Genetic and epigenetic mediated memory formation and inheritance Effects of classical and epigenetic mechanisms on memory

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Abstract: Brain function research has always been a very attractive field, among which there have been many related studies on the physiological mechanism of learning and memory. The brain is divided into several regions, many of which work together to form, store and invoke memories. The hippocampus is involved in the formation of declarative memory. The received information is processed, filed, and temporarily stored in the hippocampus, and information with sufficient repetitions or stimulation intensity can be transferred to the cerebral cortex for long-term storage. Changes in synaptic morphology and number based on long-term potentiation (LTP) are important physiological characterization of memory formation in hippocampus. This process involves a variety of cellular pathways and gene expression regulation, the role of many important protein genes and target genes on LTP is the key to study the molecular mechanism of memory. This paper will focus on the molecular mechanism of glutamate receptor gene, immediately early genes (IEGs) and cyclin AMP response element binding protein (CREB) gene. The diversity of these genes in the population may represent diversity in the ability to learn and remember. In addition to classical genetic mechanisms at the gene level, memory formation also involves epigenetics, such as DNA methylation and non-coding RNA. These epigenetic modifications also regulate memory formation by regulating the expression of related genes. But this way is more susceptible to environmental influences, which provides an evolutionary basis for the intergenerational inheritance of acquired memory.

1. Introduction

Characterizing how structure and function are associated in the brain is critical to furthering our understanding of neuroscience. The concept of functional specialization has led to key breakthroughs in neuroscience, such as the clarification of the visual system's organization. Based on Hubel and Wiesel's discoveries, research in the 1970s and 1980s identified the visual system as consisting of (at the time) 10–15 distinct regions with a high degree of specialization, including motion, color, and object processing selectivity. The functional specialization framework's apparent success is not only limited to vision, but also includes other sensory modalities, motor control, and cognition, as evidenced by standard textbooks.

Neuroscience also understands that brain areas do not exist in isolation, but rather interact and impact one another. Characterizing the prefrontal cortex's interaction with other areas of the brain, in particular, helped to reinforce the idea that brain design might enable "parallel dispersed networks." Functional integration has been highlighted in neuroimaging research over the last decade, and network science tools are now widely used to characterize regional relationships. However, given the observed level of interaction, comprehending functional specialization becomes much more difficult. Passingham and colleagues suggested the idea of a functional fingerprint, which is a multidimensional

representation of area function based on a small collection of "dimensions," in response to these challenges in structure-function mappings. They used terms like "motor coupling," "movement/muscle," and "proprioceptive/cutaneous" to describe the motor areas they studied [1].

Unlike other articles, this one describes the mechanics of learning and memory through the description of gene storage activities in brain areas, particularly the hippocampus, and the processes, as well as three different genes. Finally, epigenetic modification is thoroughly discussed, including DNA methylation and RNA alterations.

2. Human Brain and Memory

2.1 Different brain regions involved in memory

Many distinct areas of the human brain collaborate to store memories. Brain can be divided into six segments based on its memory storage capacity-neocortex, prefrontal cortex, amygdala, hippocampus, cerebellum, and basal ganglia. Memories are not stored just in one part of the brain. Instead, different types of memories are stored across interconnected brain regions. To a large extent, memories can be branch into two different types-declarative memory (explicit memory) and non-declarative memory (implicit memory). The conscious recovery of past information or experiences is known as explicit memory, whereas the accidental or unconscious retrieval is known as implicit memory [2]. The hippocampus–medial temporal lobe system is involved in explicit memories for experience, while the cerebellum, amygdala, and other systems are involved in implicit basic associative learning and remembering.

2.2 How Hippocampus was discovered

The hippocampus is one of the most extensively studied brain regions, which is highly involved in human's memory, special long-term memory. Until a study on a renowned patient, H.M., the function of the hippocampus was unknown. Following a bilateral resection of the medial temporal lobe to alleviate severe epilepsy, H.M. had substantial memory impairment. Much of the hippocampus and the neighboring parahippocampal gyrus were removed. H.M.'s memory loss was severe and affected all types of material (sceneries, words, faces, etc.), thus it came as a surprise when he demonstrated the ability to master a hand–eye coordination skill (mirror drawing) in just three days. He learned quickly and effectively but had no recollection of having previously practiced the activity [3].

