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Upregulation of PP1 Expression in Hippocampus Impairs Long-term Spatial Memory in Rats

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Author's contribution

Author YGC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript.

Original Research Article

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ABSTRACT

Aims: Either down-regulation of protein kinases or up-regulation of protein phosphatases weakens the level of neuronal protein phosphorylation and affects the function of neuron. Low-function and/or low-content of protein phosphatases in the hippocampus neurons is proposed to be a possible causative factor to the impairment of spatial memory ability. We show here that upregulation of PP1(protein phosphatase1) expression in hippocampus correlates with the decline of the spatial-memory retention ability in rat brain.
Study Design: In the present study, 30 adult Wistar rats were tested in a one trial step-down test to assess normal and impaired memory retention, examining protein phosphatases expression in their hippocampi and analysing both relationship.
Place and Duration of Study: Department of Pathophysiology, Medical College in Wuhan University of Science and Technology (WUST), between June 2010 and July 2011.
Methodology: To examine which rats have lower ability of learning and memory, thirty Wistar rats, male, 5-7 months old (360~450g), were trained and tested in step-down inhibitory avoidance task. R129d, R126d, R123d, Cdk5 (8) & CREB antibodies were respectively used to detect the content of PP1, PP2B, PP2A, Cdk5 & CREB. Densitometric analysis was used to quantitate the blots.Equal variance T test was used to analyze the escape latency and error from the two group rats, and the immunoblotting

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data from two groups of rat hippocampus proteins.
Results: 72h after the fourth trail, MMI (mild memory impairmemt) rats showed performance markedly worse than that of the NC (normal control) rats (latency shorter, <150sec; error more, ≥1 vs latency, 300sec; error, 0times; Both $P<<0.01$). From the learning and retention curves of the both groups of rats in Fig. 1, author found that in the first step-down train, the error times in MMI rats were also more than those in NC rats ($P< 0.05$), indicating that the ability of learning new material in MMI rats is worse than that in NC rats. The results showed that the contents of PP1 and PP-2B in MMI rats have increased or decreased ~50% ($P<0.01$) or ~40% ($P<0.01$) respectively compared with NC rats; Whereas the expression of PP2A in both groups of rats showed no obvious difference, implying that PP1 and/or PP2B might participate in the regulation of learning and memory in the rat brain. In addition, we all found that the middle degree of adverse correlation between the expression of PP1 and the latency of MMI & NC rats (-0.618, $P<0.001$, two tails), and the low degree of positive correlation between the expression of PP-2B and the latency of MMI & NC rats (0.381, $P<0.05$, two tails), indicating that the increase of PP1 content in itself might impress in part the memory ability of rats.
Conclusion: It is concluded that upregulation of PP1 content may mediate worsened memory retention in rat brain.

Keywords: Upregulation; protein phosphatase-1; expression; hippocampus; long-term spatial memory; rat.

1. INTRODUCTION

A number of neuronal functions, including spatial memory (long-time memory), depend on synthesis and proper regulation of neuronal proteins, many of which can being rapidly regulated by phosphorylation. Reversible protein phosphorylation is a fundamental mechanism by which many neuronal functions are regulated. Achievement of such control requires the coordinated action of the protein kinases and protein phosphatases, two major classes of proteins recognized to be largely involved in cognitive functions. Both intervene virtually in all steps of learning and memory. Protein phosphorylation and dephosphorylation are believed to functionally couple neuronal activity and synaptic plasticity [1,2,3]. In a nervous system, protein phosphatases are contained in highly dynamic complexes localized within specialized subcellular compartments and they ensure timely dephosphorylation of multiple neuronal phosphoproteins, which modulates the responsiveness of individual synapses to neural activity and controls synaptic plasticity. These enzymes in turn play a key role in many forms of learning and memory, and their dysfunction contributes to cognitive deficits associated with aging and dementias or neurodegenerative diseases. Consistent with their ability to constrain synaptic transmission and plasticity, protein phosphatases (PPs) negatively regulate learning and memory processes. Thus, PPs'activity or content needs to be controlled during and after these processes and PPs' alterations are implicated in memory disorders. Alterations in PPs are associated with Alzheimer's disease (AD), the most common cause of memory deficits and dementia [4,5,6]. PP1, one of Ser/Thr protein phosphatases, being distributed mainly in neurons of cerebral cortex, hippocampus and neostriatum [7]. Past research showed that PP1 plays important roles to the mechnisms of LTP, LTD and depotentiation (i.e. synaptic effects of the hippocampal CA1 region). Partial inhibition of PP1 during training improves spatial learning and recognition memory, and prevents age-related decline in these functions. During aging in humans and rodents, overall PP1 and calcineurin activity increases in the brain, which might contribute to learning and

