Neurobiology of Learning and Memory xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Review

The integrated role of ACh, ERK and mTOR in the mechanisms of hippocampal inhibitory avoidance memory

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ARTICLE INFO

Article history:
Received 7 February 2014
Revised 29 December 2014
Accepted 30 December 2014
Available online xxxx

Keywords:
Memory
Hippocampus
Acetylcholine
ERK
mTOR
Inhibitory avoidance

ABSTRACT

The purpose of this review is to summarize the present knowledge on the interplay among the cholinergic system, Extracellular signal-Regulated Kinase (ERK) and Mammalian Target of Rapamycin (mTOR) pathways in the development of short and long term memories during the acquisition and recall of the step-down inhibitory avoidance in the hippocampus. The step-down inhibitory avoidance is a form of associative learning that is acquired in a relatively simple one-trial test through several sensorial inputs. Inhibitory avoidance depends on the integrated activity of hippocampal CA1 and other brain areas. Recall can be performed at different times after acquisition, thus allowing for the study of both short and long term memory. Among the many neurotransmitter systems involved, the cholinergic neurons that originate in the basal forebrain and project to the hippocampus are of crucial importance in inhibitory avoidance processes. Acetylcholine released from cholinergic fibers during acquisition and/or recall of behavioural tasks activates muscarinic and nicotinic acetylcholine receptors and brings about a long-lasting potentiation of the postsynaptic membrane followed by downstream activation of intracellular pathway (ERK, among others) that create conditions favourable for neuronal plasticity. ERK appears to be salient not only in long term memory, but also in the molecular mechanisms underlying short term memory formation in the hippocampus. Since ERK can function as a biochemical coincidence detector in response to extracellular signals in neurons, the activation of ERK-dependent downstream effectors is determined, in part, by the duration of ERK phosphorylation itself. Long term memories require protein synthesis, that in the synapto-dendritic compartment represents a direct mechanism that can produce rapid changes in protein content in response to synaptic activity. mTOR in the brain regulates protein translation in response to neuronal activity, thereby modulating synaptic plasticity and long term memory formation. Some studies demonstrate a complex interplay among the cholinergic system, ERK and mTOR. It has been shown that co-activation of muscarinic acetylcholine receptors and β-adrenergic receptors facilitates the conversion of short term to long term synaptic plasticity through an ERK- and mTOR-dependent mechanism which requires translation initiation. It seems therefore that the complex interplay among the cholinergic system, ERK and mTOR is crucial in the development of new inhibitory avoidance memories in the hippocampus.

Abbreviations: 4E-BPs, 4E binding proteins; ACh, acetylcholine; AD, Alzheimer's Disease; APP, amyloid precursor protein; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CREB, cAMP response element-binding protein; DG, dentate gyrus; eEF1A, eukaryotic Elongation Factor 1A; eEF2, eukaryotic Elongation Factor 2; ERK, Extracellular signal-Regulated Kinase; GABA, gamma-aminobutyric acid; GPCRs, G protein-coupled receptors; IA, inhibitory avoidance; ICV, intracerebroventricular; IP, intraperitoneal; JNK, c-Jun N-terminal kinase; LTM, long term memory; LTP, long term potentiation; mAChRs, muscarinic acetylcholine receptors; M1, . . . ,M5, muscarinic receptor 1, . . . ,5; MAP, microtubule-associated proteins; MAPK, mitogen activated protein kinase; MEK, mitogen-activated protein kinase kinase; mTOR, Mammalian Target of Rapamycin; mTORC1, mTOR Complex1; nAChRs, nicotinic acetylcholine receptors; NBM, nucleus basalis magnocellularis; NMDA, N-methyl-D-aspartate; p38MAPK, p38 mitogen activated protein kinase; p70S6K, p70 ribosomal subunit S6 Kinase; PKA, protein kinase A; PKC, protein kinase C; STM, short term memory; TgCRND8, Transgenic Centre for Research in Neurodegenerative Diseases 8; wt, wild type.

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http://dx.doi.org/10.1016/j.nlm.2014.12.014
1074-7427/

1. Introduction

As St. Augustine wrote in the "Confessions" in the IVth Century a.d. "*And I come to the fields and spacious palaces of my memory, where are the treasures of innumerable images, brought into it from things of all sorts perceived by the senses. ... Nor yet do the things themselves enter in; only the images of the things perceived are there in readiness, for thought to recall. Which images, how they are formed, who can tell, though it doth plainly appear by which sense each hath been brought in and stored up?.*" (St. Augustine, 398). The purpose of this review is to try to answer to the question that already fascinated St. Augustine on how memories are formed, by summarizing some of the present knowledge on the mechanisms that underlie memory development in our brain.

The formation of memories is the result of cellular and molecular mechanisms activated in different structures of the brain. The ability of an animal to adapt its behaviour in response to environmental stimuli depends on the structural and functional plasticity of several brain regions. Therefore, it is of the utmost importance to understand how and where in the brain experiences are encoded into lasting memories.

A single learning experience starts a cascade of events, which can lead to different forms of memory: short-term memory (STM) that lasts minutes to hours and long term memory (LTM) that lasts days, weeks, and even a lifetime (McGaugh, 1966). A major question of memory neurobiology is whether these two forms are related or independent phenomena. Some cellular mechanisms that underlie the development of STM overlap with those of LTM, but other mechanisms are independent (Izquierdo, Medina, Vianna, Izquierdo, & Barros, 1999; Izquierdo, Barros, et al., 1998; Izquierdo et al., 2002). A unique characteristic of LTM is the need for a consolidation period during which synaptic, structural, and functional modifications occur (Igaz, Vianna, Medina, & Izquierdo, 2002). The most important is protein synthesis on which LTM, but not STM, depends (Bourtchouladze et al., 1998; Davis & Squire, 1984; Freeman, Rose, & Scholey, 1995; Quevedo et al., 1999; Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999; Tiunova, Anokhin, Rose, & Mileusnic, 1996).

