ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 3, 23 SECTIO DDD 2011

Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Poland

AGNIESZKA MICHALAK, MARTA KRUK-SŁOMKA, GRAŻYNA BIAŁA

The molecular basis of memory: mechanisms, neurotransmitters and receptors involved in cognitive processes

Molekularne podłoże pamięci: mechanizmy, neurotransmitery
i receptory zaangażowane w procesy kognitywne.

INTRODUCTION

Memory is definied as a process which includes encoding, storage and retrieval of knowledge of the world [2]. This is not a single function, but a complex of many mental effects that undergo different mechanisms in various structures in the brain [16]. The crucial device of memory is learning. While memory is "a storehouse" for information, learning enables to acquire this information. Learning can be described as a change in behaviour due to experience, which enables to adapt to recent living conditions. Because of practise or repetition, new responses to existing stimuli are generated or already existing responses are strengthened [11].

Each process of memory formation begins with encoding. During encoding new information, received from the environment, is transformed into a memory representation – memory traces (called also memory engrams) [2, 18]. This process leads to the formation of new pathways in the brain or to the strengthening of the already existing ones. Encoding phase is precisely set in motion at the time of experience and depends on the involved degree of attention and on the extent of details in particular information [18]. Sprung up memory traces are, however, very susceptible to disruption until they undergo consolidation, the process in which brand-new encoded data become reinforced and stocked up as long-term memory traces [1, 2, 8, 11]. In the course of consolidation two basic synaptic changes occur. First of all, the amount of chemical neurotransmitters released into synapses increases, making more powerful synaptic connections. Secondly, the actual existing connections succumb to reordering and their number also grows [16]. In the future, every stored memory representation can be reactivated during retrieval phase and used to accomplish some specific task [18]. That can make a consolidated memory labile again and another process is necessary. Reconsolidation takes place in order to mediate restabilization of memory traces over time and it is thought that this memory phase can be responsible for the protection against forgetting [2].

TYPES OF MEMORY

There are two main division cathegories of memory: the time over which is active and the sort of information storage [11, 14]. According to the first category, memory can be classified into three types: immediate memory, short-term memory and long-term memory. Immediate memory is a routine skill of human brain to keep current events in mind. It lasts only for about a few seconds and provides the ongoing experience at the present moment when it is happening [14]. The different situation takes place in case of short-term memory, where holding information is effective for seconds to minutes but after the moment which they are refering to. Short-term memory can be used for both remembering new information (like an address) and an old memory representation brought back from memory storage during retrieval phase. This stage of the memory process has, however, the limited capacity, e.g. the typical digit sequence for an average individual acounts 7-9 figures [14, 16]. The third and last temporal category is long-term memory, which demands consolidation phase for its presence. It is the most powerful and permanent type of memory, almost infinite and can last even entire life or at least a couple of days or weeks [14].

Taking into consideration systems of information recall and storage, researchers set apart long-term memory into declarative and non-declarative memory [6, 8]. Declarative memory, known also as explicit, concerns knowledge of the world (facts about people, places, things) and experience of it. What is distinctive, to recall declarative memory representation, consciousness is needed. Declarative memory is in charge of making links between different facts about the world, to create a cohesive overview of it. This memory system is parceled as episodic and semantic memory [6, 8, 16]. Episodic memory handles specific, personal recollection of experienced events from our life (e.g. the first day at school) and answers questions about where, when and how we undergo this experience, while semantic memory is a general collection of information from the range of conceptual and factual knowledge (e.g. information learned in school like a historic date) [6, 16]. As opposed to declarative memory, non-declarative memory includes representations, which are recalled unconsciously. It is implicit store of memories remembering reflexively and it is responsible for automatic human behavior like trained, reflexive motor or perceptual skills [11, 16]. Non-declarative memory includes: procedural memory, conditioning and priming. Procedural memory is a type of memory activity, acquired gradually over time, which allows us to gain skills how to do something (e.g. riding a bike). The classic example of conditioning is Pavlov's experiment, where two stimuli were combined together: an unconditioned stimulus – the meat and a conditioned stimulus – the bell. As a result of pairing during some period of time the meat to the bell, the researcher observed that the subject (a dog) was salivating when the sound of the bell was heard. So conditioning is an automatic response of the body to an instruction sending from the brain and this command is forming when a conditioned stimulus takes place [6, 16]. Finally, priming is a phenomenon refering to a increased sensitivity to certain stimuli due to prior experience. Previously exposed information affects on the current one, even if we do not remember we have encountered it [6, 14].

