COLOCALIZATION OF NEUROTRANSMITTERS IN THE HIPPOCAMPUS AND AFFERENT SYSTEMS: POSSIBLE FUNCTIONAL ROLE

Review

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Abstract. In neurophysiology, the transmitter phenotype is considered a sign of neuronal identity. Since the end of the last century, it has become known that a nerve cell can produce and use several different molecules to communicate with other neurons. These can be “classical” transmitters: glutamate or gamma-aminobutyric acid (or acetylcholine, serotonin, norepinephrine), as well as second messengers, mainly neuropeptides released from the same neurons. If classical neurotransmitters are released together from the same nerve cell, this is called cotransmission or coreleasing (release from the same vesicles). In this review article, the term “cotransmission” is used in a broad sense, denoting neurons that can release more than one classical mediator. Since transmitters are often intermediate products of metabolism and are found in many cells, the classification of neurons is currently based on carrier proteins (transporters) that “pack” neurotransmitters synthesized in the cytoplasm into vesicles. Here, we limit the question of colocalization of the main neurotransmitters in mammals to neurons of the hippocampus and those structures that send their pathways to it. The review considers problems affecting the mechanisms of multitransmitter signaling, as well as the probable functional role of mediator colocalization in the work of the hippocampus, which has not yet been clarified. It is suggested that co-expression of different mediator phenotypes is involved in maintaining the balance of excitation and inhibition in different regions of the hippocampus; facilitates rapid selection of information processing methods, induction of long-term potentiation, maintenance of spatial coding by place cells, as well as ensuring flexibility of learning and formation of working memory. However, the functional role of mediator colocalization, as well as the mechanisms of release of “dual” transmitters, have not been fully clarified. The solution of these

problems will advance some areas of fundamental neuroscience and help in the treatment of those diseases where a violation of the balance of excitation and inhibition is detected, for example, epilepsy, Alzheimer's disease and many others.

Keywords: *hippocampus, dentate gyrus, afferent structures, pyramidal neurons, granule cells, interneurons, transmitters, glutamate, GABA, transporters, theta rhythm, mechanisms, functional role, seizure pathology*

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INTRODUCTION

Neurotransmitter phenotype has long been regarded as a sign of neuronal identity. The classical view, formulated as the "Dale principle" [1], was that each neuron secretes only one transmitter. However, many classes of neurons have now become known to synthesize multiple transmitters. These include cells that secrete multiple fast-acting neurotransmitters that transmit signals via ionotropic receptors (e.g., glutamate and gamma-aminobutyric acid (GABA) or GABA and acetylcholine), either one fast-acting neurotransmitter and a second small molecule that transmits signals via G-protein-coupled receptors (e.g., GABA and dopamine), or a classical small-molecule transmitter and a neuropeptide (e.g., GABA and somatostatin) [2-4]. Among neuropeptides that colocalize with classical mediators, enkephalin, cholecystokinin (CSC), neuropeptide Y, neurotensin, etc. are also known).

If two neurotransmitters are released from the same neuron, we speak of cotransmission; this means that two transmitters are released from different vesicles at different locations and/or at different times. The additional term "simultaneous release" (corelating) refers to the simultaneous release of two mediators from the same vesicles. For simultaneous release to occur, two "classical" transmitters of several known transmitters (glutamate, GABA, dopamine, acetylcholine, serotonin, noradrenaline) must be stored within the same synaptic vesicle in an easily released pool. In the absence of simultaneous release from the same vesicles, cooperative transmission may involve the synchronous release of two different sets of vesicles containing different neurotransmitters (see Trudeau and Mestikawy [5] for a review and Figure 1).

Fig. 1. Schematic of the synaptic architecture of "dual" ($VGLUT3^+$ and $VGAT^+$) neurons that

release glutamate and GABA from separate synaptic vesicles at independent asymmetric or symmetric synapses. Adapted from [79], with permission from Elsevier (license no. 5922371097009 dated December 05, 2024). GABA, gamma-aminobutyric acid; $VGLUT3^+$ - vesicles - positive vesicular glutamate transporters; $VGAT^+$ -vesicles - positive vesicular GABA transporters

In this review, we will use the term "cotransmission" in a broad sense, referring to neurons that can release more than one classical mediator. Such release often occurs from the same synaptic endings, but can also occur from different varicosities formed by a given neuron.

Glutamate and GABA are classic fast-acting neurotransmitters utilized by the nervous system throughout most of phylogenesis. In the mammalian brain, by acting on different sets of ionotropic receptors, these transmitters are known to typically exert opposite effects on the target neuron, with glutamate excitatory and GABA inhibitory to the postsynaptic neuron. Because classical mediators are small molecules, they are often metabolic intermediates and thus found in many cells. On this basis, the classification of neurons is based mainly on carrier proteins (transporters) that "package" neurotransmitters synthesized in the cytoplasm into vesicles from which they are later released into the synaptic cleft [6] Mediator transporters maintain their extracellular concentration near the synapse to control the activity of the corresponding receptors.