2.3 Mechanism of the hippocampus

The hippocampus is essential for certain types of memory. It may work as a single unit during memory formation, or discrete sections may be responsible for different processes. The hippocampus appears to be functionally divided along its dorsoventral (septotemporal) axis, according to new research. The dorsal and ventral hippocampus have separate cortical and subcortical connections, with sensory information coming mostly in the dorsal two-thirds or three-quarters of the dentate gyrus. If small tissue blocks are spared within this region, rats can learn to navigate spatially, but equally large blocks at the ventral end are incapable of supporting spatial learning. For the encoding of spatial memory and certain forms of nonspatial memory, the posterior hippocampus (equivalent to the dorsal hippocampus in rodents) appears to be more significant than anterior portions in primates. In terms of intrahippocampal and extrahippocampal connections, the ventral (or anterior) hippocampal formation is somewhat disconnected from the rest of the structure, and it may be performing functions that are qualitatively different from, and independent of, those of the dorsal hippocampal formation [4].

2.4 Structure of the hippocampus

The hippocampus is a big, cashew-shaped structure in rodents that lies directly under the neocortex. The classic, textbook picture of hippocampal anatomical connectivity, the so-called "trisynaptic loop," can be seen in a cross-section of its long axis. Figure 1 illustrated this structure. The entorhinal cortex provides the majority of cortical input to the hippocampus, with the strongest projections to the dentate gyrus (DG) area via the perforant route (Synapse 1). The DG travels through the mossy fiber pathway

to the CA3 area (Synapse 2). The Schaffer Collateral route connects CA3 to the CA1 area (Synapse 3). Finally, CA1 completes the loop by projecting back to the entorhinal cortex. CA3 axons, in addition to their projections to CA1, transmit collaterals that form synapses with other CA3 neurons, which is an essential addition to the typical trisynaptic circuitry. This recurrent collateral channel sparked a slew of prominent hypotheses on CA3 as an autoassociative memory system, with attractor dynamics that are crucial for sustaining dispersed memory [5].

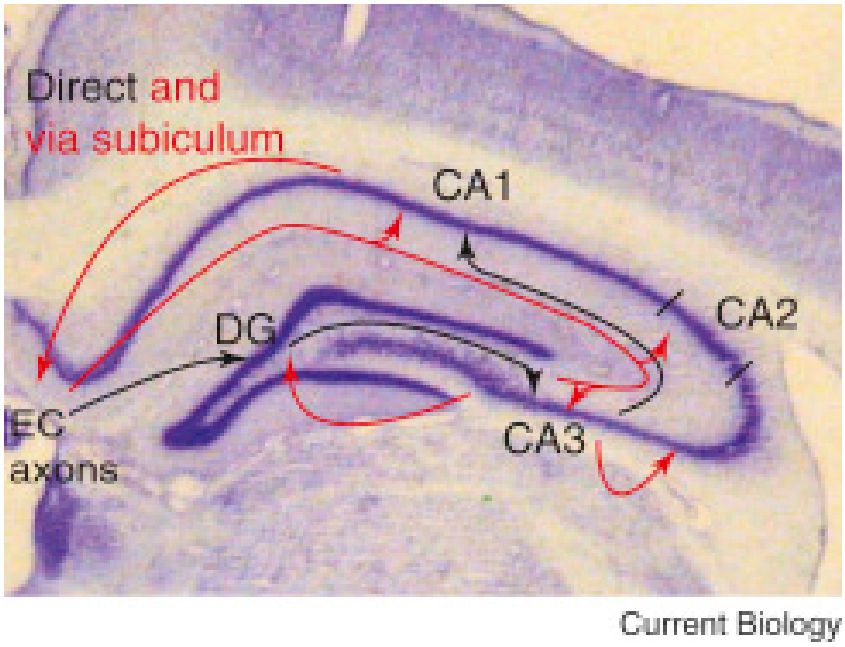

Figure 1. Trisynaptic loop in hippocampal.

2.5 The process of memory consolidation

Memory is the canvas on which we paint our lives' portraits, and study over the last half-century has revealed a great deal about it. We now know that synaptic plasticity is required for the consolidation of long-term memories, that this plasticity is dependent on important chemical signaling cascades, and that these cascades help to strengthen specific synaptic connections in distinct brain networks to consolidate memories [6].