ANATOMICAL BASICS OF MEMORY

Numerous studies of animals and humans have established which regions of the brain are involved in memory. What is significant is the fact that one brain area can be in charge of many processes of memory formation and also that one particular process can be controlled by a few brain structures. Thus, the medial temporal lobes and the diencephalon are important for episodic memory, while semantic memory is holding by the lateral temporal lobes particularly in the left hemisphere [6]. The amygdala is responsibe for emotional memory including stimulus-response conditioning such as a fear response or identifying emotions in face [8, 16], whereas motoric conditioned response (e.g. an eyeblink) is localized in the cerebellum [6, 8, 18]. The cerebellum, however, just as well as the basal ganglia, appears to be involved also in procedural memory [6]. The cortex plays an important role in formation of short-term memory, in the processing of sensory information (sounds, pictures, words) and perceptual priming [6, 8]. The hippocampus - another major brain component attending in memory, manages consolidation of information from short-term to long-term memory stage, formation of spatial memory and navigation [2, 8] (Fig. 1).

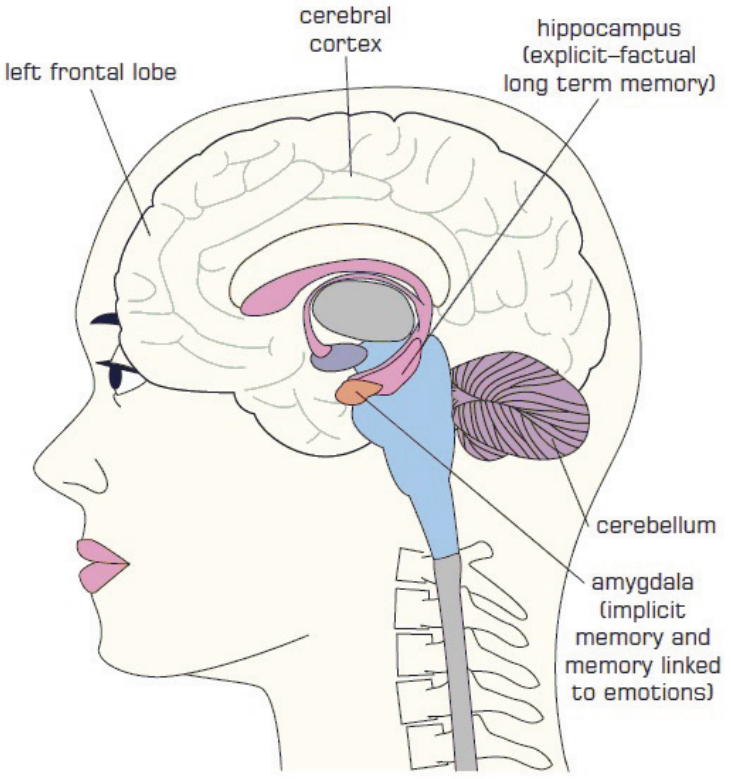

Fig. 1. Brain structures involved in memory process [8]

NEUROTRANSMITTERS AND RECEPTORS UNDERLYING MEMORY

Among many neurotransmitters modulating learning and memory performance, two of them play an especially indispensable role in cognitive function. They are glutamate and acetylcholine.

Glutamate

Glutamate is the most common excitatory amino acid in the brain, released by over 50% of all neuronal synapses. Although this agent is needed for appropriate course of all cognitive actions, its high level (e.g. raising because of neural injury) is potent toxic to the brain [3, 4].

Because glutamate is not able to cross the blood-brain barrier, it has to be synthesized in synaptic terminals from a precursor – glutamine, with a mitochondrial enzyme's participation – glutaminase. Next, molecules of glutamate are packed into synaptic vesicles and then released into the synaptic cleft during neurotransmission. Removing of glutamate from the synaptic cleft is held by a reuptake into presynaptic cell or by astrocytic transport, (however, the second mechanism appears to be more important). Glutamate is converted in glial cells via the enzyme glutamine synthetase to glutamine, which is then transported into presynaptic neurons. Then the glutamate-glutamine cycle starts from the beginning [3, 7].