Glutamatergic cells (using glutamate as a mediator) are one of the most common types of neurons not only in the hippocampus but also in the brain as a whole. Vesicular glutamate transporters (VGLUT) are expressed on the membranes of synaptic vesicles and are thought to be unique to neurons. There are three isoforms of VGLUT (VGLUT1, VGLUT2 and VGLUT3) without their significant differences in glutamate capture when tested in *in vitro* experiments [7, 8]. While the first two isoforms of the transporter do not normally colocalize with other major transmitters, VGLUT3 exhibits co-localization with other mediators such as GABA, serotonin, dopamine or acetylcholine [9]. Recent evidence suggests that neurons that release GABA together with glutamate express either VGLUT2 or VGLUT3, but not VGLUT1 [10].

The other "classical" mediator in the hippocampus is represented by inhibitory GABA. Canonically, GABA is synthesized from intracellular glutamate by one of two varieties of glutamate decarboxylase enzyme (GAD65 or GAD67), which requires the accumulation of two opposite classical neurotransmitters in the cytoplasm of most inhibitory interneurons in the CNS. GABAergic cells usually lack VGLUTs; the exception is cholecystokinin-expressing interneurons [11]. The transporters for GABA, and thus markers of GABAergic cells, are vesicular GABA transporters (VGATs). They are represented by four main types: VGAT1, VGAT2, VGAT3 and

VGAT4. The main GABA transporters in the brain are VGAT1 and VGAT3; they are expressed both by neurons and some astrocytes [12].

This review considers the issue of colocalization in mammals of the main mediators both in hippocampal neurons and in neurons of structures that send their pathways to it and have targets in the form of various subcellular compartments of hippocampal and dentate gyrus neurons. Some attention is also paid here to questions about the mechanisms of release and possible functional role of such colocalization in norm and pathology.

COLOCALIZATION OF NEUROTRANSMITTERS IN CELLS AND AFFERENT PATHWAYS OF THE HIPPOCAMPUS AS A TOOL IN BRAIN FUNCTION

It is known that the hippocampus is rich in both locally produced glutamate and VGLUT3-positive ($VGLUT3^+$) afferents [13, 14]. At the same time, $VGLUT3^+$ -synapses formed by afferent fibers are formed both on the bodies of pyramidal cells and on their dendrites [13, 15-17].

Colocalization of glutamate and GABA in hippocampal neurons. It is known that GAD-positive (i.e., GABAergic) hippocampal neurons (interneurons) also express VGLUT3; this indicates that they can release glutamate [13, 15-18]. In the work of Stensrud et al. [13], performed at the level of electron microscopy using immunogold staining, it was found that in mice and rats in the hippocampus, the terminations of GABAergic fibers in the hippocampus contain markers of both VGAT and VGLUT3. Immunoisolation of synaptic vesicles confirmed these findings and showed vesicular colocalization of VGLUT3 and VGAT.

This and other studies have revealed that VGLUT3 is often found in symmetric (i.e., inhibitory) nerve endings [11, 13, 17, 18]; this confirms that inhibitory interneurons release glutamate in addition to GABA. Thus, a known type of interneurons, basket cells expressing the neuropeptide cholecystokinin in addition to GABA, contain VGLUT3 in their synaptic endings [11, 18]; they are designated as CCK^+ $VGLUT3^+$ -cells [18]. Such interneurons are found in the CA1 and CA3 fields of the hippocampus, as well as in the dentate gyrus of the hippocampal formation (see below). Most often inhibitory glutamate-releasing interneurons are located in stratum radiatum of areas CA1 and CA3, less often they are located in the pyramidal layer and layers oriens, lacunosum-moleculare and lucidum . In the dentate gyrus, such interneurons are localized in the subgranular zone as well as in the hilus [11, 13, 19]. VGLUT3-positive hippocampal inhibitory neurons are quite heterogeneous in their targets [20]; acting mainly on hippocampal pyramidal cells, they also innervate some interneurons and principal cells of the dentate gyrus [11, 13, 20, 21] (Fig. 2; Table 1). The effects of these interneurons are predominantly

inhibitory; however, under certain conditions, the excitatory glutamatergic action can dominate over the inhibitory one, and it is realized through ionotropic receptors [13, 20].

Fig. 2. Schematic representation of neurons and axons in region CA1 of the hippocampus and of the dorsal dentate gyrus revealing neurotransmitter colocalization. Local GABAergic cholecystokinin-positive interneurons express VGLUT3 in addition to GABA; these neurons are most abundant in the stratum radiatum and send axons to pyramidal cells. $VGLUT3^+$ -afferents identified by *in situ* hybridization and immunohistochemical experiments are mainly localized in stratum radiatum and stratum lacunosum and often, but not exclusively, co-express serotoninergic markers. There are $VGLUT3^+$ $serotonin^+$ -afferents from the median suture nucleus (mNASH) to the hippocampus, and $VGLUT3^+$ $dopamine^+$from the ventral tegmental area (VTO). Denotations: CA1, CA3 - hippocampal fields; ZI - dentate gyrus; str. moleculare, str. lacunosum, str. radiatum, str. pyramidale, str. oriens - cell layers in the CA1 field of the hippocampus; granular and supra-granular cell layers - cell layers of the dentate gyrus. Explanations in the text. The figure was created based on the analysis of works [11, 13, 21, 48, 62, 70, 76, 78, 79] and uses Servier Medical Art (Servier) templates, provided by Creative Commons Attribution 3.0 unported license

Table 1. Summary of neurotransmitter colocalization data in the hippocampus and afferent structures

