Chapter 5

Synaptic Soluble and Membrane-Bound Choline Acetyltransferase as a Marker of Cholinergic Function In Vitro and In Vivo

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Additional information is available at the end of the chapter

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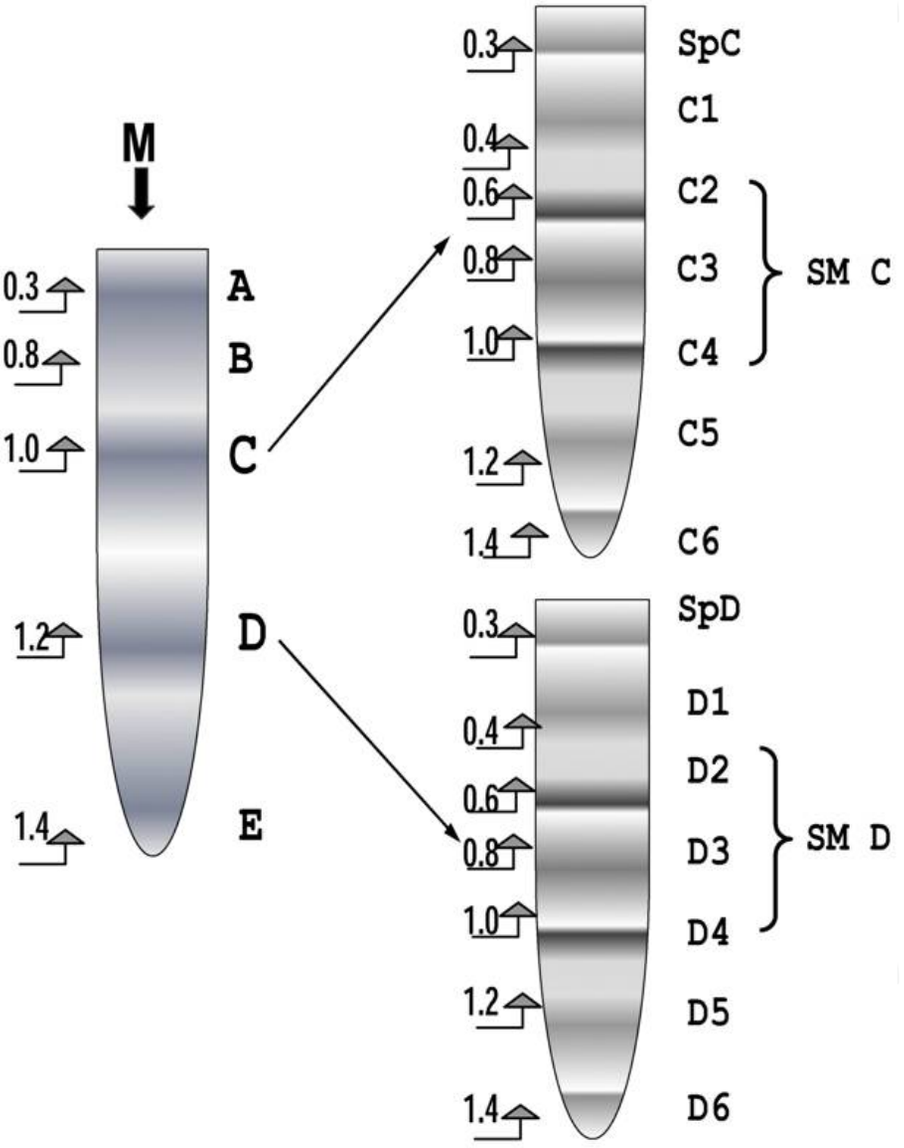
1. Introduction

1.1. Synaptosomes — Definition, a bit of history

The synaptosome is the presynaptic part of the nerve ending with, as a rule, a postsynaptic membrane in the region of the junction of the pre- and postsynaptic membranes that remains with the presynapse during homogenization and centrifugation. The presynaptic part of the synaptosome is a membrane-bound structure with a preserved cytoplasm (synaptoplasm), synaptic vesicles, mitochondria and some other cellular components. The term *synaptosome* was adopted by V.P. Whittaker and coworkers [1]. Together, V.P. Whittaker and C.O. Hebb first isolated and identified nerve endings in nervous tissue [2].

Subcellular fractionation emerged in the 1930s and 1940s and has since established itself as a major technique in experimental biology. The first attempts at the fractionation of nervous tissue were made in the early 1950s. A few years later, the fraction of synaptosomes was successful, using discontinuous sucrose-density gradient centrifugation [2-4]. After this, researchers achieved the preparation of the synaptic components, including the synaptic membranes, synaptoplasm, synaptic vesicles [1, 5, 6] (Figure 1) and membrane junction complex [7, 8]. These studies were a powerful impetus for investigations into the biochemistry of synapses and in the development of new methods of synaptic fractionation. Synaptosomes, as nerve endings, are heterogeneous in density, size and mediator specificity. Therefore, a number of the methods were developed for separating the synaptosomal fraction into two fractions [4], as well as into many fractions using a continuous sucrose-density linear gradient [9-11]. Among the many publications at this time, two books stand out. In the first one, D.J. Jones recounts the story of subcellular fractionation techniques, and presents the entire set of

modern synaptic and subsynaptic fractionation techniques and data about the ultrastructure of synaptosomes and synaptic components, their sedimentary characteristics and biomarkers [12]. In the second one, R.N. Glebov is focused on the achievements of that time in the field of the functional neurochemistry of synapses, their molecular structure as well as on the metabolism and biochemistry of "classic" neurotransmitters and on the concepts of mediator secretion [13].



To the left of the tubes are marked the density of sucrose layers (in moles). Fractions obtained from crude mitochondrion fraction (by the method of De Robertis et al., 1962): myelin (A), neuronal and glial membrane and possibly small synaptosomes (B), light (C) and heavy (D) synaptosomes, cell mitochondria (E). Subsynaptic fractions obtained from light and heavy synaptosomes fractions (by the method of Whittaker et al., 1964): synaptoplasm (Sp), synaptic vesicles (1), synaptic membranes (2, 3, 4), non-disrupted synaptosomes (5), synaptic mitochondria (6).

Figure 1. Scheme of distribution of the fractions and subfractions of synaptosomes in discontinuous sucrose-density gradients.

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2. Synaptosomes as an object of study in vitro and in vivo

Since these techniques were developed, new technologies in brain research have emerged. However, synaptosomes and their components remain a unique object of study. The reasons for this are as follows:

- the synapse is a unique structure specialized in the chemical transmission of nerve signals (chemical synapses are mainly in the mammalian brain).
- the synapse is always at the center of concepts about the adaptive properties of nervous tissue, such as learning and memory.
- the synapse is the most dynamic and labile structure of the nerve cell, and is an indicator of the reaction of the neuron to external stimuli.
- the synapse is an inherent structure of the neuron only.

It is now known that neurotransmitters and their key metabolic enzymes exist in some non-neuronal mammalian and human cells, including some cells of the neuroglia and vascular endothelium, epithelium and blood. In these cells, neurotransmitters perform specialized functions such as proliferation, differentiation, migration, organization of the cytoskeleton, cell-cell contact, secretion and transport of ions and water, blood-brain barrier maintenance and anti-inflammatory functions [14-17]. So, synaptomoses are the only object of molecular and biochemical studies that guarantees the investigation of neuronal function. Therefore, new technologies for the isolation of synaptosomes and their components continue to be developed, consistent with the purpose of science [18-20].

Using synaptosomes, one may study in vitro the molecular mechanisms of neurotransmitter secretion and the metabolism of neurotransmitter systems using the entire complexity of the molecular processes or using models of functional or pathological conditions in vitro and ex vivo.

Studies on synaptosomes in vivo are rarer but they are not less important. The brain is a very complicated organ in which a neuron exists in a permanent relationship with many other neurons. These interactions occur mainly through synapses. It is important not to forget about the signaling molecules that come into the brain through the blood, cerebrospinal fluid and intracellular matrix. The functional response of presynapses reflects the integrated response of the neuron to a stimulus. Therefore, it is important to know whether the patterns of synaptic functions are identical in vitro and in vivo.

