline transporter. Elimination of released acetylcholine is done not by transport but by enzymatic degradation. It is first decomposed by cholinesterase to choline and acetate, followed by the uptake of choline (Ribeiro *et al.*, 2007).

Mechanisms of transmitter release into the extrasynaptic space

There are several major sources of transmitters (endogenous substances) for non-synaptic release (Figure 4, Table 3).

1 Exocytotic (vesicular) release of transmitters from non-synaptic varicosities of for diffusion (Figure 4). Most typically occur via monoaminergic and cholinergic axons, which can release transmitters via exocytosis into the extracellular space of the brain (Descarries *et al.*, 1997). This release is $[Ca^{2+}]_o$-dependent, is related to axonal firing and is subject to presynaptic modulation (Table 3). This type of release has long been known to involve peptides and, in the intestine, ACh.

   The terminal axons of the cholinergic Auerbach plexus in the gut have many varicosities and are associated with the accumulations of small vesicles. These represent sites of exocytotic release of ACh; however, there are no post-junctional specializations on the smooth muscle cells (Paton *et al.*, 1971). The distance between varicosities and smooth muscle cells is about 100–200 nm, and ACh that is released from the boutons has to diffuse across these distances (Paton *et al.*, 1971). Muscarinic receptors of the M_1 subtype are evenly distributed on the smooth muscle surface and have a high affinity (EC_{50} for Ach = ~100 nM).

   Similar exocytotic release of NA for diffusion is also known to occur at different neuroeffector transmission sites in the periphery (Burnstock, 2008a). The distance between noradrenergic boutons and effector smooth muscle cells may vary considerably, and clefts range from 15–20 nm (e.g. in the vas deferens and iris) to 1–2 μm (e.g. in the large arteries). NA is released *en passage* from varicosities during the conduction of APs along the axon. The APs are propagated along the varicose axon and release transmitters from only some of the varicosities that the action potentials encounter (Jackson and Cunnane, 2001).

   Vesicular release of glutamate has been described in glial processes, which participate in the formation of the tripartite synapse (Figure 3).
2 Spillover of transmitters from the synapse (see Figures 3 and 4). Transmitters can 'escape' from the synapse (Asztely *et al.*, 1997), especially during high frequency firing, and spill over, which results in their diffusion far away from the original release sites. These 'escaped' transmitters can then hit extrasynaptic receptors, which could potentially appear as extreme activity of the synapse.
3 Transmitter release by the reverse operation of transporters (carrier-mediated release; Table 3). In certain pathological conditions, such as ischemia or epilepsy, the high Na^+ load (Sheldon *et al.*, 2004; Fekete *et al.*, 2009) can reverse the operation of the transporter for the transmitter, resulting in release instead of reuptake of transmitters as a form of 'carrier-mediated release'. This release is $[Ca^{2+}]_o$ independent, and is not related to axonal firing or subject to presynaptic modulation (Vizi *et al.*, 1986; Ikeda *et al.*, 1989). It is worth mentioning that transporters are capable of a channel-like operation. In the case of monoamine transporters, not only ions but the transmitter molecule itself is able to cross the membrane through the channel (DeFelice and Goswami, 2007) and to be released into the extracellular space. It has been proposed that reverse operation of transporters may be a normal physiological mechanism for dynamic modulation of ambient levels of certain transmitters (Richerson and Wu, 2003).