Transmitters	Hippocampus	ZI	SUM	WTO
Glutamate+ GABA	GABAergic interneurons, in addition to VGAT, also express VGLUT3 [13, 15-18]	in developing rats, glutamate and GABA are present in granular neurons [25-29]; in adults, this is detected after seizures [26, 27, 40-42]	lateral neurons (SUML), exhibit markers of the dual phenotype: VGLUT2, VGAT, and GAD65 [77,79]	About 20% of neurons express GAD, VGAT and VGLUT2 [79]
Glutamate+ serotonin	mYASH	serotonin-containing cells detect VGLUT3 [63-68]; about 50% of such cells express VGLUT3 mRNA [65, 71, 72]		
Glutamate+ dopamine	WTO	A relatively high number of dopaminergic neurons contain VGLUT2 mRNA [97, 98]		

Glutamate+ noradrenaline	blue spot	part of noradrenaline-containing neurons demonstrates VGLUT2 expression [102, 103]
GABA+ acetylcholine	MSDS and BM	GAD67 mRNA and ChAT mRNA have been detected in 24% of FFMB neurons [51] and 25% of BM cells [53-55]
Acetylcholine+ glutamate	FFM	A limited number of cholinergic neurons in the FFMDB co-express VGLUT3 [13, 57]

Note. Abbreviations used: BM, Meynert's basal nucleus; VTO, ventral tegmental area; ZI, dentate gyrus; MSDB, complex of medial septal nucleus and Broca's diagonal bundle nucleus; SUM, supramammillary nucleus.

In addition to GABA, another inhibitory mediator, glycine, is present in the hippocampus. Although glycine is an inhibitory neurotransmitter in the mammalian CNS, it can also play a key role in excitatory neurotransmission as a coagonist of glutamate when acting on NMDA receptors [22]. Recent work in mice [23] studied the transporter-mediated interaction between glycine and glutamate and determined the coexistence of glycine and glutamate transporters in hippocampal nerve terminals. Purified nerve terminals (synaptosomes) analyzed at the ultrastructural level were used as an experimental model. It was found: (1) exogenous glycine stimulated [3H]D-aspartate release, partly through activation of the VGLUT1 transporter and partly through VGLUT2; (2) D-aspartate stimulated [3H]-glycine release through a process sensitive to glutamate transporter blockers (see Cortese et al. [23]).

Colocalization of neurotransmitters in the afferent systems of the hippocampus. ***The dentate gyrus (dentate gyrus).*** The dentate gyrus is often attributed to the hippocampus, but it is a special structure closely related to the hippocampus, sending afferent fibers to it and forming together with it the hippocampal formation. The main elements of the dentate gyrus are glutamatergic granular neurons projecting by means of their axons (mossy fibers) to pyramidal cells and interneurons of the CA3 field. Granular neurons are "born" throughout the life of animals and humans, which demonstrates the ability of the dentate gyrus to neurogenesis (see the review by Kichigina et al. [24]).

At the end of the last and beginning of this century, colocalization of glutamate and GABA was found in the dentate gyrus of developing rats (up to the end of the third week) in granular neurons and/or in "giant synapses" formed by mossy fibers [25-29]. This was also found in some studies on material taken from normal adult animals and humans [30-32]. A high level of vesicular GABA transporter (VGAT) mRNA expression was also found in granular cells of the dentate

gyrus in developing [26, 31, 33] and adult rats [32]. However, in some studies, the transporter protein was not detected in mossy fibers [34, 35]. This fact and the fact that individual GABA-containing neurons do not contain VGAT [34], indicated the existence of an as yet unidentified GABA transporter [33]. It was also demonstrated that full expression of GABAergic phenotype in granular cells requires calcium influx caused by activation of glutamate receptor and neurotrophin, as well as protein synthesis [33].

Interesting results in the aspect of colocalization of glutamate and GABA in the hippocampal system were obtained by Galván and Gutiérrez [36]. In developing rats, they analyzed the occurrence and plasticity of simultaneous glutamatergic-GABAergic signaling in the mossy fiber-interneuron system of the CA3 field of the hippocampus. In this work, monosynaptic responses evoked by stimulation of mossy fibers mediated by glutamate and GABAergic receptors were recorded [36]. Many interneurons in stratum lucidum and stratum radiatum demonstrated both types of response, i.e., they received both signals (with GABAergic signaling suppressed by activation of metabotropic glutamate receptors); at the same time, interneurons in stratum lacunosum-moleculare revealed an exclusively glutamatergic response. Notably, while glutamatergic responses were subjected to long-term depression, simultaneously recorded GABAergic responses of interneurons in stratum lucidum (but not radiatum), showed long-term potentiation. Thus, this work demonstrates a special type of transmitter colocalization in which there is a "compartmentalized" (segregated or separate) release of glutamate and/or GABA from different fiber endings in the same pathway and differential plasticity of granular cells depending on the type of interneurons to which they project [36].

In a series of experiments with double immunofluorescence staining of fixed hippocampal slices from rodents, it was demonstrated that, in norm, the endings of axons of granular cells (mossy fibers) in mature rats do not simultaneously reveal markers of glutamatergic and GABAergic synapses [37, 38]. Similarly, in experiments with selective stimulation of identified single giant synapses formed by mossy fibers on the apical dendrites of dissociated pyramidal neurons of the CA3 field, adult rats showed responses mediated only by glutamate receptors [29] (see also the review by Gutiérrez et al. review by Gutiérrez [39]); on the contrary, synaptic currents mediated simultaneously by glutamate and GABA receptors were recorded in developing rats upon stimulation of mossy fibers.