In vivo models are used to investigate effects on the entire organism such as learning models and models of adapting and neuropathology. Then, the synaptosomes or subsynaptic components from the brain structures can be isolated. It is usually impossible to analyze the totality of the synaptic molecular and metabolic processes in these studies. The synaptic reaction is measured by the synaptic key indicators identified in studies in vitro. It is important that the biochemical methods allow the estimation of very fine metabolic and functional changes in synapses. The connection between nervous system function and synaptic processes has been

investigated this way. It is possible to research the reaction of certain brain structures and even certain neuronal populations to external influences. Using neuromediators as markers, one can identify the participation of neuromediator systems in the mechanisms of various brain functions.

Furthermore, the in vivo study of synaptosomes has additional scope. Using biochemical parameters, not only metabolic changes can be evaluated, but also quantitative (synaptogenesis, reduction, degeneration) and morpho-structural (transformation) reorganizations in the synaptic pool. This is possible in comparative studies on the synaptic membrane and synaptoplasm subfractions. Subfractions of synaptic membranes and the synaptoplasm are the largest integral parts of the presynapse. Therefore, a correlation between the biochemical membrane (m) and cytosolic (c) biochemical parameters may reflect the reaction of the presynapse as a structural unit.

3. Natural markers of the neuronal systems

Neurotransmitters and some molecules of neurotransmitter metabolism are used as neuronal markers. These are natural indicators of functionally specialized brain systems, given to us by nature. Therefore, neuronal markers are widely used in biochemical studies, both in vitro and in vivo.

Neurotransmitter systems are named based on the main transmitter (glutamatergic, GABAergic, dopaminergic, etc.). Each mediator system consists of several neuronal populations. The neuronal populations in the brain are distributed topographically. Depending on the locus in the brain, neurons form specific neuronal connections using a specific combination of receptors. Moreover, these neurons can have specific metabolism dependent on their functional destination. Therefore, topography determines their metabolic and functional effects. Additionally, different neuronal populations often express comediators. These comediators influence the effects of mediators and metabolic pathways of the neuron in certain ways. It seems that future prospects in the study of brain function will be the investigation of the functional, metabolic and molecular features of distinct neuronal populations. It is necessary to understand the true mechanisms of the regulation, maintenance and recovery of brain functions. It should be noted that studies on synaptic fraction levels were carried out along these lines from the beginning [4, 18, 21-31].

In particular, regarding the cholinergic brain system, studies on this neurotransmitter system have been performed on synaptic fractions in vitro and in vivo. This review will present data from investigations into the molecular properties and metabolic and functional characteristics of cytosolic (c) and membrane-bound (m) choline acetyltransferase (ChAT) and of the use of cChAT and mChAT as cholinergic markers to establish brain function mechanisms. For the sake of completeness regarding modern notion, the characteristics of the molecular forms of ChAT will be presented using data from tissue cultures as well.

But first, a brief description of the cholinergic brain system.

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4. Cholinergic brain system, cholinergic neuronal populations and their importance in health and disease

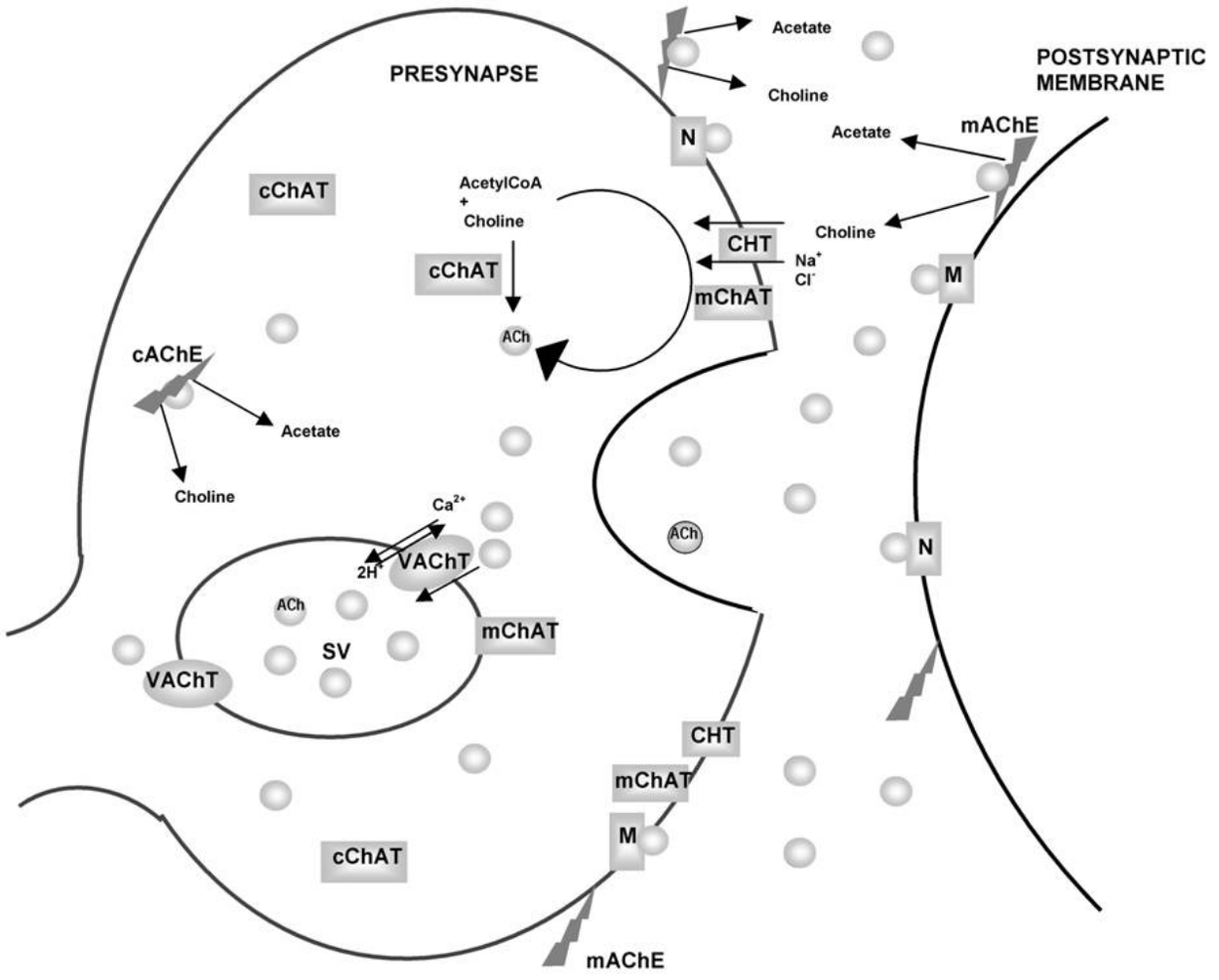
Cholinergic neurons use the classical neurotransmitter acetylcholine (ACh). ACh is a famous mediator. It was the first neurotransmitter discovered, by Otto Loewy in 1921-1926, and it proved the validity of the chemical nature of nervous communication [32]. ACh was quantified in P. Fatt's and B. Katz's experiments when the quantum nature of chemical neurotransmission was discovered [33]. S.O. Hebb and V.P. Whittaker used ACh and ChAT as indicators when they searched for and found subneuronal structures (synaptosomes) which accumulate mediators [2]. ACh was the first among the neuromediators found in the non-neuronal cells of mammals [34]. It would perhaps be helpful to add that ACh is also called "gentleman number one", for its non-neuronal function as well. It is clear that ACh is the most thoroughly examined neurotransmitter.

4.1. Metabolism of acetylcholine

ACh is an ester of acetic acid and choline with the chemical formula $CH_3COO(CH_2)_2N+(CH_3)_3$ and systematic name 2-acetoxy-N,N,N-trimethylethanaminium.The cycle of ACh synthesis, storage, release and degradation has been well-characterized at the cellular and molecular levels [26, 35, 36]. Briefly (Figure 2), ACh is synthesized in the cytoplasm of cholinergic neurons from the precursors choline and acetyl-coenzyme A by the enzyme choline acetyltransferase (ChAT), and is then taken up into synaptic vesicles for storage by the vesicular acetylcholine transporter (VAChT). Depolarization of the nerve terminal causes the fusion of synaptic vesicles with the presynaptic membrane at specialized release sites called active zones (named the junction complex in the subsynaptic fraction). Depositing and releasing ACh is a calcium-dependent process that involves the coordinated actions of many presynaptic proteins [26, 37]. When vesicles are linked up with the presynaptic membrane, ACh diffuses into the synaptic cleft where it can bind to subtypes of nicotinic and muscarinic receptors located on both post- and presynaptic membranes. ACh signaling is terminated by its diffusion away from the synaptic cleft and by its rapid hydrolysis into choline and acetate by acetylcholinesterase (AChE). The choline derived from ACh hydrolysis is recycled into the presynaptic terminal by the sodium-dependent high-affinity choline transporter (CHT) for resynthesis of ACh. After secretion of ACh, synaptic vesicles are recycled and are refilled with the neurotransmitter for another round of the depolarization-induced release. It should be noted that the details of the molecular mechanism of the regulation of these processes in both health and disease are lacking.

