Effect of Training on Tau Phosphorylation in Rat Brain

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Abstract

Numerous neurofibrillary tangles found in Alzheimer's disease (AD) brain is positively correlated to the memory impairment of the patients, whereas hyperphosphorylated tau is the major components of the tangles. To explore the correlation between tau phosphorylation and the ability in spatial learning and memory, we first trained the rats by using Morris water maze apparatus and then assessed tau phosphorylation by immunocytochemistry and Western blot. It was found that with the improvement in spatial learning and memory of trained rats, tau was partially dephosphorylated at Ser198/199/202 and Ser396/404 determined by monoclonal antibodies Tau-1 and PHF-1. The data presented in the present study demonstrated that the phosphorylation of tau is correlated with learning and memory. To our knowledge, this is the first data shown directly connection of tau phosphorylation and learning, implying that hyperphosphorylation of tau found in AD brain at least partially accounts for the memory impairment.

Keywords: Alzheimer disease, special memory, tau, phosphorylation

Introduction

Alzheimer's disease (AD) is a progressive dementia characterized clinically by a specific pattern of memory impairment followed by more global cognitive deficits[1]. The confirmed diagnosis of the disease is made histologically by the presence of numerous â-amyloid (Aâ) containing senile plaques (SPs) and neurofibrillary tangles (NFTs). Therefore, the relationship between formation of SPs or NFTs and memory impairment is one of the focuses in AD research. Recently, it has been reported that transgenic mice expressing human amyloid precursor protein, and rat infused intracerebroventricularly Aâ peptide had spatial learning and memory deficits[2]. On the other hand, although positive correlation between the degree of dementia and the amount of NFTs was observed by clinical and postmortem histological investigations, no report till now to address directly the relationship between memory and tau phosphorylation, which is considered as one of the most crucial step in NFTs formation. We have found recently that hyperphosphorylation of tau caused by over-activation of certain kinases causes memory impairment in rats (manuscript in preparation), suggesting that tau phosphorylation might be involved in learning processes. To test this hypothesis, we investigated in the present study the effect of extensive training on tau phosphorylation by using Morris water maze apparatus, which is considered as the most frequently used tool to investigate spatial learning and memory in behavioral neuroscience[3].

Materials and Methods

Materials and reagents 3 months old male SD rats were divided into two groups after first training. One group went through continues training and the other one was cultured as control. Rats were housed in groups (2–5 rats per cage) in temperature and humidity-controlled rooms with *ad libitum* access to food and water. All experiments were carried out blinded with respect to the genetic status of rats.

Behavioral test *The* spatial learning abilities of rats were assessed in Morris water maze task[4]. The water maze consisted of a metal circular pool (diameter, 120 cm; height, 80 cm), the upper part of which was surrounded by a 40-cm-high Perspex wall and filled with water (25°C) in which a circular escape platform (11 cm in diameter) was hidden 1 cm below the surface of the 60-cm-deep water. The maze was located in an experimental room rich in environmental cues. Rats were trained in six daily sessions consisting of four trials that were started from four cardinal points of the compass. Rats were given 60 s to find the escape platform in the center of the northeast quadrant of the pool, and if the rat did not find the platform within this limit, it was guided onto it. All animals were allowed to rest on the platform for 15–20s. Trajectories were monitored with a computerized tracking system[5], and swim paths and latencies to locate the platform were evaluated. Because there was a high positive correlation between the swim paths and escape latencies and no sensor motor impairment was observed, only the latter values were used for evaluation of the animals' performance.

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Tissue extractions and Western blot To prevent dephosphorylation during post mortem delay, brain was rapidly dissected and tissue homogenization was processed on ice. In brief, hippocampus were homogenized in buffer containing 0.1 mol/L 2-(*N*-morpholino) ethanesulfonic acid (pH 6.4), 0.5 mmol/L $MgCl_2$, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L dithiothreitol, 5 µg/ml leupeptin, 5 µg/ml pepstatin, 200 µmol/L phenylmethylsulfonyl fluoride, 20 mmol/L NaF, 200 µmol/L sodium orthovanadate, 1 µmol/L okadaic acid, 5 µg/ml soybean trypsin inhibitor, 1 % Triton X-100, 1 % sodium deoxycholate, and 0.1 % sodium dodecyl sulfate. Then, the homogenate was centrifuged at 12,000 *g* for 30 min at 4°C, and the supernatant was denatured immediately by adding sample buffer. Protein concentration was determined with BCA Protein Assay Reagent (PIERCE, USA).