Memory is not a unitary function. Memory depends on the integrated activity of various brain structures and neurotransmitter systems and involves multiple receptors, postsynaptic mechanisms, and signal transduction pathways (Izquierdo, Barros, et al., 1998). Among the various brain structures implicated in memory formation, the CA1 region of the hippocampus plays a major role in memory encoding (Eichenbaum, 2001; Hasselmo, Wyble, & Wallenstein, 1996; Lisman & Grace, 2005; Squire, 1992; Vinogradova, 2001).

1.1. Step-down inhibitory avoidance memory

The step-down inhibitory avoidance (IA) is a form of associative learning that is acquired in one trial through several sensory inputs. IA memory depends on the integrated activity of CA1, entorhinal and posterior parietal cortex, and is modulated by the amygdala and by the basal forebrain cholinergic neurons of the medial septum and indirectly by stress hormones (Cammarota, Bevilaqua, Medina, & Izquierdo, 2007; Izquierdo, 1989; Izquierdo & Medina, 1997). The step-down IA is a widely used task in memory studies (Gold, 1986; Izquierdo et al., 2007; McGaugh, 1966; McGaugh & Izquierdo, 2000) and relies upon the natural tendency of an animal to explore a novel environment. In the IA acquisition task, the animal is placed on an elevated platform by one wall of an arena, steps down to explore the arena and learns to associate exploration with a punishment (a foot shock delivered through the floor grid). On a subsequent exposure to the same environment (recall task), the animal increases the latency to step down onto the floor grid, or avoids stepping on the grid. The natural exploratory behaviour is repressed after the punishment is given, without affecting the exploratory behaviour while on the safe, non-aversive part of the training apparatus. IA is an emotionally-arousing paradigm (Izquierdo et al., 1997; Maren, 2001), that involves:

(i) an explicit, associative component to the context,
(ii) an operant-like conditioning component to the shock, since the animal may avoid the aversive stimulus (Wilensky, Schafe, & LeDoux, 2000),
(iii) a spatial memory component, since the animal remembers the location where the noxious stimulus was given during acquisition (Cammarota et al., 2007).

In the IA, the environment is arranged so that the animal can avoid a painful stimulus; i.e., the "escape" or avoidance is an option available to an animal that could learn and perform it. From an experimental view point, IA is a relatively simple test since it is acquired in a one-trial session. Recall can be performed at different times after acquisition, thus allowing to study both STM (Izquierdo, Barros, et al., 1998; Izquierdo, Izquierdo, et al., 1998) and LTM mechanisms (Izquierdo et al., 2002).

IA depends upon the activation of the cholinergic system, since its acquisition is impaired by pre-training (Giovannini, Bartolini, Bacciottini, Greco, & Blandina, 1999; Izquierdo, Izquierdo, et al., 1998) or post-training peripheral administration of mAChRs antagonists (Table 1) (Giovannini et al., 1999; Izquierdo, Izquierdo, et al., 1998; McGaugh & Izquierdo, 2000), and is facilitated by mAChRs agonists (Baratti, Huygens, Mino, Merlo, & Gardella, 1979; Barros, Pereira, Medina, & Izquierdo, 2002).

1.2. Short term and long term memory mechanisms: open questions

All types of novel stimuli induce the activation of the forebrain cholinergic system (Pepeu & Giovannini, 2006). In this review we shall examine how the cholinergic system participates in the formation of STM and LTM in CA1 during the acquisition and performance of the step-down inhibitory avoidance task in the rat. A key question that still remains unanswered is whether STM represents a step toward LTM only or the formation of the two memory types reflects separate processes.

According to current hypotheses, STM and LTM formation imply biochemical processes that act in parallel and on different time scales (Izquierdo, Barros, et al., 1998; Izquierdo et al., 1999, 2002). Nevertheless, to better answer to this question, it is necessary to demonstrate that STM can be suppressed without affecting LTM. The pharmacology and molecular bases of IA have been studied by us (Giovannini et al., 2005; Lana et al., 2013) and Izquierdo's group, particularly in the CA1 region (Igaz, Bekinschtein, Izquierdo, & Medina, 2004; Marti, Ramirez, Dos Reis, & Izquierdo, 2004). Moreover, for the reasons mentioned above, unlike multitrial learning tasks, IA offers the possibility to neuroscientists to distinguish the processes involved in STM and LTM by the simple modulation of time parameters after the acquisition task. In particular we shall try to shed light on the complex interplay among the cholinergic system, ERK and mTOR in IA memory formation. Among the several actors downstream of the cholinergic activation implicated in STM and LTM formation, this review will focus particularly on ERK and mTOR since they can modulate both early processes such as phosphorylation of protein substrates, implicated in STM, and later processes like immediate or *de novo* proteosynthesis in neurons, implicated in LTM formation (Alonso, Viola, Izquierdo, & Medina, 2002; Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998; Bourtchouladze et al., 1998; Cammarota et al., 2000; Davis & Squire, 1984; Freeman et al., 1995; Quevedo et al., 1999;

Please cite this article in press as: Giovannini, M. G., et al. The integrated role of ACh, ERK and mTOR in the mechanisms of hippocampal inhibitory avoidance memory. *Neurobiology of Learning and Memory* (2015), http://dx.doi.org/10.1016/j.nlm.2014.12.014