4.2. Topography and functions of cholinergic neuronal populations

Knowledge of the topography of mediator systems is basic for neurobiologists. The topography of the populations of cholinergic neurons and their projections has been studied in detail. ChAT has long been used as a marker of cholinergic structures in immunohistochemical studies. Initially, AChE was used as the cholinergic marker, but it was found that AChE coincides with ChAT only partially [38]. Later, colocalization of AChE was revealed in non-



The idea of the scheme is taken from Black and Rylett [Black & Rylett, 2011]. Abbreviations: Presynapse, presynaptic part of nerve ending; SV, synaptic vesicle; ACh, acetylcholine; AcetylCo-A, acetylCoenzyme-A; cChAT and mChAT, cytoplasmic and membrane-bound choline acetyltransferase correspondingly; mAChE and cAChE, membrane-bound (extracellular isoform) and cytoplasmic acetylcholinesterase correspondingly; CHT, sodium-dependent, high-affinity choline transporter; M, muscarinic receptor; N, nicotinic receptor; VAChT, vesicular ACh transporter.
Some details of molecular mechanisms of the regulation of these processes are set out in sub-chapter 4.1.

Figure 2. Mechanisms involved in the synthesis, storage, release and degradation of ACh at the cholinergic synapse.

cholinergic neurons. For example, 36% of AChE-positive cells are GABA-immunoreactive [39]. VAChT, discovered after ChAT, is also used as a marker [40]. However, some discrepancies between VAChT and ChAT have been found [41].

Cholinergic neurons innervate almost all areas of the nervous system, both the central and peripheral systems. These areas can be innervated by either extrinsic projective neurons or by intrinsic interneurons. A very famous and major group of cholinergic projective neurons is found in the basal forebrain, which is comprised the nucleus basalis magnocellularis, also called the Meynert nucleus in primates and humans (a large bundle of cholinergic neurons encompassing the magnocellular preoptic nucleus, substantia innominata and globus pallidus), the medial septal nucleus and the vertical limb nucleus of the diagonal band of Broca.

The cortex, amygdaloid complex, hippocampus and olfactory bulb receive their cholinergic innervation principally from cholinergic projection neurons of the basal forebrain [40-49]. It is known that basal forebrain cholinergic projective neurons play a role in attention, learning, memory and consciousness. Another group of cholinergic projective neurons is found in the upper brainstem, which is comprised the pedunculopontine nucleus of the pontomesencephalic reticular formation and within the laterodorsal tegmental gray of the periventricular area. The thalamus and medulla receive their cholinergic innervation principally from cholinergic projection neurons of these brainstem nuclei. These neurons also present a minor component of the corticopetal cholinergic innervation of the frontal and visual cortical areas [43, 49-51]. The cholinergic projective neurons of the mesopontine region play a role in the primary treatment of some sensory information and memory (in the thalamus) and, hypothetically, in the central mechanisms regulating respiration and blood circulation (in the medulla). All immunohistochemical studies indicate the topographical arrangement of cholinergic projections. On the basis of connectivity patterns, M.M. Mesulam and coworkers proposed that the central cholinergic projective neurons to subdivide into six major sectors designated Ch1-Ch6 [43]. Moreover, the rostrocaudal and layerwise topographical arrangement of the cholinergic projections is indicated in the cerebral cortex [44-46, 48].

The most famous cholinergic interneurons are localized in the striatum, and they are involved in motor function and cognition [49]. As well, cholinergic interneurons have been detected in the cerebral cortex [46, 48, 52] and in the hippocampus [53-55]. Cortical and hippocampal interneurons perform associative functions and are presumably involved in learning and memory. Numerous electrophysiological studies have indicated this, but regarding cholinergic cortical and hippocampal neurons, such data are absent. In the human cerebral cortex, ChAT-immunoreactivity was found in some of the giant Betz and Meynert's pyramidal neurons [56].Cortical pyramidal neurons carry out motor functions. The medullar reticular formation has ChAT-positive neurons and their participation in the respiratory center is assumed [50].

Finally, ACh as a neurotransmitter is widely presented in the peripheral nerve system [40, 49, 57]. Acetylcholine is one of many neurotransmitters in the autonomic nervous system and is the only neurotransmitter used in the motor division of the somatic nervous system. The parasympathetic motoneurons of as the cranial nuclei of the caudal brainstem and postganglionic neurons and preganglionic sympathetic motoneurons of the spinal cord nuclei are ChAT- and VAChT-positive. Their efferents innervate all vegetative organs and glands, parasympathetic directly and sympathetic indirectly. ChAT- and VAChT-immunoreactivity has also been detected in the cell bodies of the spinal nerve motor neurons as well as in their axons and the endplates of the skeletal muscles.

It was found recently that the vagus (parasympathetic) nerve, involved in the control of heart rate, bronchomotor tone, hormone secretion and gastrointestinal motility, is also an immunomodulator. Its stimulation attenuates the production of proinflammatory cytokines and inhibits the inflammatory process via the $\alpha 7$ nicotinic acetylcholine receptor [58, 59]. It is possible that these studies are beginning to describe a new function of the cholinergic nerve system.

4.3. Comediators and other neuroactive components in some cholinergic neuronal populations

The functional effects of ACh are unique to each cholinergic population due to its targets and chemical composition. As a whole, ACh functions more often as a modulator in the central nervous system and as a mediator in the peripheral nervous system. Some populations of cholinergic neurons co-express vasoactive intestinal peptide (VIP) or/and nitric oxide (NO) or substance P. VIP has been found in cholinergic interneurons of the cortex [48, 60, 61] and in the parasympathetic efferents to the airways [62, 63]. Substance P is present in the majority of projections to the medial frontal cortex from ChAT-positive neurons in the midbrain [48]. ChAT-VIP-, NO synthase-ChAT- and NO synthase-ChAT-VIP-immunoreactive ganglionic cells have been detected in the sphenopalatine ganglia [64, 65].

All three substances as well as ACh are well-known vasodilators. Therefore, their co-localization with ACh is connected in the first place with blood flow regulation. The vasodilator action of ACh on the vessels of the vegetative organs was one of its first described effects [66]. With respect to cerebral vessels, it was detected in (1) direct contacts with small cortical vessels with vasodilator effects of the cholinergic projective neurons and interneurons, including ACh-VIP-containing interneurons [61, 67-69]; (2) ACh-, NO-ACh- and rarely ACh-VIP- containing fibers innervate the middle cerebral arteries composed of perivascular nerves of the sphenopalatine ganglia [64]; (3) ACh induces both direct vasodilation and atypical constriction in the internal cerebral arteries [64, 70, 71]; (4) brainstem ACh indirectly induces, via the stimulation of the dorsal facial area neurons of the medulla, a vasodilator effect in the common carotid and the internal cerebral arteries [72, 73].

The third vesicular glutamate transporter (VGLUT3) is present in a subset of cholinergic projective neurons in the basal forebrain and in cholinergic interneurons in the striatum [74]. It should be noted that both these cholinergic populations have similar large neurons. VGLUT3 is one of three transporter isoforms that fills synaptic vesicles with glutamate; however, VGLUT3 is also expressed in neurons and brain regions that were not previously thought to use glutamate as a neurotransmitter. It is possible that VGLUT3, because of its ionic balance, helps to load synaptic vesicles with ACh. In addition, the cholinergic projective neurons of the basal forebrain express the nerve growth factor (NGF) receptor [75, 76]. Basal forebrain neurons are trophically responsive to NGF. Neurotrophin is important for the development and maintenance of the basal forebrain cholinergic phenotype. In these neurons, NGF markedly increases ACh synthesis, content and release [77, 78].