The situation is complicated by the fact that in adults, new granular cells are constantly emerging in the dentate gyrus [7]; therefore, it is possible that the joint release of glutamate and GABA found in adults [30-32] originates from immature granular neurons.

Interestingly, in contrast to normal brain, markers of glutamatergic and GABAergic phenotypes of granular cells/synapses of mossy fibers clearly coincided in time after convulsive

seizures in adult individuals [26, 27, 40-42] (Table 1). It is also emphasized that GABA release from mossy fibers is significantly enhanced by epileptic activity [41-43]. Treviño and Gutiérrez [42], who studied the GABAergic influence of granular cells of the dentate gyrus on hippocampal CA3 field neurons after convulsive seizures, showed that GABA released from mossy fibers in these cases acts on pre- and postsynaptic regions of neurons, affecting hippocampal activity at the network level. Another study [44] found that after generalized seizures, not only does GABAergic signaling occur in synapses formed by glutamatergic mossy fibers, but blockade of ionotropic GABA and glutamate receptors in pyramidal cells and interneurons of the CA3 field of the hippocampus also reveals M1-cholinergic depolarizing signal, which postsynaptically modulates glutamatergic and GABAergic fast neurotransmission. In contrast to cholinergic responses normally elicited by activation of associative/commissural fibers in the hippocampus, cholinergic responses to stimulation of the dentate gyrus were observed only after epileptic seizures and were suppressed by activation of type 2 glutamate receptors; however, both types of responses were suppressed by activation of M2-cholinergic receptors. Using immunohistological experiments, it was shown that the cholinergic pathway in the hippocampus runs parallel to the mossy fibers. The authors conclude that there is a complex interplay of different neurotransmitters in the hippocampal formation to maintain the delicate balance of excitation and inhibition that ensures its optimal functioning. In inhibition of glutamatergic mossy fibers has a general inhibitory effect on the CA3 field of the hippocampus, and disruption of this balance can lead to the onset of a seizure [44].

Medial septal complex (medial septal nucleus and Broca's diagonal band nucleus, MSDB). The MSDB is a nuclear structure standing at the entrance to the hippocampus from the brainstem side. It plays an important role in the modulation of hippocampal activity, being a pacesetter of theta oscillations generated in it [45-48]. Three types of cells have been found in the MSDB: cholinergic, GABAergic, and glutamatergic; axons of neurons of all these types form pathways to the hippocampus [49-52]. Colocalization of two major neurotransmitters, namely acetylcholine and GABA, was first identified in MFDB cells projecting to the hippocampus in 2003. [51]. The authors detected glutamic acid decarboxylase 67 (GAD67) mRNA in these neurons, which was sometimes localized together with choline acetyltransferase (ChAT) mRNA. Neurons simultaneously detecting GAD67 and ChAT accounted for 24% of the total cellular composition of the FFMDB. The results showing colocalization of acetylcholine and GABA confirmed data from earlier experiments performed on dissociated MFDB and/or Meynert's basal nucleus cell culture, where colocalization of ChAT and GAD67 mRNA was detected in 25% of neurons [53-55] (Table 1). Approximately the same number of FFMB neurons were found to have the ability to simultaneously synthesize and secrete two other transmitters, namely acetylcholine

and glutamate. This was revealed in MFDB cell culture using an electrophysiological method with pharmacological identification of the released neurotransmitters [56]. Interestingly, the capacity for simultaneous release of these two transmitters increased with the use of neuroinflammatory growth factor (NGF): after its exposure, the number of neurons releasing both mediators, acetylcholine and glutamate, increased from 29% to 48%. The colocalization of these two main neurotransmitters in the neurons of the MSDB was confirmed later, when it was revealed that a limited number of cholinergic neurons of this structure coexpress VGLUT3 [13, 57] (Table 1). All these data indicate that the MFDB neurons, which form part of the anterobasal region of the brain and project to the hippocampus, have the molecular capacity to co-express several transmitters.

Suture nuclei. The suture nuclei are the main source of serotoninergic pathways to the hippocampus; the median suture nucleus (mNSH) and the dorsal suture nucleus (dNSH) project to the CA1 and CA3 fields of the hippocampus, as well as to the dentate gyrus. The main afferent pathway from the mNASH goes to the dorsal part of the hippocampal formation [58, 59], while the dNASH projects to the ventral hippocampus and forms much less significant projections [60, 61].

The *median raphe nucleus (median raphe nucleus)* is known for its serotonin content, the marker of which is the serotonin transporter (SERT). Anatomical data show that mNASH projections form classical synapses on GABAergic interneurons in the hippocampus [62], potentially providing a substrate for rapid neuromodulation of the hippocampal network.