In the mid-twentieth century, reductionist methods used a variety of model systems to determine the chemical mechanisms of synaptic consolidation. The marine snail Aplysia californica proved particularly useful in shedding light on the distinctions between short- and long-term memories. Short-term memories involve enhanced pre-synaptic glutamate release as well as alterations in post-synaptic glutamatergic receptor activity, both of which are mediated by covalent modification of pre-existing proteins at pre-existing synapses [7, 8]. Long-term memories, on the other hand, necessitate the transcription of new genes, the translation of novel proteins, and synaptic development at pre- and post-synaptic terminals [8]. Findings indicating both mitogens activated protein kinase (MAPK) and protein kinase A (PKA) operate in concert on cAMP response element binding protein (CREB) in the nucleus to consolidate an LTM were critical. PKA alters synaptic transmission in short-term memory via acting in the cytoplasm. PKA's catalytic component translocate to the nucleus in long-term memory to phosphorylate CREB-1, which then controls the transcription of genes containing cAMP response elements. This transcriptional pathway recruits a slew of other genes, including the immediate-early gene CCAAT box/enhancer-binding protein (C/EBP), which stimulates the transcription of synaptic growth genes (e.g., elongation-factor 1) by dimerization with an activating protein. Importantly, MAPK indirectly regulates CREB-1 by removing CREB-2, a protein that in the baseline state represses CREB-1 activity. MAPK is also involved in the internalization and redistribution of neural cell adhesion molecules to new synaptic development sites. These investigations revealed how an active

synapse signal activates a unique intracellular signaling cascade that changes nuclear function and synaptic connections in order to consolidate an LTM [9].

3. The classical genetics of learning and memory

3.1 Genes associated with learning and memory

LTP is the functional basis of learning and memory, and its formation is regulated by a variety of genes, including glutamate receptor gene, kinase gene, calcineulin gene, cyclin AMP response element binding protein (CREB) gene, immediately early genes (IEGs), Aitra activation factor gene, heat shock protein 70 gene, drosophila olfactory memory mutant gene and genes that affect cell growth, development and other functions [10]. Among them, glutamate receptor gene, immediate early gene and CREB gene are more closely related to the key steps of LTP formation, and have received more attention than genes with lower tissue specificity.

3.1.1 Glutamate receptor gene. Glutamate is an important neurotransmitter.

Glutamate receptor is a double gated channel with voltage and receptor. N-methyl-D-aspartic acid (NMDA) receptor is a type of glutamate receptor. Before LTP formation, NMDA receptor is competitively bound by Mg^{+}, and Mg^{+} can be removed only after AMPA receptor assisting membrane depolarization. NMDA receptors that are unrestricted from Mg^{+} can bind to NMDA, opening channels for Ca^{2+} influx. Ca^{2+} as a second messenger activates calcium/calmodulin-dependent protein kinase II (Ca/CaMKII), cAMP-dependent protein kinase and so on. Then synaptic response to premembrane signals is enhanced by increasing the number of receptors on the membrane. Tsien et al. found that LTP could not be formed in CA1 region after conditionally knocking out NR1 gene of NMDA receptor in mouse hippocampus, and the spatial learning ability of mice was severely impaired. Correspondingly, studies by Tank et al. suggest that enhanced NMDA receptor function may enhance learning ability.

3.1.2 Immediately early genes (IEGs).

Immediate early genes are proto-oncogenes that can be induced to be transcribed minutes after synaptic being stimulated, and by encoding a number of factors with different functions, such as transcription factors, secretory proteins, postsynaptic proteins, and signaling molecules [11]. These factors act as third messengers on other target genes in the nucleus to regulate their expression, thus coordinating the formation of LTP or LTD. The IEGs mainly include C-Fos, C-Jun, C-Myc and EGR family [12]. The most studied IEGs is C-Fos gene, it can be activated by Ca^{2+}, cAMP, phospholipid inositol and other second messengers [13], and then produce Fos protein. After post-translational modification, Fos protein binds to homologous gene product C-Jun in the form of leucine zipper to form heterodimer nuclear protein complex. The complex binds to the AP-1 binding region of the target gene DNA with high affinity, thereby affecting the expression of the target gene and ultimately cell proliferation. At the same time, Fos protein in turn inhibited the expression of C-Fos gene [14], this may be the reason why the transcription of C-Fos gene only lasts 15~20 minutes [15]. Due to the tissue specificity and transient nature of C-Fos expression, its expression product can be used as a marker of neural activity. In many studies, high-frequency electrical stimulation that induces LTP and training behaviors that promote long-term memory formation can induce the expression of a large number of *IEGs*, suggesting that the immediate early genes play a crucial role in the formation of LTP and learning and memory.