4.4. Cholinergic functions and brain diseases

The brain cholinergic system is of permanent interest for neuroscientists because of its important role in cognitive, attention and motor functions. Dysfunction of cholinergic neurotransmission in the central nervous system is revealed in a number of neurological disorders. Dysfunction and degeneration of the cortical and hippocampal cholinergic projections from the basal forebrain nuclei is the basis of the pathogenesis of diseases such as Alzheimer's disease and Lewy body dementia, as well as diseases with other etiologies such as schizophrenia, Parkinson's disease and cerebral ischemia, in some cases aggravated by

cognitive impairment [79-88]. The leading role of cholinergic afferent dysfunction in the development of ischemic pathology was suggested by data on the sensitivity of cortical and hippocampal cholinergic projections to ischemic exposure and a correlation between the development of cholinergic dysfunction, the delayed death of pyramidal neurons and cognitive impairments in rodents [89-91]. Dysfunction of cholinergic interneurons of the striatum is partly responsible for involuntary movements in Harrington's disease [80, 92]. Low expression of ChAT in the cholinergic neurons of the motor nuclei of the spinal cord is a specific early sign of amyotrophic lateral sclerosis [80, 92]. Multiple abnormalities in cholinergic function in the motor nuclei of the spinal cord a responsible for congenital myasthenic syndrome [93].

4.4.1. Synaptic soluble and membrane-bound choline acetyltransferase and their participation in cholinergic function in vitro and in vivo

Choline acetyltransferase (ChAT, E.C. 2.3.1.6) is a key enzyme in ACh synthesis and a marker of cholinergic neurons. It catalyzes the transfer of an acetyl group from acetyl-CoA to choline to form ACh. Studies in recent decades have revealed (1) the significant role of ChAT in the regulation of ACh synthesis and secretion and (2) that disturbances in the catalytic properties of ChAT may be the origin of some neuropathologies.

5. Forms of ChAT

It has been shown that ChAT has both a hydrophilic (cChAT) and hydrophobic state (stationary mChAT) in nerve endings. It has also been shown that ChAT is able to translocate from the cytosol to the synaptic membrane and to turn reversibly into the hydrophobic state associated with the synaptic membrane by ionic links (ionic-bound mChAT) [92, 94-96]. All this presupposes the existence of multiple forms or isoforms of the enzyme. Also, differences in the optimum pH, substrate specificity, sensitivity to the selective inhibitor 4-(1-naphthyl) pyridine (NVP) and some other molecular characteristics of the synaptoplasm and synaptic membrane fractions indicated this [97-99].

Research has revealed only one ChAT gene that encodes the multiple forms and isoforms of the enzyme [80, 83]. High homology has been detected between ChAT gene nucleotide sequences in the mouse, rat, pig and human brains with differences in the 5'-noncoding region. Polymorphisms of ChAT mRNAs are due to alternate splicing and various use of at least of five non-coding exons in the promoter region of the gene [100].

Five types of mRNA have been isolated from the rat brain ChAT (R1/2-, N1/2- transcripts and M-) [101] and six types from the human brain (R1/2-, N1/2-, S- and M-transcripts) [83]. All five ChAT transcripts generate ChAT with a molecular weight of 69 kDa (ChAT-69). This is the major form of ChAT in the CNS. In addition, the human M and S transcripts generate minor forms of ChAT with a molecular weight of 82-83 kDa (ChAT-82) and 74 kDa (ChAT-74) [80, 83, 93, 100, 102, 103]. Also, ChAT-69 and ChAT-82 are subdivided into a number of isoforms with differences in the isoelectric point [104].

The cytoplasm and plasma membranes of cholinergic neurons express only ChAT-69 [80, 83, 100]. In the human brain, ChAT is also found in the cell nucleus. Initially, ChAT-82 was selectively found in the nucleus in some brain structures [82, 95, 104, 105] and, later, ChAT-69 was also found [83]. In rat ganglia in the central nervous system at the level of the medulla oblongata, ChAT is expressed with a molecular mass of about 50 kDa and is called peripheral ChAT (pChAT). pChAT also exhibits alternative splicing of the mRNA [106].

The physiological significance of such a large number of isoforms of ChAT is not clear at present. Also, the relationship between of ChAT-69 isoforms in subsynaptic compartments is not known. Polymorphisms in ChAT transcripts suggest that ChAT isoforms or transcripts may vary in stability or translation efficiency or may be differentially expressed in response to trophic or pathological factors. Thus pChAT is not expressed in cholinergic neurons of the parasympathetic dorsal motor nucleus of the vagus nerve and nucleus ambiguus in the medulla of intact rats but pChAT- positive neurons were detected in these nuclei after axotomy against the background of almost disappearance of ChAT-69-positive neurons [107]. Furthermore, targeting of the enzyme to the cell nucleus suggests that ChAT may be able to perform other functions in addition to its essential role of synthesizing ACh in nerve terminals [102].

6. Features of ChAT phosphorylation

It is known that the genome does not provide the variety in the protein forms presented in a cell. In this regard, the post-genomic protein modifications are of special significance. Phosphorylation is one of the most studied pathways of the post-translational influence on the molecular properties of enzymes. Covalent modifications to serine, threonine and tyrosine residues in protein molecules can dynamically change their physicochemical nature, as well as regulate protein function and interactions with cellular components. This has been shown for the key enzyme in the synthesis of dopamine (tyrosine hydroxylase) and serotonin (tryptophan hydroxylase) and for glutamate decarboxylases GAD65 and GAD67, two synthetic enzymes of gamma-aminobutyric acid (GABA) [108].

For a long time, ChAT was not related to rate-limiting enzymes on the basis of kinetic calculations. It was believed that the ChAT synthesis rate dependents only on fluctuations in the levels of the substrate and the product of the synthesis, although ChAT is not saturated by choline and acetyl-CoA in their physiological concentrations [35]. However, in recent decades, other intracellular factors have been revealed to regulate the activity of the enzyme. These data suggest an important regulatory role of ChAT in the synthesis and secretion of ACh [36, 82, 103, 108, 109]. It is assumed that the cause of several diseases is spontaneous point mutations in the molecule of ChAT or of its regulatory proteins which lead to dysregulation of the enzyme or to changes in its ability to communicate with regulatory factors [93, 108].

As a rule, the different effects of phosphorylation on synaptic soluble (hydrophilic) cChAT and membrane-bound (hydrophobic) mChAT occur even with non-specific stimulation by the

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substrates ATP or phosphorus (Pi). Increased ATP markedly affects the specific activity of mChAT compared with cChAT [37]. At rest, cChAT but not mChAT is phosphorylated in incubation medium enriched with Pi. Under these incubation conditions, veratridine depolarization selectively activated and dephosphorylated mChAT but had no influence on either the degree of phosphorylation nor the activity of cChAT. Removal of Ca^{2+} from the incubation medium significantly inhibited the phosphorylation of cChAT and the specific activity of mChAT [110].

It has been shown that, in vivo, ChAT exists as a phosphoprotein [36, 111]. In vitro, phosphatase inhibitors activate cChAT and mChAT a little, even under non-phosphorylation conditions (ATP absent in the incubation medium) [37]. ChAT is a substrate for certain protein kinases. The amino acid sequence of the enzyme suggests the existence of multiple sites for phosphorylation by protein kinases such as protein kinase C (PKC), α-Ca^{2+}/calmodulin-dependent protein kinase II (CaM2), casein kinase II (CK2) and some others [108]. ChAT-69 is phosphorylated by the serine/threonine kinases CK2, PKC and CaM2 [92, 95, 105, 112].

It should be noted that PKC and CaM2 are the well-known and important regulators of neuronal functions. CaM2 is an obligatory component of the cholinergic vesicular mechanism [37], and PKC plays an important role in the regulation of ChAT molecular properties [36]. The authors also make the conjecture that oxidative stress can alter the phosphorylation-dependent regulation of ChAT expression and ACh synthesis in the aging brain and in the early stages of vascular and Alzheimer's disease and related disorders. Both of these protein kinases interact with serine/threonine residues which the protein kinases use for ChAT phosphorylation [92, 95, 108, 112]. In different studies, PKC activated cChAT and mChAT with variable efficacy [37, 95, 104]. It has been shown that different protein kinase isoforms have distinct patterns and ChAT phosphorylation by PKC isoforms has a hierarchical construct [92, 95, 108, 112]. Thus, phosphorylation of Ser-476 had no effect on the molecular properties of ChAT but allows the possibility of phosphorylating other serine residues, such as Ser-440 and/or Ser-346/347 which are necessary to maintain the catalytic activity of ChAT under basal and stimulated conditions. Also, Ser-346/347 modulates ChAT phosphorylation at other amino acid residues, and Ser-440 initiates the translocation of soluble ChAT to the cellular membrane and the formation of ionic-bound ChAT.