There are two main groups of glutamate receptors: metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors, including ligand-gated ion channels: NMDA receptors (N-methyl-D-aspartic acid receptors), AMPA receptors (α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid receptors) and kainate receptors (kainic acid receptors) [3, 7]. All of these ionotropic receptors allow the passage of Na^+, K^+ and in case of NMDA receptors also the entry of Ca^{2+}, which can act as a second messenger. NMDA receptors are special for more than this one reason. In order to open the channel, the following conditions must be met: the presence of a co-agonist (glycine) and the removal of Mg^{2+} block by the depolarization of postsynaptic cell membrane. These characteristics are believed to underlie synaptic changes in the process of memory [3].

Glutamate and glutamate receptors modulate learning and memory processes such as encoding, formation and retrieval of memories, spatial recognition and the maintenance of consciousness [7]. The abundant concentration of glutamate is observed in the cortex and the hippocampus, which are the most important areas involved in the mechanism of memory. Presumably, glutamate participates in cognitive function (especially long-term stage of memory), by influencing the cholinergic pathways precisely in cortical and hippocampal pyramidal neurons. Moreover, it has been established that NMDA and AMPA receptors play an important role in long-term potentiation, a process believed to be fundamental to the mechanism of memory [3, 7, 12].

Acetylcholine

The synthesis of acetylcholine (ACh) takes place in nerve terminals at neuromuscular junctions, in the ganglia of the visceral motor system and in the central nervous system (CNS). The reactants of this process are: choline and synthesized from glucose – acetyl coenzyme A. However, in order to start the course of the reaction a catalyzer is needed, and choline acetyltransferase (ChAT) functions as this constituent. After the release from presynaptic sites (for what the presence of extracellular Ca^{2+} is required), and binding with cholinergic receptors, ACh is removed from the synaptic cleft in

a less common way, compared to other neurotransmitters, which undergo reuptake. Namely, ACh is decomposed by a hydrolytic enzyme, acetylcholinesterase (AChE), into acetate and choline, which then is taken up from extracellular space into neurons by Na^+/choline transporter, making a new circuit of Ach synthesis possible to start [3, 17].

ACh acts on pre- and postsynaptic receptors, which can be classified into two groups: nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChRs) [3, 12]. The first group contains ligand-gated, cation-selective ion channels, which bind two molecules of ACh. Within the nicotinic receptors two subtypes are found: muscle-type nicotinic receptors and neuronal-type nicotinic receptors. Although both of them consist of five subunits arranged around a central pore, they differ from each other in a number of subunit types. And so in muscle two copies of α subunit are combined with the other three: β, γ and δ, while in neuronal nicotinic receptors only two subunit types are present, but with a greater range of variety (α2-α10 and β2-β4) [3, 5, 17].

In contrast to nicotinic receptors, muscarinic receptors belong to metabotropic, seven transmembrane-spanning receptors, binding with protein G. There are five types of muscarinic receptors: M1 – present mainly in CNS, M2 – detected in mammalian heart, M3 – found in smooth muscle and glands, M4 and M5 – localized in several brain structures. M1, M3, and M5 receptors cause stimulation of phospholipase C, leading to the synthesis of two second messengers: inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) through the hydrolysis of phosphatidylinositol 4,5-bisphosphate. As a consequence of these changes the intracellular level of Ca^{2+} increases and activation of cell function occurs. Meanwhile M2 and M4 receptors inhibit adenylyl cyclase leading to decreased cAMP formation (the second messenger) and regulation of K^+ channels [17].

There is no doubt that memory is strongly dependent on cholinergic neurotransmission in CNS [4, 12]. The release of ACh in many brain areas (such as the cerebral cortex, the hippocampus, the striatum) seems to be essential for many cognitive functions, especially for processes of attention (highly required during early stages of memory formation), detection of novelty or salience and for consolidation process [12]. To prove the cholinergic hypothesis of learning and memory, many pharmacological studies have been undertaken [4, 8, 17]. Especially valuable were investigations of cholinergic antagonists influence on memory performance, which have confirmed that this group of drugs (such as muscarinic receptor antagonist – scopolamine) can be used to induce amnesia [4]. Significant was also the evidence, indicating a correlation between the decline of cholinergic transmission and dementia [4, 8, 17]. Moreover, in the early stage of Alzheimer's disease significant loss of nAChR is observed. It may indicate that those acetylcholine receptors play crucial role in memory formation (especially subtypes containing the α7 subunit). It has led to the development of a new, potential treatment target for dementia and other cognitive disorders [5].