3.1.3 Cyclin AMP response element binding protein (CREB) gene.

CREB is similar to Fos protein in that it can form homologous dimer through leucine zipper structure and then bind target genes to regulate their transcription. In contrast, CREB needs to be phosphorylated by kinases, which can originate in a variety of signaling pathways. In general, a classical pathway of action of CREB is roughly as follows: neurotransmitter acting on the postsynaptic membrane mediates the production of a large amount of cAMP, which activates PKA, and then the

subunit of PKA activates CREB in the nucleus. CREB acts on the immediately early gene, and the expressed product modulates some of the late expressed genes to express the proteins required by LTP [16, 17].

The formation of LTP involves a variety of genes, RNA and protein interactions. Several important genes with high LTP correlation are introduced above to roughly describe the classical genetic basis of LTP. In addition, recent studies have shown that LTP formation may also involve epigenetics, such as histone methylation and DNA methylation, and many important genes are involved in these pathways. There is no clear boundary between classical genetics and epigenetics in the formation of LTP, but for evolution, epigenetics provides a new perspective on the inheritance of acquired memory.

3.2 Diversity and SNP analysis of learning and memory ability in population

For a specific gene, there are often some single locus differences in the population, which reflects the single nucleotide polymorphism (SNP) of the gene. SNP analysis of LTP-related genes may explain differences in learning and memory between individuals. Some studies have analyzed the correlation between SNP of three proteins in KAT3 family (EP300, CERBP and PCAF) and human learning and memory ability. These three proteins are important histone acetyltransferases that regulate the expression of long-term memory-related genes individually or in complexes. Through SNP analysis of people with different degrees of mental retardation and normal population, the study found that EP300 and CERBP may affect episodic memory in humans, and a new unreported single base mutation in EP300 may be associated with impairment of memory ability and intelligence [18].

4. Epigenetic

Analyzing genetic memory and its cases from the perspective of epigenetics can be explained from many aspects. Table 1 lists some classical epigenetic regulatory mechanisms. This paper will analyze from two aspects: DNA methylation and RNA modification.

Table 1. Different regulation mechanisms of epigenetic.

Different regulation mechanisms of epigenetic	
DNA methylation	Convert cytosine to 5-methylcytosine, add modification group to DNA
RNA modification	RNA, especially microRNA, related to memory will increase or decrease
Histone modification	Affect the affinity with DNA, change the agglutinated state of chromatin
Chromatin remodeling	Chromatin remodeling complex mediates nucleoside changes
Genomic imprinting	Cells with identical genes can have different and heritable ciliated stria

4.1 DNA methylation

DNA methylation refers to the covalent binding of methyl (-CH3) with cytosine 5 carbon position of CpG dinucleotide in the genome under the action of DNA methyltransferase (DNMT). This process is reversible [19]. As a form of DNA chemical modification, it can change genetic properties without changing DNA sequence. That is, without changing the structure of the gene, only the heritable change of gene function will eventually lead to the change of phenotype. These are the basic genetic information of DNA methylation.

DNA methylation plays a pivotal role on memory. DNA methylation is dynamically regulated in the central nervous system, and this cellular mechanism is a key step in memory formation. One of the most important is DNA methylation in the hippocampus. The hippocampus is a brain subregion that is known to be necessary for the establishment of long-term spatial and episodic memory. Thus, some studies focused on the hippocampus, hippocampal synaptic plasticity, and hippocampal neuron function. The inhibitors of DNMT activity can change DNA methylation in brain and change the DNA