In addition to serotonin, cells of this nucleus may contain glutamate [15, 63-69]. This is evidenced by the data showing that VGLUT3 is present in mNASH neurons (and/or in their axons) along with SERT [63-68], as well as by the facts obtained in anatomical and electrophysiological studies [66, 69]. Interestingly, however, the markers $SERT^+$ and $VGLUT3^+$ are often co-expressed but can also occur separately [15, 63-67]. Although initial studies suggested that the majority of serotoninergic neurons in the dorsal and median suture nuclei express VGLUT3 mRNA [63, 70], subsequent work has shown that only about 50% of cells express VGLUT3 mRNA [65, 71, 72] (Table 1). Based on this, some authors believe that many neuronal populations in the brain utilize glutamate in addition to their "core" neurotransmitter [5]. Some studies [67, 68] have shown that most varicosities along axon trunks going to the hippocampus from mNASH neurons express both SERT and VGLUT3 markers, but other studies [65] showed the opposite results: these two markers (SERT and VGLUT3) were hardly expressed simultaneously in the hippocampus in the same varicosity. It is not yet known whether these contradictions are the result of differences in the methods used or whether this represents a physiologically important observation related to the functional state of neurons.

Along with SERT and glutamate, GABA was also detected in mNASH neurons [71, 72].

As for the possibility of serotonin and GABA colocalization in the projection pathways from the mNASH to the hippocampus, there are works confirming [21] and refuting this assumption [20, 73], i.e., question remains open.

The supramammillary nucleus (Supramammillary nucleus). The SOM is a thin structure lying above the mammillary bodies in the hypothalamus; this nucleus sends essential projections to the hippocampal formation. The AMU is composed of several cell populations that differ in their neurochemical composition and specificity of connections. The neurons of the AMS send their axons to the CA2/CA3a field of the dorsal and ventral hippocampus, as well as to the dentate gyrus (see reviews by Pan and McNaughton [74], Vertes and Kocsis [75]). At the cellular level, it has been found that neurons of the lateral portion of the SUM (SUML) are a distinct population. They intensively innervate the supragranular layer of the dorsal dentate gyrus, increasing the activity of this part of the granular neurons, and to a lesser extent project to the ventral dentate gyrus [76]. At the same time, SUML neurons show markers of a dual phenotype: VGLUT2, VGAT and GAD65, i.e. they are simultaneously capable of releasing glutamate and GABA [77-79] (Table 1). There is morphological evidence of the dual action of SUML on dentate gyrus neurons in rats and mice: they establish asymmetric (presumably excitatory) and symmetric (presumably inhibitory) contacts on different neurons of this structure [76, 78, 79] (see Fig. 1). In the work of Billwiller et al. [76], it was also shown that one axon bud formed by a SUML neuron can form an asymmetric synapse on some granular cells and a symmetric synapse on others. At the same time, stimulation of axon ends of SUML neurons innervating the dorsal dentate gyrus induces cotransmission of GABA and glutamate on almost all granular cells [76, 80, 81].

Importantly, the REM controls the hippocampal theta rhythm [75, 82, 83] and is involved in learning and emotional memory formation [74, 84-88]. The recent study mentioned above [76] showed that during rapid-eye-movement (REM) sleep in mice, the power of theta and gamma rhythms in the dentate gyrus can increase under the influence of optogenetic stimulation of the RMSL. Furthermore, during slow-wave sleep, activation of the sUML-tentate gyrus pathway caused mice to awaken, switch from delta to theta activity, and increase the power of gamma oscillations. Thus, supramammillary neurons colocalizing GABA and glutamate may be involved in the control of theta and gamma oscillations in the dentate gyrus during REM and slow-wave sleep, contributing to cognitive functions such as learning and memory.

Ventral tegmental area (VTA) (ventral tegmental area). The VTO or A10 is the main source of dopamine in the ventral hippocampus. The use of immunochemical methods and fluorescent histochemistry showed that dopaminergic axons are distributed in the dentate gyrus and, to a lesser extent, in the CA1 field of the rat hippocampus; an insignificant number of fibers was also found in the CA3 field [89]. In monkeys, the density of dopaminergic fibers is moderate

and diffuse throughout the hippocampus [90]. Numerous studies using electrophysiological and optogenetic methods have shown that in many cases dopamine-producing neurons also release glutamate [91-94] and acetylcholine [95, 96]. A study of the distribution of VGLUT2 mRNA in the WTO revealed a relatively high number of dopaminergic neurons containing VGLUT2 mRNA in the rostro-medial region of the WTO [97, 98]. A recent study found that WTO neurons containing VGLUT2 (i.e., releasing glutamate) also reveal GABAergic markers, GAD and VGAT [79]; more than 20% of neurons with this phenotype were found to be present in the WTO (Table 1).

The locus coeruleus (locus coeruleus). The locus coeruleus is a small nucleus located deep in the brainstem that is the main source of noradrenaline for the hippocampus [99-101]. In general, the pattern of noradrenergic innervation is similar at all septo-temporal levels of the hippocampal formation; the densest plexuses are found in the hilus of the dentate gyrus, str. lacumosum CA3, str. moleculare of the entire hippocampus, and the subiculum [99]. Some noradrenaline-containing neurons demonstrate a glutamatergic phenotype and VGLUT2 expression [102, 103] (Table 1). In this case, the activity-dependent concentrations of noradrenaline are locally modulated in the target structure due to the released glutamate; the plastic transformations (potentiation or depression) realized in the hippocampus depend on the local concentrations of noradrenaline [103]. Interestingly, the neurons of the locus coeruleus can also release dopamine [104-106]; its release in the hippocampus depends on changes in the activity of the noradrenaline transporter triggered by high levels of local glutamate, as well as on the activation of NMDA receptors on the terminals of noradrenergic neurons [107, 108]. The possibility of co-release of dopamine and noradrenaline in the presence of high levels of local glutamate in the hippocampus is an important mechanism, and its deciphering may improve our understanding of the influence of the locus coeruleus on plastic processes in the brain and on unraveling the nature of attention and memory.