BRAIN PLASTICITY

Brain plasticity is a unique ability of the brain to be functional and morphological remodeled [8]. According to "dual-process theory" of learning, habituation and sensitization underlie these plastic changes. Habituation as well as sensitization can demonstrate both short-term and long-term

forms. Short-term form of plasticity (depression and potentiation) alludes to short-term memory and its modifiability potential, but long-term changes may also occur. On a molecular level at the bottom of long-term habituation long-term synaptic depression (LTD) lies and analogically long-term sensitization is characterized by long-term synaptic potentiation (LTP) [13]. Both mechanisms are discussed in the next paragraph.

Habituation

Usually, habituation is caused by a continuously delicate stimulus, such as gentle touch, but stimulation can be also divided with a variable interstimulus pause. Presumably this type of brain plasticity is induced by homosynaptic depression in the stimulus-response circuit, but also other processes, such as increased inhibition and decreased neuronal excitability, are assumed to be involved in habituation mechanism [13]. The most well-known example of habituation refers to the gill withdraw reflex of the sea slug – Aplysia californica. After mechanical stimulation of the animal siphon, the slug withdraws the gill, what is a simple reflex protection mechanism. However, when the siphon is stimulated repeatedly, a response becomes significantly less strong and finally ceases. This gradual reduction in strength of response to a given stimulus is called habituation [10, 11]. Possibly a decrease in the number of vesicles containing dopamine, what is observed during long-lasting stimulus action, may play an essential role in mechanism of habituation [11].

Sensitization

Sensitization is a process of enhancing in the strength of response to a primary stimulus in one pathway through treating another one with repeated applications of an extrinsic, noxious stimulus such as forceful touch or electrical shock [11, 13]. Once again a suitable example comes from experiments in Aplysia californica – the sea slug. Stimulation of the siphon results in activation of a sensory neuron, which leads to a turn-activation of a motoneuron and to the gill withdrawal. But when the siphon is stimulated immediately after stimulation of the animal tail, the response becomes stronger and quicker (the gill withdrawal reflex). It happens because during stimulation of the tail by a noxious stimulus excitatory postsynaptic potential (EPSP) in the motoneuron increases, making it more sensitive to the following stimulus [11]. Other examples of sensitization are hyperalgesia (hyperresponsiveness to noxious stimuli) and allodynia (hypersensitivity to normal innocuous stimulus), mechanisms of which are believed to be analogous to sensitization forms of learning [13].

In the mechanism of sensitization, serotoninergic, axo-axonic synapses are involved. Serotonin (5-HT) released by the facilitatory axon binds to receptors on the sensory neuron (the presynaptic terminal in the gill withdrawal circuit), which are linked with G protein. It leads to the synthesis of the second messenger called cyclic adenosine monophosphate (cAMP) and the activation of protein kinase A (PKA), which phosphorylates K^+ channels, making them closed. Next it causes opening of Ca^{2+} channels and the influx of Ca^{2+} into the sensory neuron. As a result, a greater amount of neurotransmitters is released into the synaptic cleft, which brings on a response of the motor neuron (the postsynaptic cell in the gill withdrawal circuit) [11, 13] (Fig. 2).

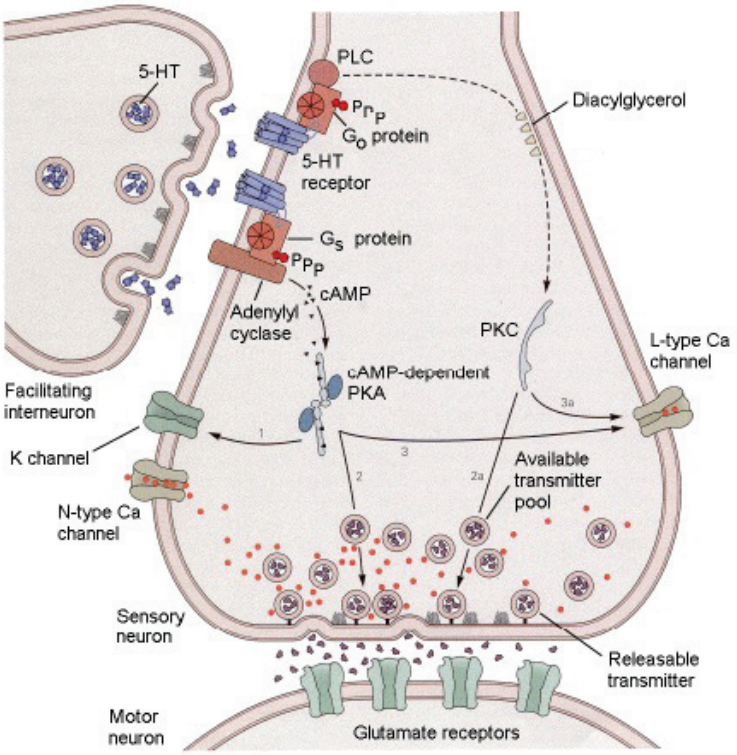

Fig. 2. Molecular mechanism of short-term sensitization [11]