memory failures, cognitive decline and altered synaptic plasticity [8,3]. In 2002, Genoux et al [9] found that the physiological importance of PP1 as a suppressor of learning and memory, and as a potential mediator of cognitive decline during aging. To explore the correlation between upregulation of PP1 expression (implicating dephosphorylation of functional proteins) in hippocampus and MMI (mild memory impairment), we have examined the memory behavior of rats 72h (decided by my pre-experimental results) after training sessions of 4 times by one trail step-down test (electric jump plateform task). At meantime, the content of three protein phosphatases (esp.PP1) in rat hippocampus has been determined by Western blot.

2. MATERIALS AND METHODS

All 30 animal experiments were performed according to the "Policies on the use of Animals and Humans in Neurosience Research", revised and approved by the Society for Neuroscience in 1995. The animals were individually housed in a room with constant temperature and a 12-h light, 12-h dark cycle. The animals were given their normal food and drink.

2.1 Experiment Materials

Primary antibodies to the relative proteins are listed in Table 1. Secondary antibodies for Western blot were from Amersham Pharmacia Biotech (Little Chalfort, Buckinghamshire, England).

Table 1. Primary antibodies used for this study

Antibody	Dilution	Type[a]	Specificity[b]	Epitope[c]	Resource
R123d	1:1000	Poly	NP	PP2A (catalytic unit)	Wang, China
R126d	1:1000	Poly	NP	PP2B (catalytic unit)	Davis, USA
R129d	1:1000	Poly	NP	PP1 (catalytic unit)	Bindle, USA
Cdk5	1:300	Poly	NP	Cdk5 (α-unit)	Iqbal, USA
CREB	1:1000	Poly	NP	unp-CREB	Wang, China

a: Poly: polyclonal. b: NP, not-phosphorylated epitope. c: unp, unphosphorylated epitope.

2.1 Behavioural Experiment Procedures

To examine which rats have lower ability of learning and memory, thirty Wistar rats, male, 5-7 months old (360~450g), were trained and tested in step-down inhibitory avoidance task. The experimental device is a 30-cm×30-cm×30-cm electronic avoidance-response chamber, made of Plexiglas on its three sides and hard black plastic on the other. The chamber has a bottom of parallel 0.5-cm stainless steel bars spaced 1cm apart. A rubber platform (5cm high, 5cm in diameter of its top surface) was fixedly placed at a corner on the bottom of the chamber, providing rats a shelter from the electronic attack. Before normal test, rats were continually trained in an one-trial step down inhibitory avoidance task for four times (one time/per day, conducted between 19:00~21:00), and tested for their memory retention onto the escape platform from electronic attack at the same time 72h after training. Rats were placed on the platform, and their latency to step down first, placing their four paws on the grids, was measured. At the meantime, the times of placing their four paws on the grids (errors) were recorded. In training sessions, immediately upon stepping down, the rats received a 0.3mA, 2-s, scrambled foot shock. No foot shock was given in test session. Test session step-down latencies and errors (during 5min) were taken as a measure of memory

retention [10]. 72h after the last training, 15 rats showed performance markedly worse than that of the other 15 rats (latency shorter, <150sec; error more, ≥1; Both P<<0.01; The 15 rats were viewed as MMI rats). We elected 15 rats that had more excellent memory retention in step-down electronic inhibitory avoidance task as normal control (NC) rats (latency=300sec; error=0).

After the last trail, all rat hippocampus were homogenized at 4□, and boiled for biochemical analysis (see below).