CaM2 and its inhibitors selectively regulate mChAT activity without affecting the activity of cChAT [37]. These data were also confirmed indirectly by experiments with total ChAT (actually cChAT), in which CaM2 phosphorylated but did not activate the enzyme [104]. Further investigations showed that CaM2 activated total ChAT in terms of the combined phosphorylation of Thr-456 by CaM2 and of Ser-440 by PKC [112]. It is assumed that this PKC feature of the potentiation of CaM2 action in cholinergic projection neurons of the hippocampus and the cortex is dramatically implicated in the pathogenesis of Alzheimer's disease [112]. Likewise, PKC inactivation of Ser-440 phosphorylation is implicated in the pathogenesis of myasthenic syndrome in the motor nuclei of the spinal cord [94, 113].

7. Role of cChAT and mChAT in regulation of acetylcholine synthesis and secretion – In vitro studies

In neurons, the principal place of synthesis of ACh is in the nerve endings. ChAT has long been recognized as a cytoplasmic enzyme, even after its detection on synaptic membranes in the 1960s [1, 114]. Later, it was shown that ChAT exists as a structural membrane protein [95, 115-117]. The long-term study of the properties of synaptic soluble (c) and membrane-bound (m) ChAT in vitro has shown that the relationship between ChAT activity and the secretion of ACh depends on the compartmentalization of the enzyme.

7.1. Functional properties of synaptic cChAT

Soluble cChAT activity is the prevalent activity of synaptic ChAT. cChAT regulates the dynamic equilibrium between the synthesis and degradation of ACh in the resting state [35, 99, 118, 119]. Under physiological conditions, cChAT is activated during stimulation by depolarizing agents such as K^+ and/or veratridine [37, 120, 121]. Another regulator of the level of free cytosolic ACh is AChE, the enzyme that mediates ACh splitting. A close interaction takes place between cChAT and soluble cAChE [120]. Thus, in calcium-free medium conditions, the quantum release of ACh is blocked, the activity of cChAT is not changed and cAChE is activated and cleaves an abundance of ACh [120, 122].

From these experiments, it follows that non-quantum, Ca^{2+}-independent "leak" of acetylcholine and its decay products, choline and acetate, is in direct dependence on the ratio of the activity of these two cytosolic enzymes [120, 122, 123]. In these studies, (1) K^+ stimulation in calcium-free medium causes the release of cytosolic choline due to disruption of cytosolic ACh by cAChE and (2) veratridine stimulation can cause the release of both choline and cytosolic ACh. (3) In mAChE and cAChE inhibition conditions by a tertiary inhibitor such as paraoxon coming through the plasma membrane, the release of choline is blocked under veratridine stimulation in calcium-free medium and its extracellular level is decreased. Instead of choline, the release of cytosolic ACh is observed. (4) Under cChAT and mChAT inhibition conditions by the selective inhibitor NVP, cChAT is selectively activated and the release of newly synthesized ACh is increased directly from the cytosol under veratridine stimulation in physiological medium (in the presence of Ca^{2+}). (5) A similar output of ACh is observed under the same conditions in calcium-free medium.

The choline and ACh concentrations could increase by 40-60% in the extracellular medium in such a non-quantum manner. Choline is a selective agonist of α-7 subtype of ACh nicotinic receptors [65, 124]. Thus, the "leak" of cytosolic choline and/or ACh, as well as changes in their relationship in the extracellular environment may have independent signaling effects in intercellular interactions.

7.2. Functional properties of synaptic mChAT

The functional purpose of mChAT has long been unclear [80, 125]. Investigation of this problem was difficult in the absence of selective inhibitors of cChAT and mChAT. Their

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separation is possible only by subsynaptic fractionation in combination with methods that destroy the synaptosome. The contribution of mChAT to general ChAT activity is low, i.e. 4-15% [1, 94, 95, 97, 119]. Therefore, for a long time, it was assumed that the association of ChAT with neuronal membranes was an artifact as the result of synaptoplasm contamination [1, 35, 114].It has now been shown that mChAT exists (1) as stationary membrane protein [99, 115, 117] and (2) as ionic-bound mChAT, a reversible form of cytosolic ChAT [95, 126].

In vitro, mChAT like cChAT are activated in response to K^+ or veratridine stimulation in physiological medium [37, 110, 120, 121]. Compelling data have accumulated regarding the direct involvement of mChAT in the mechanisms of quantum secretion of acetylcholine. This is indicated by a number of ultrastructural and functional characteristics of the enzyme.

mChAT is localized to synaptic vesicles [127]. Its activity, unlike cChAT, depends on the specific factors of ACh transfer into the vesicles, VAChT and the proton gradient, and on CaM2 activity, which is the main kinase associated with synaptic vesicles [37]. Activation and inhibition of mChAT are fully coupled with the activation or, respectively, blockade of ACh quantum release [120].

The non-vesicular Ca^{2+}-dependent pathway of the quantum secretion of ACh has been revealed [128-131]. It was shown that this pathway provides fast secretion of ACh by a synaptic membrane structural protein [132] called mediatophore [128]. It was found that mediatophore is functional linked to ChAT [133]. This suggests that mChAT located on the synaptic membrane participates in the regulation of the quantum secretion of ACh, similar to vesicular mChAT. This agrees with the preferential sensitivity of mChAT to the functional state of CHT that is selectively localized to the neuronal membrane of cholinergic neurons [94, 134].

mChAT is selectively sensitive to the balance of ions. It is known that ions are important regulators of quantum neurotransmitter release and other transmembrane functions. Control of quantum ACh release is carried out by the interaction of the Ca^{2+} and H^+ balance (vesicular Ca^{2+}/H^+ antiporter), Zn^{2+} and K^+ (K^+ channels) [131, 135-138]. mChAT activity is selectively or preferably (1) inhibited in calcium-free medium [118, 120, 121], (2) is increased at a high concentration of Ca^{2+} and/or K^+ [37, 110, 121, 123, 139], (3) is dose-dependently inhibited by the intracellular concentration of Cl^- [118, 125] and increases in conditions of a high Cl^- concentration and chloride conductivity stimulation [125]. (5) Zn^{2+} regulates both pathways of the quantum secretion of ACh. High concentrations of Zn^{2+} block ACh release from vesicles and through mediatophore [135, 140]. Similarly, the direction of ChAT translocation depends on Zn^{2+} ions. Zn^{2+} blocks the "anchoring" of ChAT on the membrane [126]. The last argument indicates the involvement of ionic-bound mChAT in the quantum release of ACh.

So, the catalytic properties of cChAT and mChAT depend on phosphorylation and possibly on the type of splicing. Moreover, the specific activity of mChAT, unlike cChAT, also depends on the ionic environment and on other factors affecting the quantum secretion of ACh. The functional significance of mChAT is not nearly as clear as cChAT. The relationship between cChAT and mChAT and their dependence on external influences are poorly understood [138]. Nevertheless, it seems that the compartmentalization of the enzyme ensures the involvement

of cChAT and mChAT in different functional-metabolic cycles, which may contribute to the fine regulation of the mediator actions of ACh.

8. cChAT and mChAT as markers of functional and structural reorganization in cholinergic nerve endings following external exposure — In vivo studies

The synaptosomal subfractions of the synaptic membranes and synaptoplasm of the cortex, hippocampus and some other rat brain structures are used for research in vivo cholinergic mechanisms of brain functions by biochemical methods (radiometric and spectrophotometric). Subsynaptic fractions gave according to the scheme shown in Figure 1. Respectively, mChAT and cChAT activity and the m-protein and c-protein content have been measured to estimate cholinergic function. In addition, in some experiments, mAChE and cAChE and Na^+/K^+-ATPase activity was measured. The Na^+/K^+-ATPase activity and content of synaptic proteins, as universal synaptic parameters, as well AChE activity were correlated with ChAT activity in those cases when the cholinergic reaction following exposure was dominant in the synaptosomal fraction. Generally, models of acute (3 hours) and chronic (11-14 days) brain ischemia (bilateral occlusion of the carotid arteries, the 2VO model) or acute hypobaric hypoxia with variable intensity (10% O_2, 60 min; 6.5% O_2, 15 min; 4.5% O_2, 1-3 minutes or 10-20 minutes) were used as the exposure methods.