MOLECULAR MECHANISM OF MEMORY FORMATION

Many intracellular, signaling pathways participate in several stages of memory (encoding, storage, retrieval). On the other hand, there are also molecular mechanisms that are involved only in one very specific stage of memory formation. It is suggested that the molecular events underlying both acquisition and consolidation are similar, but with a greater extension in case of consolidation, whereas retrieval seems to be subject to the different, unrelated molecular processes [1].

LTP, a form of synaptic plasticity of the brain, plays the main role in memory storage during acquisition and consolidation [1]. During the early phase of LTP, stimulation of NMDA receptor occurs and Ca^{2+} becomes allowed to enter the postsynaptic neuron. Growing of intracellular calcium level activates calmodulin-dependent kinase (CaMK), protein kinase C (PKC), calcineurin and tyrosine kinase. As a result of action of CaMK, non-NMDA channels are phosphorylated, which increases their sensitivity to glutamate and makes neuronal junctions more stable. When LTP extends (in the late phase), the high level of Ca^{2+} triggers adenylyl cyclase and increases cAMP kinase level. That causes activation of PKA, and its translocation into the nucleus, where phosphorylation of cAMP response element binding protein (CREB) takes place. Following gene expression leads to protein synthesis and to structural changes, that are believed to be essential in long-term memory formation [1, 10, 11] (Fig. 3).

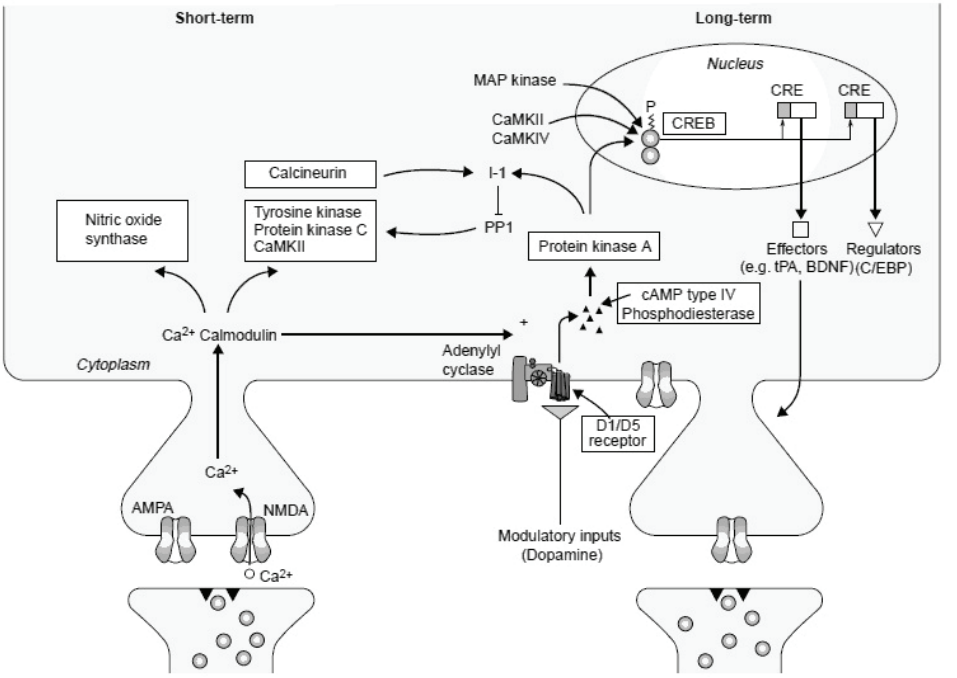

Fig. 3. Molecular mechanism of long-term potentiation [1]

Synapses have some level of maximum efficacy, above which storage of new information is not possible any more. Thus, to prevent this synapse overuse as a result of LTP, the different process – LTD has to arise. The balance between LTP and LTD is necessary for the proper course of learning and memory and is the basis of brain plasticity. Although LTD is the reverse of LTP, also this process requires activation of NMDA receptors. When the amount of Ca^{2+} is low, synaptic depression is determined [10]. Activation of protein phosphatases like calcineurin (CaN) leads to dephosphorylation of synaptic receptors including type-A GABA receptor and make specific sets of synapses weaker [10, 11, 13].