2.3 Immunoblot Analysis

Rat hippocampus was quickly dissected out in cold homogenizing buffer containing 50-mM Tris-HCl (pH, 7.4), 10-mM β-mercaptoethanol, 1.0-mM EDTA, 1.0mM EGTA, 0.1-mM phenylmethylsulfonyl fluoride, and 2.0μg/ml each of aprotinin, leupeptin, and pepstatin A. Then samples were homogenized in the same buffer at a ratio of 8.0ml of buffer/1.0g tissue with phosphatase inhibitor mixture containing 20-mM β-glycerophosphate, 2.0-mM Na3VO4, and 100-mM NaF (pH, 7.0). The homogenate was spun at 18000 × g for 10min at 4□ to remove precipitated material. The supernatants were mixed with 4× sample buffer (200mM Tris, pH6.8; 8% SDS, 20%β-ME、 40% glycerol) and boiled for 8 min. After cooling at room temperature, samples were stored at-70□. Protein concentration of the samples was determined by BCA kit (Pierce Chemical Co., Rockford, IL). The expression of proteins in the above samples was analyzed by Western blot using 10% SDS-PAGE as described originally by Laemmli. Molecular weights were verified by using prestained molecular weight marker (Bio Rad). Proteins were transferred to 0.45μm pore nitrocellulose (Bio Rad) membranes using electroblotting (280mA for 1h). Following the transfer, membranes were blocked against nonspecific antibody binding in 5% milk/TBS for 1h at room temperature, and then incubated in primary antibody diluted in 3% milk/TBS over night at 4□; R129d, R126d, R123d, Cdk5 (8) & CREB antibodies were respectively used to detect the content of PP1, PP2B, PP2A, Cdk5 & CREB. After incubation with the appropriate alkaline phosphatase-conjugated secondary antibody, the blots were developed using NBT/BCIP (Nitro blue tetrazolium/5-Bromo-4-chlor-3indolyl phosphate salt) system. Densitometric analysis was used to quantitate the blots.

2.4 Statistical Analysis

Unequal variance T test was used to analyze the escape latency and error from the two group rats, and the immunoblotting data from two groups of rat hippocampus proteins.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Performance of the rats in step-down electronic inhibitory avoidance task

72h after the fourth trail, MMI rats showed performance markedly worse than that of the NC rats (latency shorter, <150sec; error more, ≥1 vs latency, 300sec; error, 0times; Both *P*<<.01). From the learning and retention curves of the both groups of rats in Fig. 1, author found that in the first step-down train, the error times in MMI rats were also more than those in NC rats (*P*< .05), indicating that the ability of learning new material in MMI rats is worse than that in NC rats (Fig. 1).