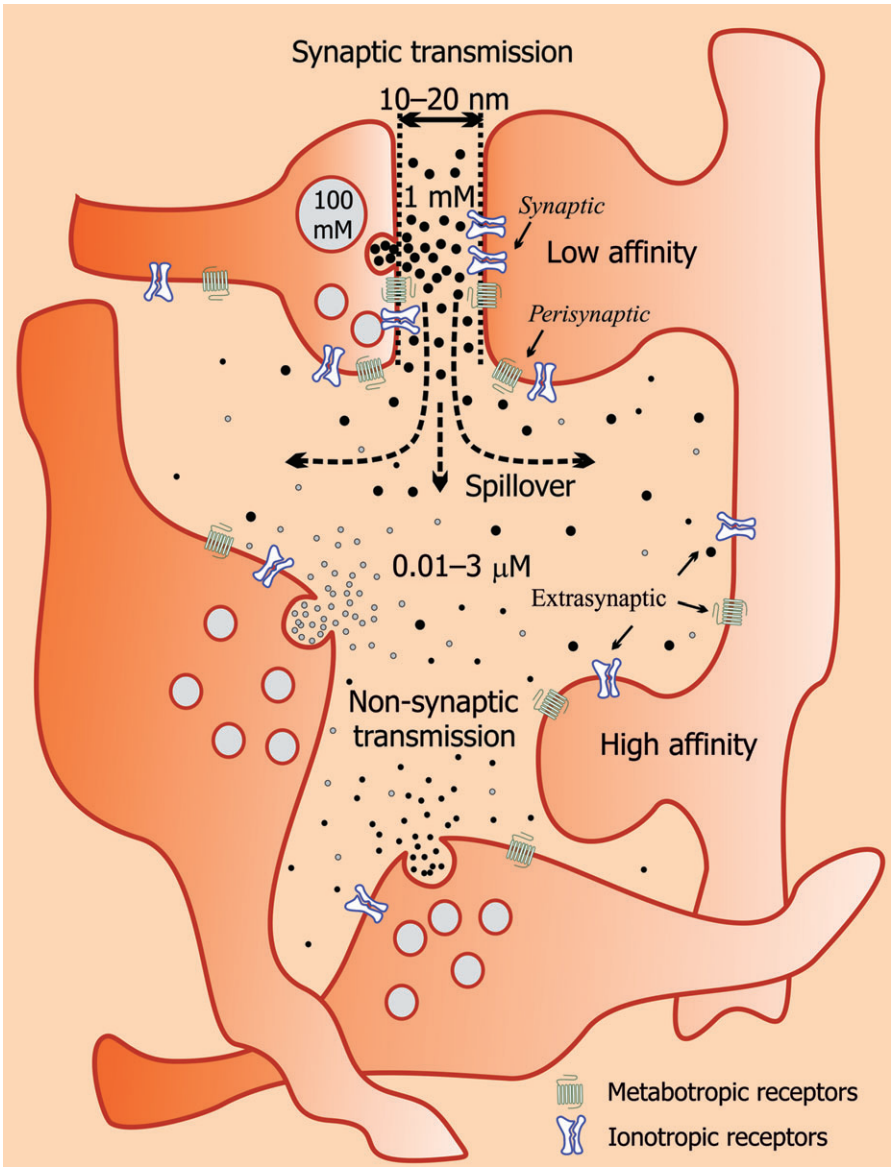

Figure 4 Scheme of different types of chemical communication, including synaptic transmission when the transmitter released into the synaptic gap acts on postsynaptic low affinity receptors located in the synaptic gap, and non-synaptic communication when the transmitter spills over from the synaptic gap or is released from a bouton without making synaptic contact and reaches its target non-synaptic (perisynaptic or extrasynaptic) receptors by diffusion. The distance could be from a few hundred nm to a few hundred μm. Note the significant difference in concentration in the vesicle (~100 mM), in the synaptic gap (~1 mM) and in the extrasynaptic space (~0.01–3 μM). Concentrations in the figure are approximate values from the literature (see Table 4).

4 Other non-exocytotic mechanisms (Vizi and Mike, 2006; Malarkey and Parpura, 2008; van der Zeyden *et al*., 2008) can also significantly contribute especially to ambient levels of certain transmitters. Included among these mechanisms are *release through gap junction hemichannels* (Ye *et al*., 2003), *P2X7 purinergic receptors* (Duan *et al*., 2003), *cystine-Glu exchange* (Cho and Bannai, 1990), *Ca^{2+} ion-dependent release* (Bezzi *et al*., 1998), *volume-sensitive organic anion channels* (Del Arco *et al*., 2003) and *swelling-induced release* (Kimelberg *et al*., 1990). ATP-induced activation of the homomeric $P2X_7$ receptor releases Glu from astrocytes into the extracellular space via a pore that is able to allow molecules as large as 900 Da to pass through (North, 2002). This type of release is $[Ca^{2+}]_o$-independent (Papp *et al*., 2004; Malarkey and Parpura, 2008).