8.1. Biochemical equivalents of activation and inhibition of cholinergic mediator function

In in vivo investigations, ChAT activity was found to be the most mobile parameter. So, ChAT has become the main landmark for analysis of the cholinergic reaction to exposure.

cChAT activation was observed under acute ischemia or hypoxia at all intensities [27, 29, 141]. mChAT or both mChAT and cChAT activation was revealed under acute and chronic ischemia and only in severe hypoxia (4.5% O_2) [27-29, 141]. When the activation of ChAT was observed (165-170%), extracellular mAChE (the predominant isoform of mAChE) was simultaneously activated [141]. cChAT activation positively correlated with the activation of Na^+/K^+-ATPase and negatively correlated with the decrease in the c-protein content [27]. All these reactions of the synaptic biochemical parameters and their combinations are regarded as the activation of cholinergic synaptic function, because they conform to the characteristics of synaptic activation.

Compared to cChAT, the selective activation of mChAT has been revealed (1) under equal experimental conditions (3 hours of ischemia) in rats less resistant to hypoxia [141], and (2) under hypoxic conditions with variable intensity only in severe hypoxia [27] and was not observed in the subcritical and moderate hypoxia (6.5% or 10% O_2) [27, 29]. A parallel study of the ultrastructure of the synapses in the cortex revealed the dependence of swelling synapses and synaptic mitochondria on the duration of severe hypoxia [27]. Taken together, these data suggest that the activation of mChAT in vivo occurs due to an imbalance of synaptic $Ca2^+$,

while cChAT activation is apparently initiated in the natural physiological way, under neuronal influences [27].

The inhibitory reactions of ChAT under ischemic/hypoxic conditions were revealed as well. It was found that these conditions decrease cChAT or mChAT or both cChAT and mChAT activity [27-29, 141]. Also, a negative correlation has been found between cChAT activity and the c-protein content and a positive correlation has been found between mChAT activity and the m-protein content [27, 29, 141].

A parallel study of the ultrastructure of the synapses in the cortex revealed a significant decrease in the number of vesicles docked to the presynaptic active zone in the rat with a profound decrease in both cChAT and mChAT activity in acute hypoxia [27, 142].Taken together, these data suggest that such a decrease in ChAT activity in vivo may reflect the deep inhibition of cholinergic synaptic function as result of the superexcitation, the equivalent of the well-known "depression of neurons" in electrophysiology, i.e. reduced neuronal excitability due to the depletion of mediator substrates.

Selective inhibition of mChAT, as well as its activation, is likely a consequence of a disturbance in the ion balance. Based on the dominance of the hypoxic factors in these experiments, it is supposed that the decrease in mChAT activity is due to the accumulation of H^+ ions in the presynapses [27, 29]. It can be induced (1) by acidosis in the case of severe hypoxia and (2) by the weak increase in H^+ ion concentrations as the primary response to hypoxia in the case of moderate hypoxia (10% O_2). It has been shown that such primary H^+ ion accumulation is subthreshold for the initiation of cellular acidosis and can disrupt the function of the Ca^{2+}/H^+ antiporter [143].

Finally, cAChE activation has been detected under acute ischemia [141]. This is probably another means of regulating the abundance of free cytosolic ACh during the inhibition of ACh quantum transmission. The simultaneous increase in the c-protein content in the same synaptosomal fraction corroborates this supposition. It is well-known that numerous fibrillar synaptic proteins are soluble at rest and quickly form a structure under stimulation conditions.

So, the high reactivity as cChAT and mChAT and the peculiarities in the manifestation of ChAT (and AChE) activity according to the compartmentalization of the enzyme and to the experimental situation in vivo testify to the naturalness of functional properties cChAT and mChAT (and also c- and mAChE) revealed in vitro.

8.2. Biochemical equivalents of the quantitative changes in the cholinergic synaptic pool

The correlations in the activation or inhibition of cChAT and mChAT may reflect changes in a number of cholinergic synapses, namely synaptogenesis (the growth of new synapses) or their elimination, retraction or another means of reduction in the quantity of nerve endings. As was described above, the correlation between the biochemical synaptic membrane and cytosolic parameters may reflect the reaction of the presynapse as a structural unit. The most reliable criterion of the quantitative reorganization of cholinergic synapses is the positive correlation between ChAT activity and the c-protein content, since their functional changes have contrasting directionality.

A reduction in the number of presynapses was provoked by acute hypoxia of variable severity [27, 29]. It was shown by various methods, including non-invasive video technology, that a reduction in the number of synapses can occur within minutes or tens of minutes [144-148].

Sprouting as well as destruction with the swelling of neurons and their terminals, including cholinergic neurons, predominates in late brain ischemia or postischemic reoxygenation (over days and months) [149-152]. In biochemical studies, the activation of ChAT was observed in the majority of the synaptic subfractions of the cortex and hippocampus following chronic brain ischemia [28]. The correlated increase between mChAT activity and m-protein content could indicate synaptogenesis and hyperfunction of the cholinergic synapses, whereas the correlated increase between cChAT/mChAT activity and the c-protein content indicates synaptogenesis only.

8.3. Biochemical equivalents of the morpho- structural reorganization in the cholinergic synaptic pool

Under the influence of moderate hypoxia (10% of O_2, 60 min), an increase in the activity of cChAT and the c-protein content was observed in the “light” synaptosomes from the caudal structures of the brainstem [29]. This indicated an increase in quantity of the corresponding synapses; however, synaptogenesis was impossible in such a brief period. Additional analysis revealed a decrease in the activity of mChAT and the m-protein content in the "heavy" synaptosomal fraction of the same brain structures. This decrease in the “m” biochemical parameters in the "heavy" fraction negatively correlated with the increase in the corresponding “c” biochemical parameters in the "light" fraction.These data indicate the transformation of presynapses from one morphological type to another.

This phenomenon of the transformation of synapses was found in electron microscopic experiments during 90 minutes of severe hypoxia [144, 145]. It was shown that the change of a morphological type occurs due to the changes in the area, density and configuration of the network elements of the presynapses and in their configuration [153]. Almost all of these parameters can affect the density of the presynapses. Therefore, it is possible that some population of the cholinergic presynapses from the "heavy" synaptosomal fraction transformed into presynapses with the less density and was located in the “light" fraction of the sucrose density gradient. Apparently, this transformation resulted in a morphological type more resistant to hypoxia.

In such studies on synaptic subfractions in vivo, various cholinergic synaptic reactions have been revealed in response to hypoxic/ischemic exposures. The responsiveness of synaptic cChAT and mChAT allows the study of synaptic reactions depending on the exposure conditions, the functional specificity of different brain structures and neuronal populations. It was noticed that a reaction to the hypoxia had phase type of change in the course of intensification of hypoxic exposure [27]. As well a diversity of the plastic possibilities of the brain is detected. For example, under the same moderate hypobaric hypoxic conditions (10% O_2, 60 min) which initiated an increase in resistance to hypoxia, three alternative cholinergic adaptive pathways were obtained in the same brain structure (the caudal brainstem) in three different groups of rats. Transformation and activation of the presynapses was seen in one of the rat

groups and the inhibition of cholinergic activity in different populations of presynapses (in the light or heavy synaptosomes) was seen in the other two groups [29]. The mechanisms behind this plastic diversity are unknown, although it is clear that it is associated with individual neuronal organization of brain functions.

8.4. Cholinergic organization of brain functions under normal conditions and patterns of adaptive reorganization under the influence of stress stimuli or pathological conditions

Synaptic ChAT activity can be used as an instrument to study the cholinergic mechanisms of brain functions in vivo. Biochemical (radiometric) methods to estimate synaptic ChAT activity are very sensitive and allow for assessing fine individual differences between experimental animals. In turn, this method allows for successful correlation analysis between ChAT activity and indicators of brain function and performance. Moreover, it is possible to study certain populations of cholinergic neurons using, for example, the synaptic fractions of the cortex and hippocampus.

As mentioned above, according to immunohistochemical data, both the cortex and the hippocampus have two basic sources of cholinergic innervation. The first major source is neuronal projections from the forebrain nuclei. The second minor source is interneurons (intrinsic neurons). The third source to the frontal and visual cortical areas from the mesopontine region is weak and biochemical methods can detect it only when the frontal or visual area is assessed separately. In these brain structures, ChAT activity was estimated in the fractions of the light and heavy synaptosomes (isolated as in [4]), and it appeared that these fractions both in the cortex and in the hippocampus differ in terms of functional activity. From this, it follows that in both brain structures, the cholinergic presynapses from different sources are isolated in different synaptosomal fractions during preparation in the sucrose density gradient.