Proteins were separated by SDS-PAGE. After transfer of proteins to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA), the blots were probed with monoclonal antibody PHF-1 (1:500)[6] to P-Ser396/404 or tau-1 (1:30,000) to non-P-Ser198/199/202 of tau[7] and developed with HRP-labeled antibodies to mouse IgG (0.5 g/ml, Amersham Pharmacia) as secondary antibody. Images were processed with the aid of a computerized Kodak Imaging System.

Immunohistochemistry Rats were anesthetized and transcardially perfused with paraformaldehyde (4 % in phosphate-buffered saline, PBS). Hippocampus was immersion-fixed overnight and then cut to vibratome sections (40 µm). Endogenous peroxidase was blocked with 0.2 % H_2O_2 in absolute methanol for 30 min and non-specific binding sites were blocked with 10 % fetal calf serum (GIBCO, USA) in 0.1M PBS for 30 min at room temperature. Sections were then incubated overnight at 4°C with one of the following mouse monoclonal antibodies: Tau-1, PHF-1, 12E8 to P-Ser262 and M4 to P-Thr231 and P-Ser235. The immunoreactions were visualized by using biotinylated secondary antibody (1:200) (Amersham, Pharmacia) and the extravidin-peroxidase conjugate (1:200) (Sigma, Saint Louis, MO, USA). Diaminobenzidine tetrachloride (500ìg/ml) (Sigma, Saint Louis, MO, USA) was used as substrate.

Statistic analysis Student's t-test was used for statistical analysis.

Results

As shown in Figure 1A, there was no significant difference in performance between control and trained rats after the first training. Then, we divided these rats randomly into two groups. After 6 days training, the performance of the trained rat significantly improved, significantly short swim distances and latencies required to locate the submerged platform compared with control mice (Figure 1B, Figure 2). It is suggested that the training improved the ability of spatial learning and memory.

To detect the effect of training on tau phosphorylation, phosphorylation-dependent monoclonal antibody tau-1, PHF-1, M4 and 12E8 were used to detect the phosphorylation state of tau. Compared to the control (Figure 3A), Tau-1 reacted more strongly with the mossy fiber at stratum lucidum of the CA3 sector after training (Figure 3B). On the other hand, more enhanced staining with PHF-1 at the same fibers from the same sector was observed in control rats (Figure 3C and 3D). Immunoreactivity with M4 was observed in control rats (Figure 3E and 3F), but only weak background staining was seen in trained rats (not shown). No positive stain was detect with 12E8 in both control and trained rats (not shown). The results indicated that tau was dephosphorylated at Ser198/199/202 (Tau-1), Ser396/404 (PHF-1) and Thr231/ Ser 235 (M4) after training.

The dephosphorylation of tau was further confirmed by Western blot. Similar results as seen by immunocytochemistry were obtained with antibodies tau-1 (Figure 4A, C), PHF-1 (Figure 4B, D) and 12E8 (not shown). Different from immunoreactivity, no positive stain was observed with M4 by Western blot (not shown).