Possible functional significance of mediator colocalization in the hippocampus. Speculatively, we can assume that cells with two or more transmitters may contribute to the reduction of metabolic costs and signaling errors, as well as increase the information capabilities of neuronal networks and ensure the accuracy and flexibility of their operation.

When investigating the question of what are the functions of the joint release of glutamate and GABA in the hippocampal formation, it is interesting to consider the following experimental facts: when stimulating the internal systems of connections from the dentate gyrus to the CA3 field of the hippocampus (at slightly suprathreshold currents), an inhibitory response was most often observed, and only when the current intensity was further increased, a neuronal discharge occurred [109]. Taking into account the possibility of joint release of glutamate and GABA from mossy fibers in the dentate gyrus [32, 110], this phenomenon can be explained in the following way: at

weak signals, the synapse in which the two mediators are released "works" as a purely GABAergic synapse, and at their amplification - as a glutamatergic synapse. Analysis of extracellular responses of hippocampal neurons and related structures shows that the initial phase, often regarded as a latent period, actually includes a brief inhibitory "reset" (reset) when correlated with the preceding spontaneous activity. This can achieve a significant increase in the signal-to-noise ratio and synchronization of responses, improving the effect of weak signals [46, 111]. In the absence of inhibitory reset, the signal-to-noise ratio decreases sharply, often to zero, and responses in the hippocampus are blocked [47, 112]. Similar facts were obtained when activating the fibers of the perforant pathway and registering responses in the dentate gyrus and CA1 field of the hippocampus [113]. In addition to the hippocampus, the significance of the inhibitory "resetting" or "zeroing" effect has been shown in related systems both in signal processing and in preparation for movement [114, 115]. Regarding the mechanism of these events, experimental data and computational modeling show that inhibition for several milliseconds in close proximity to excitatory glutamate receptors effectively reduces the amplitude of the postsynaptic calcium response [116-118]; the spatial specificity of calcium dynamics may also be enhanced [118]. Recently, it was also found that in somatostatin-positive hippocampal interneurons, calcium influx via NMDA receptors selectively enhances inhibition from a subset of inhibitory synapses (see Chiu et al. [117]). In addition, VGLUT expressed in axon endings can lead to enhanced uptake of GABA into synaptic vesicles (vesicular synergy) [32, 119], thereby increasing GABA release. Moreover, combined effects on glutamate and GABA receptors at the postsynapse may provide a faster and more targeted form of short-term inhibition [4].

In addition to the hippocampus itself, the dentate gyrus, which belongs to the hippocampal formation, is another structure where we can trace the action of mediators of opposite orientation, released at the same stimulus and having the same target. Indeed, granule cells and their axons (mossy fibers) express in a regulated manner all markers of both phenotypes: glutamate and GABA, as well as GAD, VGLUT and VGAT; their activation elicits in postsynaptic target cells synaptic responses mediated by both glutamatergic and GABAergic receptors. It has been observed that the dynamics of neurotransmitter phenotype expression in granular cells are as follows: initially GABAergic, then dual glutamatergic-GABAergic, then glutamatergic only, but after a period of hyperexcitability it can temporarily become glutamatergic-GABAergic (see Gutiérrez [27] for a review). The expression of these phenotypes, which depends on the brain activity in which the dentate gyrus is involved, seems to be involved in maintaining the balance of excitation and inhibition in the contacts between the dentate gyrus and the CA3 field (see Gutiérrez [39] for a review).

On the other hand, the activity of dentate gyrus cells is also modulated by SUML neurons

through the release of glutamate and GABA [77, 79]. In the work of Ajibola et al. [120] in mice using optogenetic, electrophysiological and pharmacological approaches demonstrated that the SUML differentially regulates the activity of different dentate gyrus neurons through different synaptic mechanisms. Although activation of the sUML results in synaptic excitation and inhibition of all postsynaptic cells, the ratio of these two effects is variable and cell type dependent. In particular, those interneurons in the dentate gyrus that receive projections to dendrites experience predominantly synaptic excitation, whereas other interneurons that receive projections to soma as well as granular cells only decrease the latency period of responses to excitatory impulses and increase fidelity of triggering. In addition, excitation of the SUML enhances glutamate release from granular neurons in response to cortical input signals, thereby promoting induction of long-term potentialization at synapses from the neocortex to granular neurons. Taken together, these data indicate the important physiological significance of glutamate and GABA cotransmission by SUML neurons for the functioning of the dentate gyrus network [120].

Regarding the role of cotransmission in the generation of the theta rhythm in the hippocampus (which is necessary for information processing, learning, and memorization [45]), it is assumed that GABAergic cells of the MSDB, by phasically influencing cholinergic neurons of this structure via GABA receptors, involve a larger population of septal neurons in synchronous activity [45]. Considering that glutamatergic neurons of the ISDB also project to the hippocampus [51, 52], and some cholinergic neurons of this structure co-express VGLUT3 [13, 57], it can be assumed that glutamate released from septal neurons plays a significant role in the generation of the hippocampal theta rhythm.