THE LATEST REPORTS – THE ROLE OF PROTEIN KINASE M ZETA (PKMZ)

PKC is a family of enzymes consisting of many isozymes and one of them is PKCζ. It has been established, that the gene responsible for encoding PKCζ is also in charge of encoding one more autonomously active isoform – PKMζ. This N-terminal truncated kinase is a completely independent catalytic domain, which causes changes at synapses. Significance of PKMζ elevated after reports, suggesting that PKMζ is needed to maintain long-term memories [9, 15].

PKMζ plays an obligatory role in maintaining the late phase of LTP and is required for keeping up spatial memory in hippocampus. Furthermore, PKMζ is also responsible for sustaining memory in neocortex [9, 15]. These properties have been proved during studies with a PKMζ inhibitor, called zeta inhibitor peptide (ZIP). ZIP was shown to be able to erase memory in conditioned taste

aversion test in rats. During the test the rats were exposed to a new taste – saccharin. Quick after that, they were given an injection of lithium chloride, what made them sick and in consequence animals avoided drinking from bottles with saccharin solution. But when the rats received a dose of ZIP, the taste aversion memories were deleted. This effect was achieved even for three-month-old memories. On the other hand, injections with the PKMζ inhibitor had no influence on short-term memory in tested animals [15].

CONCLUSION

Despite the fact that countless number of attempts has been taken to get to know how human brain does work, we are still very uncertain in this field of our life. It concerns also mechanisms which underlie memory. Processes involved in memory formation are very complicated and still unclear; therefore carrying on tests on animals and humans is highly demanded. Received results can be very useful in working out new courses of treatment of dementia and other different types of cognitive disorders. In the context of rapid population aging it can bring many invaluable benefits especially on economic and social levels.

REFERENCES

1. Abel T., Lattal K.M.: Molecular mechanisms of memory acquisition, consolidation and retrieval. Curr Opin Neurobiol, 11, 2, 2001.
2. Alberini C.M.: The role of reconsolidation and the dynamic process of long-term memory formation and storage. Front Behav Neurosci, 5, 12, 2011.
3. Augustine G.J. (2004). Neurotransmitters, receptors, and their effects. In: Neuroscience third edition. Purves D et al. (editors). Sunderland: Sinauer Associates, Inc.; p.129-164.
4. Blokland A.: Acetylcholine: a neurotransmitter for learning and memory? Brain Res Brain Res Rev, 21, 3, 1995.
5. Buckingham S.D. et al.: Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection. Pharmacol Rev, 61, 1, 2009.
6. Budson A.E., Price B.H. (2001). Memory: Clinical disorders. In: Nature Encyclopedia of Life Sciences. London: Macmillan Publishers Ltd; p.529-536.
7. Daikhin Y., Yudkoff M.: Compartmentation of brain glutamate metabolism in neurons and glia. J Nutr, 130, 4, 2000.
8. Edwards R. et al. (2010). Mechanism of memory foundation. In: Oxford VCE Psychology Units 3 & 4 Student Book. DeBaest I, Salter O (editors). Melbourne: Oxford University Press Australia and New Zealand; p.267-300.
9. Hernandez A.I. et al.: Protein kinase M zeta synthesis from a brain mRNA encoding an independent protein kinase C zeta catalytic domain. Implications for the molecular mechanism of memory. J Biol Chem, 278, 41, 2003.
10. LaMantia A.S. (2004). Plasticity of mature synapses and circuits. In: Neuroscience third edition. Purves D et al. (editors). Sunderland: Sinauer Associates, Inc.; p.575-612.