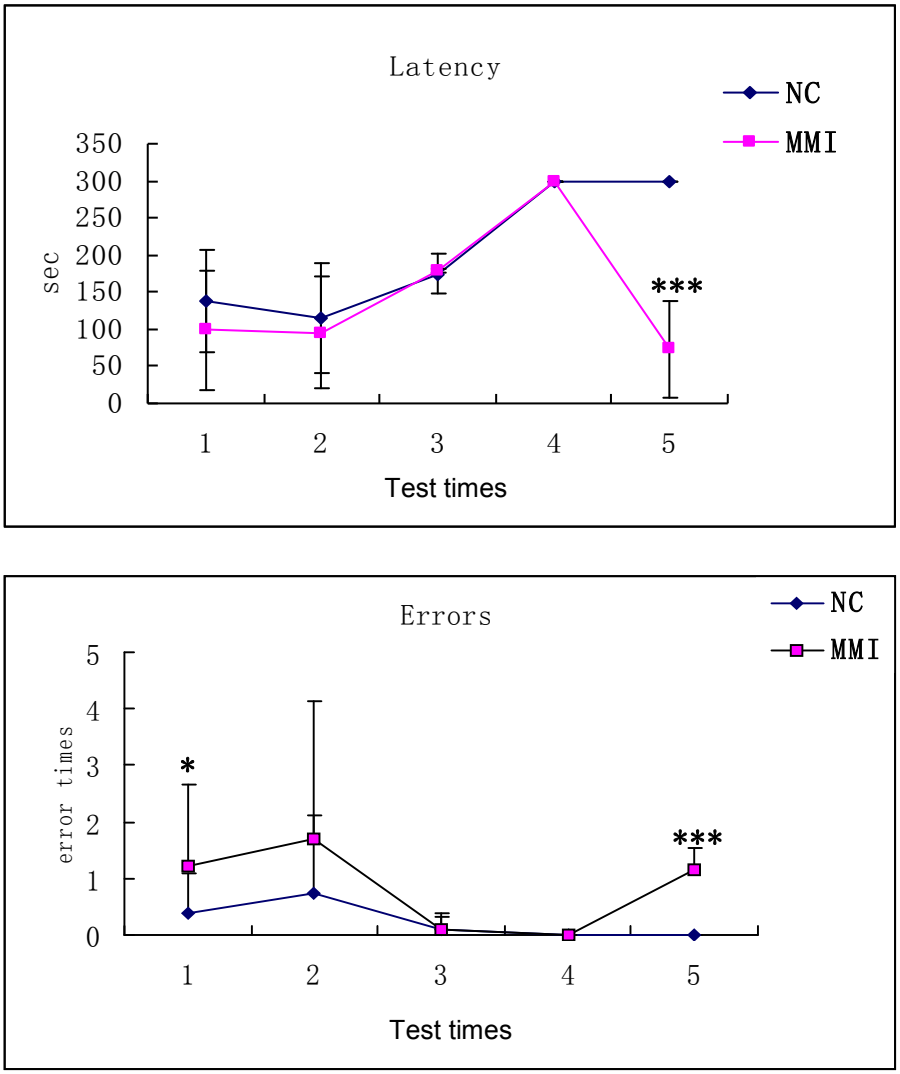

Fig. 1. Step-down latency and errors of rats during training and testing (n=15, respectively; Mean±2SD)

**P<0.05 & ***P<0.001 represent the difference significance, compared MMI rats with NC ones respectively at the same time*

Mean ± S.D. = Mean values ± Standard difference of means of fifty of rats

3.1.2 Enzyme expression

To explore whether phosphatases in relation to dephosphorylation of the relative functional proteins have changed, we detected the content of PP1, PP2B and PP2A. The results showed that the contents of PP1 and PP-2B in MMI rats have increased or decreased ~50% (*P*<.01) or ~40% (*P*<.01) respectively compared with NC rats; Whereas the expression of PP2A in both groups of rats showed no obvious difference (Fig. 2), implying that PP1 and/or PP2B might participate in the regulation of learning and memory in the rat brain. In addition, we all found that the middle degree of adverse correlation between the expression of PP1

and the latency of MMI & NC rats (-0.618, *P*<.001, two tails; Fig. 3), and the low degree of positive correlation between the expression of PP-2B and the latency of MMI & NC rats (0.381, *P*<.05, two tails; Fig. 3), indicating that the increase of PP1 content in itself might impress in part the memory ability of rats.