In addition to the hippocampus and dentate gyrus, the structure where two neurotransmitters of opposite action, released at the same stimulus and having the same target, can work is the mNASH, where such mediators are glutamate and serotonin. Glutamate in mNASH is released from $VGLUT3^{+}$ -vesicles of those cells in which it is the main neurotransmitter; however, this mediator is also found in serotoninergic neurons and, in a small percentage in GABAergic cells [21, 71, 72]. In contrast to the slow modulatory effect usually associated with ascending systems, electrical stimulation of this nucleus or pathway from the mNAS causes a rapid and robust modulation of network activity in the hippocampus, the IADB, and other underlying structures [75, 121, 122]. In the hippocampus, this is accomplished by classical synapses formed by mNASH cells on GABAergic interneurons, potentially providing a substrate for rapid neuromodulation of the hippocampal network [16]. Thus, the demonstration of rapid synaptic activation of hippocampal interneurons by mNASH afferents via co-transmission of glutamate and serotonin shows that the view of subcortical control of cortical activity as slow and diffuse state-dependent modulation [123, 124] is now complemented by evidence for the ability of subcortical afferents to

exert synaptic influences with high ***temporal*** and spatial resolution [125-127]. This likely contributes to the rapid formation and selection of specific local representations or modes of information processing in the hippocampus [128, 129]. It has been suggested that this may be accomplished by rapid changes in the relative contribution of different classes of hippocampal interneurons to rhythmic population activity [130, 131]. This assumption is supported by other, particularly more recent work [20, 21, 132, 133].

It is known that different types of inhibitory interneurons in the hippocampus are specialized for innervation of certain classes of pyramidal cells and, within them, various subcellular compartments (in particular, specific parts of dendrites) [132, 133]. One remarkable type of interneurons in the hippocampus are "dual" CCK-VGLUT3-positive basket cells that have the ability to send signals using both glutamate and GABA; in addition, they contain presynaptic cannabinoid type 1 receptors (CB1R), indicating their close bidirectional interactions with postsynaptic pyramidal cells. The functions of these neurons have been studied in detail by simultaneous studies of two groups of authors [18, 21]. It was shown that in mice with absence of VGLUT3 in CCK^+ -neurons ($VGLUT3^{-/-}$ line) a marked increase of GABAergic transmission to pyramidal cells of CA1 field is detected. This suggests that the inhibition exerted by GABAergic CCK^+ -neurons is VGLUT3-dependent [21]. In the work of Del Pino et al. [18] revealed that in the absence of the tyrosine kinase receptor ErbB4 during animal (mouse) development, the normal integration of CCK-VGLUT3-positive basket cells into neuronal networks is impaired and the number of inhibitory synapses formed by them is reduced. Accordingly, the inhibitory effect they exert on pyramidal neurons already in adult animals is reduced. In such mice, as well as in the absence of VGLUT3 in mice [21], a shift of synaptic plasticity in the Scheffer-field CA1 collateral system and impaired theta oscillations were observed [18, 21]. It was also shown that a decrease in the number of synaptic contacts formed by basket CCK-VGLUT3-positive interneurons impaired the spatial encoding of hippocampal place cells. Thus, in object location recognition tests and in the Morris water maze, it caused selective changes in spatial learning and memory [18]. These results suggest that normal integration of $VGLUT3^+$ GABAergic basket cells into neuronal networks is key to maintaining theta oscillation-regulated spatial encoding by place cells in the hippocampus.

The importance of cotransmission in learning and memory. Several studies have identified the involvement of colocalizing neurotransmitters, including glutamate, GABA, acetylcholine, and serotonin, in the formation of contextual fear memory [134-138]. Subpopulations of neurons and fibers in the hippocampus, amygdala, and prefrontal cortex that release more than one neurotransmitter [5, 119] are known to express an atypical vesicular glutamate transporter type 3, VGLUT3, when producing fear-evoked responses [21, 139-142].

Several studies have shown that the absence of this transporter in neurons resulted in blockade of glutamatergic currents mediated by muscarinic glutamate receptors in the hippocampus [21], while others have shown blockade of glutamatergic ionotropic currents [126]. Interestingly, VGLUT3$^{-/-}$ mice show persistent hyperresponsiveness to stress [139] and impaired regulation of the hypothalamic-pituitary system [143]. Several studies have shown that VGLUT3-deficient mice have a stronger contextual memory for fear and tend to "transfer" fear developed in a certain situation to other situations (so-called generalization) [143] without other serious memory impairments [144].