Extracellular space as a communication channel

The extracellular space, which accounts for 15–30% of the total brain volume, has been shown to be an important communication channel (Sykova and Nicholson, 2008). It is filled with interstitial fluid that has a composition similar to the cerebrospinal fluid. In addition to its ion content, the extracellular space has an ambient level of various endogenous substances.

In the normal adult brain, the extracellular space may be reduced to 5% during ischemia or epilepsy (Elsen *et al.*, 2006). This type of reduction in the extracellular space can result in a significant increase in cell volume (Franco *et al.*, 2008) and an increase in the concentration of Glu, ions and neuroactive substances that have all been released into the extracellular space (Sykova, 2004).

Levels of transmitters in the extracellular space

The determinants of extracellular transmitter concentrations include the rate of release, diffusion (regulated by properties of the extracellular space, such as geometry, viscosity, matrix molecules, charged, or transiently binding molecules) and clearing processes (uptake by glia and neurons, enzymatic degradation, and removal through the blood-brain barrier; Sykova and Nicholson, 2008; Sykova and Vargova, 2008). The ambient levels of extrasynaptic transmitters can tonically inhibit or enhance both the synaptic and non-synaptic neuronal functions (release, neuronal excitability; Carmignoto and Fellin, 2006; Semyanov, 2008). A prerequisite of these functions is that neuronal networks are equipped with extrasynaptic receptors.

Although the concentration of transmitters is about 100 mM in the vesicles and 1–3 mM in the synaptic cleft following its release and dilution, the extrasynaptic concentration of transmitters is much lower, about 0.01–3 μM (Figure 4, Table 4). When a transmitter is released extrasynaptically by a varicosity, it can freely diffuse into all directions of the space, thus the concentration drops as a cubic function of the distance (Vizi, 2000).

Using Glu microsensors in the hippocampus, the *in vitro* basal extracellular level of Glu was found to be 1.7 μM (van der Zeyden *et al.*, 2008). There are, however, large differences in the level of Glu across brain regions and between different studies ranging from 1 to 45 μM, which may be partially due to methodological differences (van der Zeyden *et al.*, 2008). In contrast to these studies, Cavelier *et al.* (2005) estimated extrasynaptic, resting Glu concentrations to be much smaller (0.03–0.08 μM at 25–35°C) by measurement of the size of tonically activated NMDA receptor (AP-5 suppressible) current. The *in vivo* concentration of Glu was reported to be 18.2 μM in the striatum (van der Zeyden *et al.*, 2008), while Rahman *et al.* (2005) found it to be only 1.4–2 μM. The basal levels of extracellular GABA and glycine were found to be 0.27, and 4.95 μM, respectively (Kennedy *et al.*, 2002). Whereas the resting extracellular adenosine concentration in the brain is 30–300 nM (Rudolphi and Schubert, 1997), it can reach 10–50 μM following a 15 min period of ischemia (Hagberg *et al.*, 1987; Pugliese *et al.*, 2009). The extracellular concentration of ATP is as low as 100 nM (Llaudet *et al.*, 2005). Basal extracellular levels of DA, NA and 5-HT are given in Table 4. Uptake inhibitors are able to produce an increase in the extracellular levels of transmitters, showing that transporters have a tonic effect on ambient transmitter level (Segovia and Mora, 2001; Wu *et al.*, 2007).