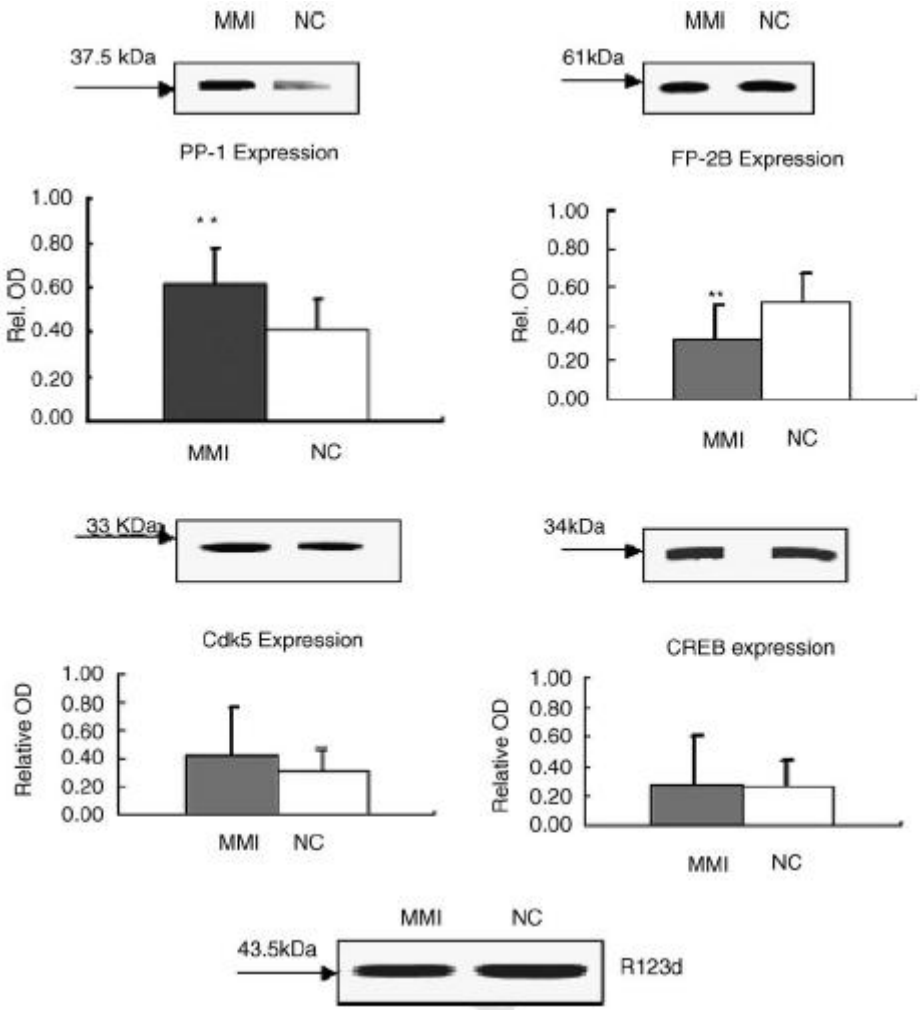

Fig. 2. The levels of PP1, PP2B, Cdk5, CREB & PP2A probed by R129d, R126d, Cdk5, CREB & R123d antibodies respectively. Western blot (upper panels) and quantitative analysis (lower panels) were done based on 30μg of hippocampal proteins per lane. The molecular mass markers are shown at the left of blots

*Each blot is respresentative of 3 separate experiments of 15 rats; **represents P<0.01, MMI rats compared with NC rats*

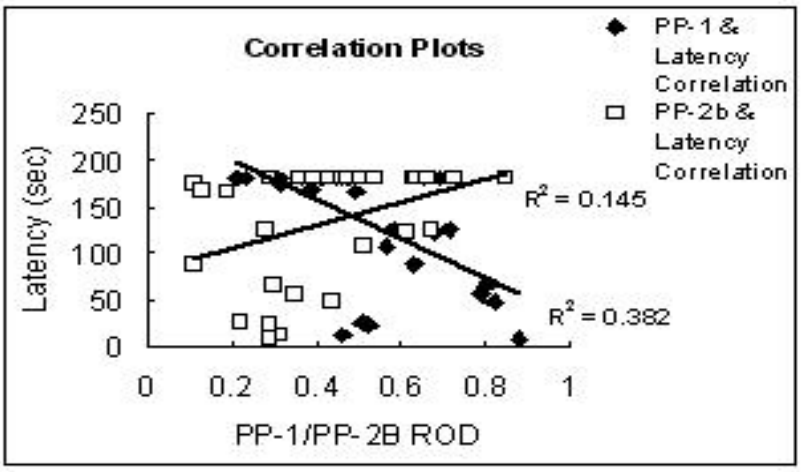

Fig. 3. Plot of correlation between PP-1/PP-2B ROD and latency of one trail step-down test from all 30 rats (MMI & NC rats)

r = -0.618, P<0.001 & r =0.381, P<0.05, respectively (two tails)
Bradford staining was used to ensure the same content of the proteins loaded for all the Western blot study.

3.2 Discussion

The results presented here show that rats showing impairment in memory retention in the step-down inhibitory avoidance task also exhibited significantly increased expression of PP1, as determined by western blots. It is concluded that upregulation of PP1 content may mediate worsened memory retention in rats. Both no obvious difference in the expression of PP2A in both groups of rats and the low degree of positive correlation between the expression of PP-2B and the latency of MMI & NC rats indicated directly that the increase of PP1 content in itself might impress in part the memory ability of rats.

Learning and memory are highly complex processes whose mechnisms are extremely intricate. It is well known that spatial learning and memory is hippocampus-dependent [10]. Inhibitory avoidance learning is an example of instrumental learning, in which shock delivery is contingent on the animal's response in our step-down avoidance task that is a kind of hippocampus-dependent test related with learning and memory. In our step-down avoidance task, rats would learn to use contextual (place) cues to escape from the detrimental shock on the shelter by repeated training. Past evidence indicated that the hippocampus is necessary for acquisition and retrieval of spatial information as well as for consolidation/storage [11]. And our result of one trail step-down test is in consistent with the data of Morris Water Maze from the other co-workers of our college. There are at least three constituent aspects to spatial learning and memory: memory capacity (the number of locations remembered), memory persistence (the duration over which a location is remembered), and spatial resolution (the least distance at which remembered locations can be discriminated). Among the three aspects memory persistence is much associated with longer-lasting spatial memory. And, one trail step-down task is very associated with special resolution (the least distance between the escape platform and electric attack field can be resolved).