Next, it was revealed that the ratio of ChAT activity in the light and heavy synaptosomal fractions corresponded to the ratio of the immunoreactivity of the enzyme in the projections and interneurons [46, 48, 52, 53, 154].This and some other data promoted the conclusion that, in the cortex and hippocampus, the presynapses of cholinergic projections from the forebrain nuclei accumulate mainly in the light synaptosomal fractions, whereas the presynapses of cholinergic interneurons accumulate mainly in the heavy synaptosomal fractions [27, 27, 155].

This differential approach was used to study rat and cat brain cholinergic synaptic organization of cognitive functions such as learning, different forms of memory and inherited abilities in some experimental situations. mChAT and cChAT activities of the light and heavy synaptosomes of the hippocampus and/or cortex were used as markers of forebrain projections and interneurons, respectively. These studies revealed some patterns in the relationship between cognitive functional mechanisms that have not been sufficiently analyzed or defined by any other methods.

Thus, under normal brain conditions, it was shown that (Figure 3, a):

1. Both the cholinergic projective systems and interneurons of the rat cortex and hippocampus are actively involved in learning and memory processes in the Morris water maze

model [28]. The presynapses of cholinergic interneurons in the cat temporal associative cortical area do not participate in inherited abilities for the analysis of images [25] but all of other associative cortical areas (frontal and parietal) active participated in cognitive processes [153].

2. The cholinergic system participates not only in the mechanisms of learning and working memory, which has been repeatedly observed [151, 157-160], but also in the mechanisms of long-term memory [28]. The involvement of cholinergic projective systems in the mechanisms of long-term memory is usually denied [161-164] or has been discussed in only a few studies [165-167].
3. Each form of memory has an individual cholinergic synaptic composition [28]. This conclusion agrees with the results of investigations into cholinergic and monoaminergic systems obtained in the Morris water maze and some other behavioral models [165, 168-170].
4. Cholinergic projective neurons and interneurons of the rat cortex and hippocampus can have both positive and negative connections with cognitive functions [28]. Identical results were obtained in all cat cortical areas except the temporal zone. Cholinergic projections in the temporal area had only negative connections with inherited cognitive functions. The number of cholinergic presynapses may be more than doubled in this brain area of cats with weak cognitive abilities as compared with cats with strong abilities [25]. Negative connections with cognitive functions are not specific for only the cholinergic system. In morphological research on hippocampal mossy fibers (glutamatergic) in the rat and mouse brain, feedback was also found between the quantity of synapses which mossy fibers create and learning [171].

Taken together, these data demonstrate that the cholinergic mechanisms of learning and memory are more complex than is currently perceived.

Stress and pathological stimuli initiate a considerable reorganization of the normal cholinergic synaptic connections in cognitive functions. As an illustration, it was revealed during chronic 2VO conditions in the Morris water maze models that [28] (Figure 3, b):

1. The majority of normal cholinergic connections are lost and new connections arise.
2. Cholinergic link was considerably reduced in mechanisms of cognitive functions and proportion of negative connections increased.
3. In addition to reduction, the structural isolation of cholinergic links in cognitive functions and performance takes place; cortical cholinergic influences are completely removed from spatial contextual functions as are hippocampal influences from spatially cued functions.

In general, cholinergic synaptic influences disappear in some forms of cognition. It is clear that the consequences of different exposures on the cholinergic composition of cognitive functions are individual; however, the itemized consequences of 2VO are general for other stress stimuli such as acute severe hypoxia (4.5% O_2, Figure 3, c) [141, 156] and changes in season from warm to cold [25] (Figure 4).

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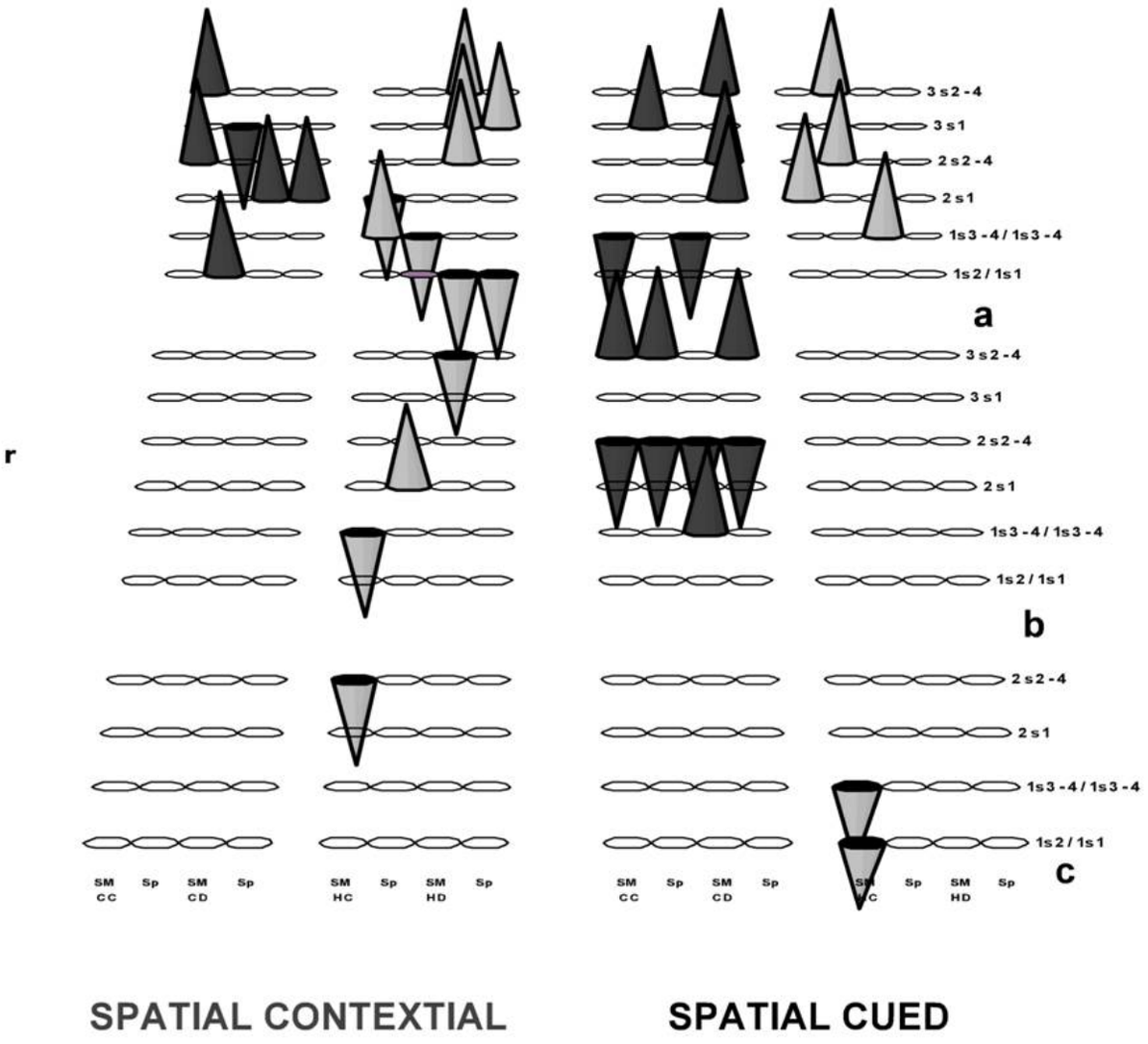
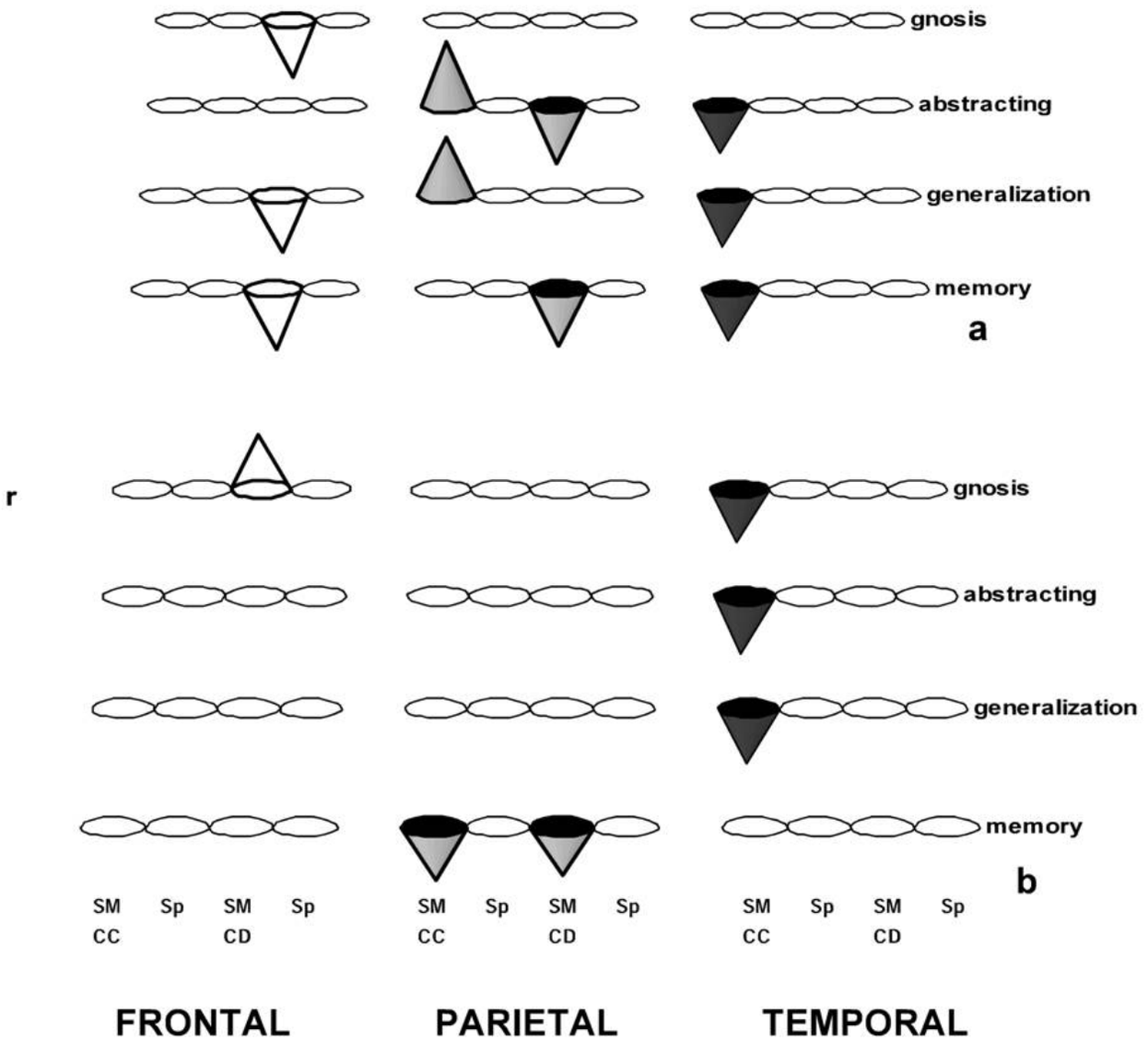