Neural stimulation results in an increase in the concentration of neurotransmitter level. Local electrical stimulation (20 pulses, 100 Hz) evoked a DA efflux of 315 nM within approximately 1 s in the striatum. In the dorsal raphe, 5-HT peaked at 16 nM under the same stimulation parameters, whereas in the ventral bed nucleus of the stria terminalis, the NA concentration peaked at 78 nM (Tso *et al.*, 2004). Using fast-scan cyclic voltammetry, the concentration of extracellular DA during somatodendritic activation in guinea pig brain slices was site specific, with a significantly higher level in caudal slices (0.48 μM) than in rostral slices (0.16 μM). In the ventral tegmental area, the stimulated DA levels peaked at 0.74 μM (Cragg *et al.*, 1997). Cragg *et al.* (2001) calculated that DA, which is released into the extracellular space in the substantia nigra pars compacta and can diffuse up 200 μm from the release site, reached a concentration of 14 nM. This was high enough to activate high-affinity DA receptors (EC_{50} for D_2 receptor activation = 1–20 nM; Levant, 1997).

Drug concentrations in the extracellular space

As a general rule, it has been observed that non-synaptic receptors have higher agonist affinity than their synaptic counterparts. This does not mean that they also will have higher affinity to drug molecules as well. However, the general assumption that drugs preferentially affect non-synaptic receptors is nevertheless true, simply because the majority of CNS drugs affect transmitter levels, for example, by acting on the modulation of release (e.g. haloperidol), on the reuptake process (e.g. fluoxetine) or on enzymatic degradation (e.g. selegiline, vigabatrin, moclobemide or galantamine). The effect on transporters and degrading enzymes minimally affect concentrations within the synapse at the time after the release, but has a major role in determining how far the transmitter will diffuse (see next section), that is the relative contribution of non-synaptic receptors.

While neurotransmitters are typically hydrophilic molecules, and therefore are largely confined to the extracellular space, CNS drug molecules are typically amphiphilic or lipophilic (since this is a requirement for blood-brain barrier penetration). Diffusion of these molecules therefore is not restricted to the extracellular space, but involves membrane-water phase partitioning, as well as intramembrane diffusion. For this reason, apparent diffusion coefficients are lower than that of hydrophilic molecules by one to two orders of magnitude (Chesney *et al.*, 2003; Gredell *et al.*, 2004), and free extracellular concentration can be several orders of magnitude lower than total brain concentration (Summerfield *et al.*, 2007; Friden *et al.*, 2009).

Diffusion of transmitters to reach non-synaptic receptors and transporters

The principal question in the case of any receptor is whether the transmitter can reach the receptors at sufficiently high concentration. The simple question: 'How far can transmitter molecules get within a given time?' addresses three aspects of transmitter diffusion: space, time and concentration.

In order to investigate these questions for different transmitters, we performed simulations of transmitter diffusion. A detailed description of the simulations and a presentation of some typical examples are given in the Supplementary Information (Appendix S1). The files used for simulation are also available.

For a typical transmitter at 1 ms after the release of a single vesicle, 95% of all released molecules will be within a sphere with a radius of ~2 μm. At the highest point of this diffusion wave, the concentration of the transmitter will be ~5 μM. In order to increase the distance 10-fold (~20 μm), even if no transporter activity is present, 100-fold this much time (100 ms) will be needed, and the concentration will drop 1000-fold (~5 nM). For the spillover of transmitters to perisynaptic receptors, a two dimensional model may be more appropriate. In this case concentration drops as a quadratic, not cubic function of distance (Slide S1).

We found that differences in the diffusion coefficient, tortuosity and the number of released molecules only had a minor effect on the pattern of diffusion (Slide S2). The most important determinants of the diffusion pattern were the density and binding kinetics of transporters (Slide S3). The third major determinant of the effective distance of non-synaptic transmission was the affinity of the receptor (Slide S4).