methylation status of plasticity-promoting gene reelin and brain-derived neurotrophic factor (Bdnf) [20]. Taking the conditional fear memory model of mice as an example. The rats were given punishments when they smell a specific kind of odor. After training, the rats will make connection between the specific odor and fear in their memory. Rats are sensitive to specific odors. Their offspring which were giving birth after trained are also sensitive to this odor, even if they are not punished. This classical model simply proves that fear can be inherited [21]. The further research found that after fear conditioning experiment, the expression of de novo *DNMT* was upregulated in the hippocampus of rats. By the way, fear conditioning is related to rapid methylation, transcriptional silencing of the memory-suppressor gene protein phosphatase 1 (PP1), demethylation of the PP1, and transcriptional activation of the plasticity gene reelin. These conclusions prove that active DNA methylation and demethylation are involved in the consolidation of long-term memory in the central nervous system. At the same time, Bdnf gene locus is affect by memory-associated changes in DNA methylation as well. This effect is regulated by N-methyl-D-aspartic acid receptor. Neuronal DNMT-deficient animals have deficits in fear conditioning experiment and hippocampal long-term potentiation (LTP) [20]. The above context illustrates that DNA methylation mainly occurs in the hippocampus has a far-reaching impact on memory consolidation.

The fear conditioned experiment continued to be used to observe DNA methylation. The role of DNA methylation in the anterior cingulate cortex (ACC) is equally important for the acquisition and maintenance of associative memory. Test the DNA methylation level of rats in ACC at different experimental stages: during the consolidation of neuronal system, about one hour after training, it was found that there was no obvious abnormality in the DNA methylation level; However, during memory maintenance, that is, four weeks after training, a large number of differential methylations can be detected in cortical neurons, which is the stage of associative memory formation. The same experiment was carried out in the hippocampus, and the results were exactly the opposite: during the consolidation of the neuronal system, the level of DNA methylation was very active, but during the maintenance of the neuronal system, the level of DNA methylation was relatively stable. This proves that there is an obvious spatiotemporal correlation between DNA methylation and learning and memory process [22].

4.2 RNA modification

RNA modification, another major epigenetic modification method, also plays an important role in genetic memory. There are many types of RNA, and many RNA can play a role in memory. But here we only focus on micro-RNA. Micro-RNA is a short non-coding RNA, which means RNA that does not encode proteins. They can be transcribed from the genome, but they can perform their specific biological functions at the RNA level without being translated into proteins [23].

It is involved in neuron development, synaptic transmission, the function of learning and memory. One of the important miRNAs is miR-132. MiR-132 mainly affects neural development. It has been found that the downregulation of miR-132 will promote the pathogenesis of Alzheimer's disease (AD), because it destroys the S-nitrosation balance of AD and guides the occurrence of tau phosphorylation, thus affecting the function of learning and memory. Some studies have proved that knocking out the expression of miR-132 can significantly reduce the spatial memory and learning ability of mice. Similarly, using the method of inhibiting miR-132 will also decline the memory and learning ability of experimental subjects. Firstly, the expression of miR-132 in adult rat and older rat was compared. It was found that the expression of miR-132 in older rat was down regulated compared with adult rat. This really shows that learning and memory ability will decline with age. Rat was injected with miR-132 inhibitor, and Morris water maze experiment was carried out to test rat's memory and learning ability by letting rat swim and cross the underwater platform. The results showed that compared with the control group rat without inhibitor injection, the escape latency of rat injected with miR-132 inhibitor increased significantly and the number of crossing the platform decreased significantly, indicating that the inhibition of miR-132 made it difficult for rat to complete the task and the decline of its learning and memory function [24].

Caenorhabditis elegans' fear is inherited through RNA. We also use the fear memory model for elegans, which is similar to the fear conditioned experiment about rats. The parental response affects

the gene expression of the next generation through heritable micro-RNA molecules. Among them, small RNAs regulates small interfering RNAs and germline gene expression for multiple generations. This enables genetic memory. It is worth mentioning that such a mechanism will slowly decline without specific conditions, which will take about five generations [25].

5. Conclusion

This paper systematically introduces the basic factors of memory formation from three aspects: physiology, classical genetics and epigenetics. Although the existing research on the mechanism of memory have reached the molecular level, and the pathways and epigenetic modifications of some key genes have been studied in depth, the formation process of memory is too complicated, and there are certain polymorphisms of genes in the population, so the importance of many genes cannot be quantitatively measured. Meanwhile, although the sites of epigenetic modifications have been identified, the specific mechanisms by which the environment causes these modifications to have yet to be studied. If the mechanisms of action of environmental factors can be elucidated at the molecular level, the role of acquired inheritance in facilitating active evolution may be emphasized.

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