In the work of Fasekas et al. [144] tested the hypothesis that vesicular glutamate transporter 3 (VGLUT3) deficiency is associated with cognitive impairment. Male mice with genetic knockout of VGLUT3 (KO) and wild-type (DT) mice were subjected to a series of behavioral tests based on spontaneous exploratory behavior as well as reinforcement. Reverse learning was used to test cognitive flexibility. KO mice showed some learning ability; for example, social recognition memory was intact in these mice. The Y-maze test revealed a weaker working memory in KO mice, but again no serious learning impairment was observed. There were also no severe learning impairments in the operant conditioning or discrimination paradigm. In avoidance-based learning tests (Morris water maze and active avoidance), KO mice showed slower learning compared to DT mice, but not a complete learning impairment. In tests based on simple associations (operant conditioning, avoidance learning) KO mice showed impaired cognitive flexibility. Thus, genetic knockout of VGLUT3 leads to mild impairments in working memory and learning flexibility. The authors conclude that this glutamate transporter is not a major player in learning and memory formation in general. It is noted that further research is needed to identify the role of local VGLUT3-positive neurons and their terminals in the processes required for different types of declarative memory [144].

The available data on the functional role of neurotransmitter colocalization are summarized in Table 2.

Table 2. Functional role of mediator colocalization in hippocampus and afferent structures

Transmitters	Functional value
Glutamate+ GABA	increasing signal-to-noise ratio and synchronization of responses that improve exposure to weak signals [46, 109, 111]; ensuring synaptic plasticity, generation of theta oscillations in the hippocampus, improving spatial learning and memory [18, 21]; maintaining the balance of excitation and inhibition in the hippocampal

	CA3 ZI-field system [39]; induction of long-term potentialization in the pathway from the neocortex to the granular neurons of the ZI [120].
Glutamate+ serotonin	rapid and reliable modulation of the network activity of the hippocampus, MSDB, and other forebrain structures [75, 121, 122]; maintenance of synaptic influences with high ***temporal*** and spatial resolution in the mNASH-hippocampus system [125-127]; improving spatial coding by place cells in the hippocampus [18], information processing in general [128, 129]
Glutamate+ dopamine	providing reward-based learning (operant conditioning with WTO) [144]; improving learning flexibility and working memory formation [144]
Glutamate+ noradrenaline	regulation of plastic transformations in the hippocampus (potentialization or depression) by local concentrations of noradrenaline [103]
GABA+ acetylcholine	providing the mechanism of theta rhythm generation in the hippocampus involved in information processing, learning, and memory [45]

Note. Abbreviations used: VTO, ventral tegmental area; ZI, dentate gyrus; MSDB, complex of medial septal nucleus and nucleus of diagonal Broca's bundle; SUM, supramammillary nucleus.

CONCLUSION

It is now known that a nerve cell can produce and use several different molecules, including several classical mediators, to communicate with other neurons, which can have opposite effects on the target. Colocalization of neurotransmitters in hippocampal cells and its afferent systems presumably enhances the information capacity of neuronal networks, as well as the accuracy and flexibility of their operation. The mechanisms of release of colocalizing transmitters and their functional role in hippocampal function have not yet been definitively elucidated. It is assumed that the effects of weak signals to the hippocampus may be enhanced by the release of GABA and glutamate with strictly defined ***time*** delays determined by the generated theta rhythm. As a consequence, this may significantly increase the synchronization of responses of the main neurons and the signal-to-noise ratio in the neural network. It is also hypothesized that expression of different mediator phenotypes in the hippocampus contributes to rapid information processing selection, induction of long-term potentialization, and spatial place coding by cells. In addition, evidence has been obtained indicating that colocalization of transmitters may provide flexibility for learning and working memory formation.

It is important that colocalization of neurotransmitters in hippocampal cells and afferent systems is involved in maintaining the balance of excitation and inhibition in its separate regions, which is necessary for the normal functioning of the whole brain.

Several questions remain open and must be resolved to fully understand the functional significance of the joint release of neurotransmitters from the same neurons. First, although there is evidence indicating that two mediators of opposite action released at the same stimulus and sharing the same target may work in the brain, it remains unknown whether they can simultaneously affect the same neuronal locus of that target. There are other questions not fully resolved. Are both transmitters used to perform similar or different functions (optogenetic stimulation does not unambiguously answer this question because it usually causes the release of both transmitters)? In the case where distinct populations of vesicles are present in the active zone (see Root et al. [79]), are these vesicles directed to different release sites? Does each synaptic vesicle contain only one or more classes of transporters? Is there selective control of the release of each neurotransmitter? The existence of another, unidentified, GABA transporter (other than VGAT) (see Gomez-Lira et al. [33]) has also not yet been confirmed, but not disproved either.

The question of how the dentate gyrus-Ca3 field system in the hippocampus of the adult brain after a seizure becomes similar to that in the developing brain has not yet been answered. Is there an initial colocalization of GABA and glutamate in granular cells of the dentate gyrus and the possibility of their simultaneous release from mossy fibers in the mature brain that is clearly realized only in the postconvulsive period? If so, due to what inhibitory control existing in the norm, this mechanism is suppressed?

These questions should be answered by future studies using the latest advances in neuroscience (in particular, approaches using optogenetics combined with super-resolution microscopy and visualization of pre- and postsynaptic proteins (see, for example, the comparative study by Dani et al. [145] and the work of Chang et al. [146]). The solution of these problems will advance some areas of basic science, as well as help in the therapy of those diseases where the balance of excitation and inhibition is found to be disturbed, such as epilepsy, Alzheimer's disease, and many others.

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CONFLICT OF INTERESTS

The author declares that there is no conflict of interest.

ETHICS DSCLARATION

The review is written in compliance with ethical norms accepted by the Russian Federation and

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