In mammalian cells, four major classes of Ser/Thr-specific phosphatase catalytic subunits have been identified, comprising two distinct gene families. The high degree of homology among members of the same family, PP1, PP2A and PP2B and the high degree of evolutionary conservation between organisms as divergent as mammals and yeast, implies that these enzymes are involved in fundamental cell functions. Neural function of PP1 remains unclear except that it might participate in the formation of long-term depression (LTD) [11,12,13]. Increasing data suggest the role of PP2A and PP1 in learning and memory [11,14,12,13]. The fact that inhibition of PP2A and PP1 leads to dysfunction in learning and memory is not a novel one. But because we found the correlation between both changes of PP1 content and the memory ability of rats in the study, the processes of one-trial step-down inhibitory avoidance task needs protein phosphorylation and special enzyme participation, and the increment of PP1 content itself could cause the decrease of memory ability due to the persistent dephosphorylation (inactivation) of CREB (associated with long term memory) [15]. It has also been reported that the duration of CREB phosphorylation is regulated by nuclear PP1 [16]. Interestingly, Foster et al. [8] reported that there is an increase in PP2B activity in the hippocampus during aging, which could explain the upregulation of PP1 expression in rat hippocampus in this study.

PP2B (Calcineurin) is a serine-threonine specific Ca^{2+}-calmodulin-activated protein phosphatase. Neuronal cells are very rich in PP2B and multiple neuronal processes depend on its activity. The potential modulator of both memory function and cell degeneration is the Ca^{++}/Calmodulin-dependent protein phosphatase 2B (calcineurin, CaN), the most abundant phosphatase in the CNS [17]. Downregulation of CaN by an autoinhibitory peptide improves memory in rodents [18]. PP2A is a heterotrimeric enzyme that comprises one catalytic subunit, one non-catalytic A subunit, and one of several structurally distinct, regulatory B subunits [19]. It is mainly a cytosolic enzyme involved in various regulations of cell functions.

Memory appears to be the product of dynamic interactions among multiple systems in the brain, and the interaction between hippocampal and neocortical systems has turned into a topic of importance in studying the hippocampal function in learning and memory [20, 21, 22, 23, 24]. Memory consolidation would therefore involve a transitory interaction between the hippocampus related structures of the medial temporal lobe and the neocortex [25]. Consequently, lesions directed to the hippocampus or related temporal structures would lead to a weak representation of the learned material in the neocortex, thus impairing the long-term retention of information. Activated PP2B dephosphorylates the PP1 inhibitor protein, thereby activating PP1. When the content (activity) of PP1 increased, active Cdk5 can phosphorylate DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of Mr 32 kDa) on Thr75: Phospho-Thr75 DARPP-32 not only becomes unresponsive to phosphorylation by PKA, but also directly inhibits PKA activity, leading to the insult of LTM (long term memory, because of deactivation of CREB) [26, 15]. However many studies have alsoindicated that the effects of neuropharmacological agents on MWM (Morris water maze) and step-down performance as well as the involvement of the different neurochemical systems in spatial learning may be more complex than initially thought.

4. CONCLUSION

In conclusion, our studies suggest that the up-regulation of PP1 content may be much responsible than the deregulation of PP2B expression for the deficiency of learning and memory in rats. Our results are also important in relation to human memory dysfunctions,

because the deficits of recall or retrieval are common in depression [27] and Alzheimer's disease [28,29].

CONSENT

Not applicable.