Abbreviations: 1s2(Contextual)/1s1 (Cued), the inherited abilities, the first non-casual attempt of making decision in the task (respectively, the second/ the first trial in the first session, "s " inclued in the abbreviations of the forms of cognition); 1s3-4 (Contextual)/1s2-4 (Cued), the working memory (averaged from the following trials in the first session); 2s2-4 and 3s2-4, the learning (average of trials in the second and the third sessions respectively); 2s1 and 3s1, the long-term memory (the first trials on the next days after the first and the second sessions of training respectively). SM and Sp, subfractions of the synaptic membranes and the synaptoplasm, respectively, of the cortical light (CL) and heavy synaptosomes (CH) and hippocampal light (HL) and heavy synaptosomes (HH).
Pyramids towards up indicate a positive correlation between behavioural performance and ChAT activity; inverted pyramidы indicate a negative correlation between behavioural performance and ChAT activity. In the rat groups n=4-7. Correlations between behavioural performance and ChAT activity are represented with only valid values and with the Bonferroni correction ($p<0.02$-0.001).

Figure 3. Values of *r*-criterion by the Pearson's test of behavioural performance and ChAT activity in rats in the Morris water maze in the spatial contextual and the spatial cued behavioural models under control (a, sham operated rats), ischaemic (b, 2VO operated rats) and one month after a single severe hypobaric hypoxia (c, sham operated rats) conditions.

It is logical to assume that the reduction in cholinergic links in cognitive mechanisms is a consequence of the degeneration of cholinergic fibers. However, the reverse was actually



Abbreviations: SM, Sp, CL, CH, the same abbreviations of the subfractions as on Figure 3.
As on Figure 3, the pyramids towards up indicate a positive correlation between behavioural performance and ChAT activity; inverted pyramids indicate a negative correlation between behavioural performance and ChAT activity. In the cat groups n=6-8. Correlations behavioural performance and ChAT activity are represented with only valid values and with the Bonferroni correction ($p<0.02$-0.001).

Figure 4. Values of r-criterion by the Pearson's test of behavioural performance and ChAT activity in cats in the inherited abilities of generalisation, abstracting and gnosis of images tested on the basis of the food reflex (memory) in the summer (a) and winter (b).

observed. As was noted above, activation of ChAT was detected in the majority of the synaptic subfractions, and this may reflect cholinergic hyperfunction or synaptogenesis in the 2VO rat brain [28]. Moreover, ChAT activity showed a five- to ten-fold increase in the winter as compares with the summer in synaptic subfractions of both projection and interneurons in the temporal [25] as well in other cat cortical areas [156]. No quantitative distinctions in ChAT activity were found in the synaptic subfractions of the cortex and the hippocampus of rats in a month after a single acute hypoxic stress [156].

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The new cholinergic connections with cognitive functions that arise after 2VO are not necessarily the consequence of degeneration or dysfunction in the presynapses of key cholinergic populations, i.e. these new connections could arise for other, indirect reasons. Therefore, it was assumed that noticeable weakening of cholinergic synaptic influences on cognitive processes is a consequence of adaptation. It seems that the cholinergic synaptic components of the highest brain structures, besides their participation in cognition, are necessary for the functions connected with survival under stress conditions.

At the same time, some cognitive functions were not affected by cholinergic reduction after 2VO [28]. All the more the inherited cognitive processes are preserved with annual seasonal cholinergic reorganization [25, 156]. From this, it was concluded that, during stress conditions, other mediator systems replace the cholinergic system in cognitive processes.

At least four questions follow from these data:

1. In what nervous functions are the cholinergic neuronal populations of the cortex and the hippocampus involved, both projective and intrinsic, for the maintenance of viability of an organism? Is it a function of regulation of the regional blood vessels or some other factor?

2. Why are cholinergic synaptic influences lost from cognitive mechanisms? Is it a negative dependence between vital and cognitive functions or low resistance of this neuronal population to stress conditions?

3. Is the structural isolation of cholinergic links in cognitive functions presumes a functional disbalance between the cortex and hippocampus? Is it a consequence of loss of cholinergic modulating influences?

4. What mediator systems mediate the execution of cognitive functions instead of the cholinergic system?

The answers to these questions are important for the restoration, maintenance and regulation of cognitive and vital brain functions under stress and pathological conditions.

9. Conclusion

The synapse is a unique and the most dynamic and labile structure specialized in the chemical transmission of nerve signals, an inherent structure of the neuron only.

Cholinergic system is essential constituent of the mammalian brain. Due to research using the synaptosomal and other synaptic fractions, knowledge behind the metabolism and secretory function of ACh and some new notion concerning the cholinergic mechanisms of cognitive functions under normal conditions and stress stimuli were gained. Today, however, accumulated data does not provide answers to the all questions as many more questions are asked. We have tried to outline some of the outstanding problems in the course of presenting the material.

The experimental cell physiology began to develop after the invention of the microscope by A. van Leeuwenhoek and his discoveries in the middle of the 17th century. Experimental synaptology began to develop after the invention of the electron microscope in the 1930s by M. Knoll and E. Ruska, ie three centuries later! Prior to this, researchers for the longest time, following two great neurohistologists, S. Ramón y Cajal and C. Golgi, in general could not reach a consensus, whether the brain is a cellular structure or syncytium? Only the electron microscope proved that a synapse exists.

Thus the science of the synapse is very young neuroscience.

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