11. Michael D., Mann M.D.: Learning and memory. http://www.unmc.edu/physiology/Mann/index.html
12. Miranda M.I. (2007). Changes in neurotransmitter extracellular levels during memory formation. In: Neural Plasticity and Memory: From Genes to Brain Imaging. Bermúdez-Rattoni F (editor). Boca Raton: CRC Press; p.129-146.
13. Poon C.S., Young D.: Nonassiociative learning as gated neural integrator and differentiator in stimulus-response pathways. Behav Brain Funct, 2, 29, 2006.
14. Purves D. (2004). Memory. In: Neuroscience third edition. Purves D et al. (editors). Sunderland: Sinauer Associates, Inc.; p.733-754.
15. Shema R. et al.: Boundary conditions for the maintenance of memory by PKMzeta in neocortex. Learn Mem, 16, 2, 2009.
16. Squire L.R. (2007). Learning and memory. In: The Dana Guide to Brain Health: A Practical Family Reference from Medical Experts. Floyd E, Bloom FE, Flint Beal M, Kupfer DJ (editors). New York: Dana Press; p. 217-225.
17. Taylor P., Brown J.H. (1999). Acetylcholine. In: Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition. Siegel GJ, Agranoff BW, Albers R.W. et al., (editors). Philadelphia: Lippincott-Raven; p.185-209.
18. Wagner A.D. (2007). Encoding and retrieval from long-term memory. In: Cognitive Psychology: Mind and Brain. Smith EE, Kosslyn SM (editors). New Jersey: Prentice Hall; p.192-238.

SUMMARY

Memory is a consequence of plastic abilities of brain. It is a dynamic, multi-phase process, which allows adaptation to recent living conditions. The ability to learn resulting from memorisation is an essential human skill that is needed throughout the whole life span. Memory formation consists of the three following phases: encoding, consolidation and retrieval. The molecular mechanism underlying memory is based on the cascade of signaling pathways, which leads to changes in the structure of neurons through gene expression and protein synthesis. The activated brain areas during memory formation are very diverse and depend on memory formation phase, but basically they include the cerebral cortex and the limbic system, especially the hippocampus and the amygdala. Two neurotransmitters: acetylcholine and glutamate play the critical role in the memory process. The process of memory formation is highly complicated and therefore still not explored completely. All over the world, scientists put a lot of effort into making this subject more comprehensible. The latest reports indicate, that PKMζ is an especially critical component involved in the storage mechanism of long-term memory. Better understanding of the mechanisms involved in memory formation may contribute to the development of a new, more effective treatment of cognitive disorders, what is especially important in the face of population aging.

Keywords: acetylcholine, glutamate, brain plasticity, memory and learning

STRESZCZENIE

Pamięć jest konsekwencją właściwości plastycznych mózgu. To dynamiczny, wielofazowy proces, który pozwala przystosować się do obecnie panujących warunków środowiskowych. Nierozerwalnie związana z pamięcią zdolność do uczenia się stanowi podstawową właściwość ludzkiego organizmu, niezbędną przez cały okres życia. Proces powstawania pamięci składa się z trzech następujących faz: kodowania, konsolidacja i odtwarzania. Na poziomie molekularnym pamięć jest wynikiem szeregu reakcji wewnątrzkomórkowych, które prowadzą poprzez ekspresję genów do syntezy nowych białek oraz do powstawania zmian strukturalnych w obrębie połączeń synaptycznych. W trakcie procesu pamięciowego zaangażowane są różnorodne anatomiczne struktury mózgu, do których należą między innymi kora mózgu oraz układ limbiczny a przede wszystkim hipokamp i ciało migdałowate. Szczególnie ważną rolę w procesach uczenia się i pamięci odgrywają dwa układy neuroprzekaźnikowe: cholinergiczny i glutaminianergiczny. Pamięć do skomplikowany proces, który pomimo trwających od wielu lat badań na całym świecie nie został w pełni poznany i zrozumiany. Ostatnie doniesienia wskazują, że szczególnie istotny wpływ na procesy pamięci długotrwałej wywiera enzym – kinaza białkowa M zeta. W dobie gwałtownie starzejących się społeczeństw krajów rozwiniętych utrzymanie wysokiej sprawności intelektualnej do późnych lat starości jest kwestią priorytetową. Dlatego też, w celu zgłębienia mechanizmów leżących u podstaw procesów uczenia się i zapamiętywania konieczne jest prowadzenie dalszych badań. Otrzymane w ten sposób wyniki mogą przyczynić się do opracowania nowych, skuteczniejszych metod leczenia chorób neurodegeneracyjnych, takich jak choroba Alzheimera, oraz innych schorzeń przebiegających z towarzyszącą demencją i zaburzeniami pamięci.

Słowa kluczowe: acetylocholina, kwas glutaminowy, plastyczność mózgu, pamięć i uczenie się