ETHICAL APPROVAL

I hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Naqib F, Sossin WS, Farah CA. Molecular determinants of the spacing effect. Neural Plast. 2012;581 91.
2. Lee H-K. Synaptic plasticity and phosphorylation. Pharm Therap. 2006;112:810-32.
3. Jouvenceau A, Dutar P. A role for the protein phosphatase 2B in altered hippocampal synaptic plasticity in the aged rat. J Physiol (Paris). 2006;99:154-64.
4. Braithwaite SP, Stock JB, Lombroso PJ, Nairn AC. Protein phosphatases and Alzheimer's disease. Prog Mol Biol Transl Sci. 2012;106:343 79.
5. LaFerla FM. Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. Nat Rev Neurosci. 2002;3:862-72.
6. Mattson MP, Chan SL. Neuronal and glial calcium signaling in Alzheimer's disease. Cell Cal. 2003;34:385-97.
7. Greengard P. The neurobiology of slow synaptic transmission. Science. 2001;294:1024-30.
8. Foster TC, Sharrow KM, Masse J R, Norris CM, Kumar A. Calcineurin links Ca2+ dysregulation with brain aging. J Neuroscie. 2001;21:4066-73.
9. Genoux D, Haditsch U, Knobloch M. Protein phosphatase 1 is a molecular constraint on learning and memory. Nature. 2002;418(6901):970-75.
10. Ramos J. Long-term spatial memory in rats with hippocampal lesions. Eur J Neurosci. 2000;12:3375-84.

11. Bennett PC, Zhao W, Ng KT. Concentration-dependent effects of protein phosphatase (PP) inhibitors implicate PP1 and PP2A in different stages memory formation. Neurobiol Learn Mem. 2001;75:91-110.
12. Mulkey RM, Endo S, Shenolikar S. Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature. 1994;369:486-88.
13. Thiels E, Kanterewicz BI, Knapp LT. Protein phosphatase-mediated regulation of protein kinase C during long-term depression in the adult hippocampus in vivo. J Neurosci. 2000;20:7199-207.
14. Mulkey RM, Herron CE, Malenka RC. An essential role for protein phosphatases in hippocampal long-term depression. Science. 1993;261:1051-55.
15. Gupta A, Tsai LH. A kinase to dampen the effects of cocaine? Science. 2001;292(5515):236-37.
16. Bito H, Delsseroth K, Tsten RW. CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimutus duration-dependent switch for hippocampal gene expression. Cell. 1996;87:1203-14.
17. Mansuy IM. Calcineurin in memory and bidirectional plasticity. Biochemi. Biophys Res Commun. 2003;311:1195-208.
18. Malleret G, Haditsch U, Genoux D. Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell. 2001;104:675-86.
19. Sontag E, Nunbhakdi-Craig V, Lee G. Mocular interactions among protein phosphatase 2A, tau, and microtubules: implications for the regulation of tau phosphorylation and the development of tauopathies. J Biol Chem. 1999;274:25490-98.
20. Eichenhaum H, Stewart C, Morris RG. Hippoeampal representation in place learning. J Neurosci. 1990;10:3531-42.
21. Maguire EA, Mummery CJ, Buchel C. Patterns of hippocampal-cortical interaction dissociate temporal lobe memory subsystems. Hippocampus. 2000;10:475-82.
22. Nadel L. Multiple memory systems: what and why, an update. In: Schacter DL, Tulving E, editors. Memory systems.1st ed. Cambridge, MA: MIT. 1995;39-64.
23. Redish AD, Touretzky DS. The role of the hippocampus in solving the Morris water maze. Neural Comp. 1998;10:73-101.
24. Wilson MA, Tonegawa SS. Ynaptic plasticity, place cells and spatial memory: study with second generation knockouts. Trends Neurosci. 1997;20:102-06.
25. Bontempi B, Laurent-Demir C, Destrade C. Time-depedent reorganization of brain circuitry underlying long-term memory storage. Nature. 1999;400:671-75.
26. Bernerbu R, Bevilaqua L, Ardenghi P. Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. Proc Natl Acad Sci. USA. 1997;94:7041-46.
27. Ilsey JE, Moffoot AP, O'Carroll RE. An analysis of memory dysfunction in major depression. J Affect Disord. 1995;9:1-9.

28. Backman L, Andersson JL, Nyberg L. Brain regions associated with episodic retrieval in normal aging and Alzheimer's disease. Neurology. 1999;52:1861-70.

29. Mitrushina M, Drebing C, Uchiyama C. The pattern of deficit in different memory components in normal aging and dementia of Alzheimer's type. J Clin Psychol. 1994;50:591-96.

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