The relationship between non-synaptic diffusion and receptor affinity can be characterized by two useful terms: (i) 'maximum effective radius' is defined as the distance within which release of one quantum of transmitter reaches the EC_{50} concentration of the receptor, and (ii) 'maximum active lifetime' is the time during which the transmitter concentration remains above EC_{50} (Rice and Cragg, 2008). For example, for low affinity (EC_{50} between 1 and 18 μM; Neve and Neve, 1997) synaptic (mainly D1) dopamine receptors, the transmitter concentration that is enough for significant activation is present for ~1–2 ms (maximum active lifetime) and within ~1–2 μm (maximum effective radius) from the release site (Slide S4). In contrast, high affinity (EC_{50} between 10 and 32 nM; Neve and Neve, 1997) non-synaptic (mainly D2) dopamine receptors can detect release of single quanta from as far as ~4–5 μm, diffusion takes around 10–20 ms to reach this far (see supplementary files). For synaptic AMPA receptors (EC_{50} between 300 and 5000 μM; Erreger *et al.*, 2004), the signal is effectively terminated within the synaptic cleft (maximum effective radius 300 nm, with a maximum active lifetime up to ~200 μs – as judged from the two-dimensional model). In contrast, for perisynaptic and extrasynaptic NMDA receptors (EC_{50} between 0.9 and 13 μM; Erreger *et al.*, 2004), even single quanta can be detected from ~1.5 μm distance, with a maximum active lifetime of ~1 ms (Slide S4). Similar maximum effective radius and maximum active lifetime values apply to extrasynaptic mGlu receptors as well, because their affinity is within the same range (Cartmell and Schoepp, 2000). The effect of non-instantaneous release was also simulated (Slide S5). Interestingly, the non-instantaneous nature of the release increased, not decreased intrasynaptic concentration within the first 0.2 ms.

Summary

After slow acceptance, non-synaptic signalling in both the peripheral and central nervous systems is a rapidly expanding field of neuroscience. The results presented in this review may alter the way we think about separate synaptic and non-synaptic functions. Synaptic functions are thought to be responsible for definite, accurate tasks that require high speed and precision, such as visual pattern recognition or motor pattern execution. Conversely, non-synaptic functions are thought to be responsible for the modulation and tuning of these processes, such as determining the level of arousal, the focus of attention or the emotional background. Note that almost all pharmacological manipulations of the CNS are restricted to this latter realm, predominantly involving non-synaptic functions.

This type of interaction assumes that the released transmitter is able to diffuse within the extracellular space and reach distant targets at relatively low concentrations. The conditions under which this system is efficient imply that the affinity of the targeted receptors for the neurotransmitter is very high, since the concentration of molecules decreases as a cubic function of distance (Vizi, 2000). The existence of non-synaptic receptors and transporters that have affinities in the nanomolar range agrees well with such an arrangement. Moreover, the fact that a transmitter that is released from different terminals diffuses in three spatial dimensions means that the affected receptors and transporters might be located on any part of the surrounding neurons, including the somata, dendrites, axons and varicosities. The non-synaptic localization of receptors and transporters provides an anatomical basis for potential therapeutic implications. These structures, which have no synaptic arrangements, are promiscuous and accessible to chemicals that are released from numerous synapses, non-synaptic boutons or both. In addition, the non-synaptic receptors and transporters are certainly accessible to medications that have an availability in the nanomolar and micromolar concentration range in the brain. Drugs that are given for neuropsychiatric diseases could therefore exert their effects via these structures after diffusion through the extracellular space. Recognition of this general principle indicates that any future drug research should take into account the localization and properties (e.g. affinity, efficacy, etc.) of these non-synaptic receptors and transporters.

Acknowledgements

This review is dedicated to the memory of Sir William Paton, who was my supervisor (E. S. Vizi) at the Oxford University Department of Pharmacology.

We thank all of our colleagues in the Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.

We are grateful to Z. Nusser for the critical reading of this paper. This work was supported by grants by the Hungarian Research Founds OTKA NK 72959 and Jedlik II. OM-00131/2007. Arpad Mike is the recipient of Bolyai-fellowship.

Conflict of interest

The authors state no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Description of simulations.
Slide S1 3D and 2D modeling of glutamate release.
Slide S2 Dopamine and glutamate.
Slide S3 The effect of transporters.
Slide S4 Sensitivity ranges of low- and high-affinity receptors.
Slide S5 The effect of non-instantaneous release.

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