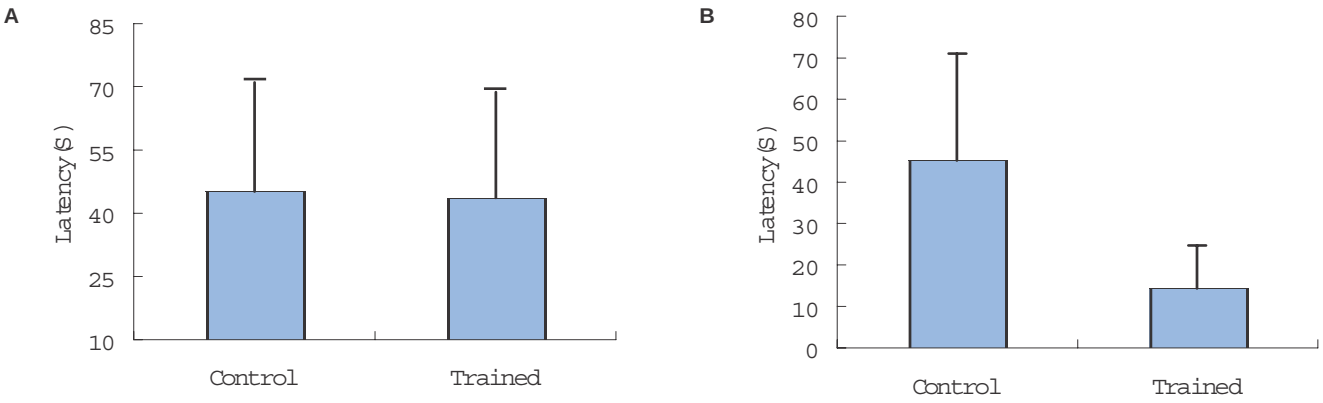

Figure 1.

Latency to find the hidden platform in Morris Water Maze from control and trained rats
A. Latency of control and trained rats in the first training **B.** Latency of control rats and trained rats in last training (*P < 0.01).

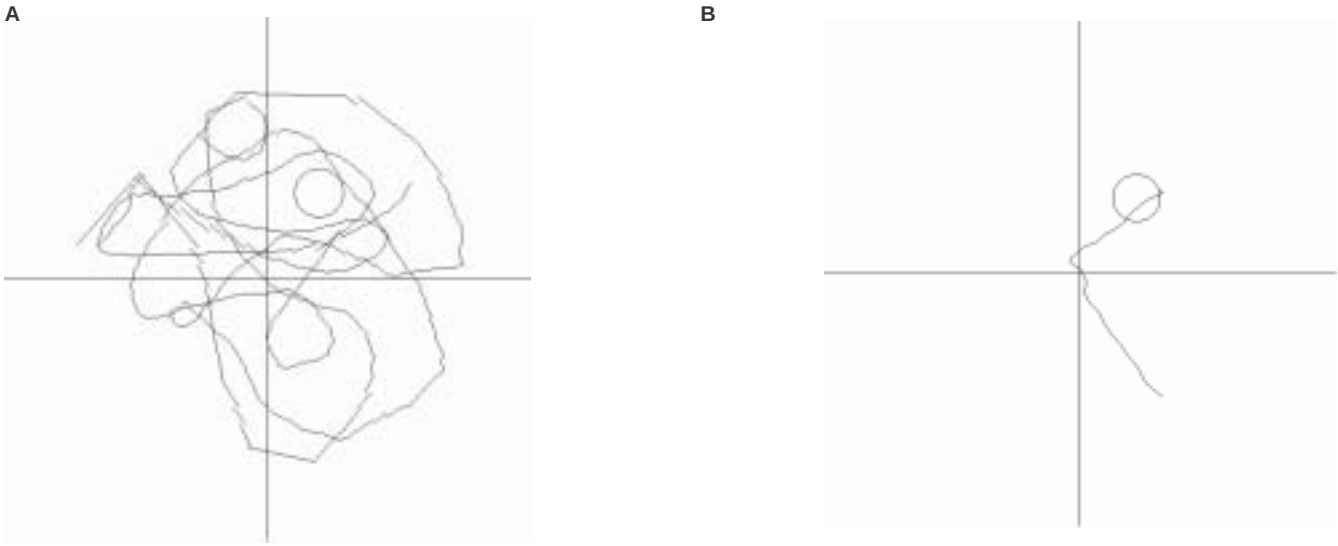

Figure 2.
Swimming path taken by control (A) and trained rats (B) from their starting position at southeast to the hidden platform at northeast in the Morris Water Maze.

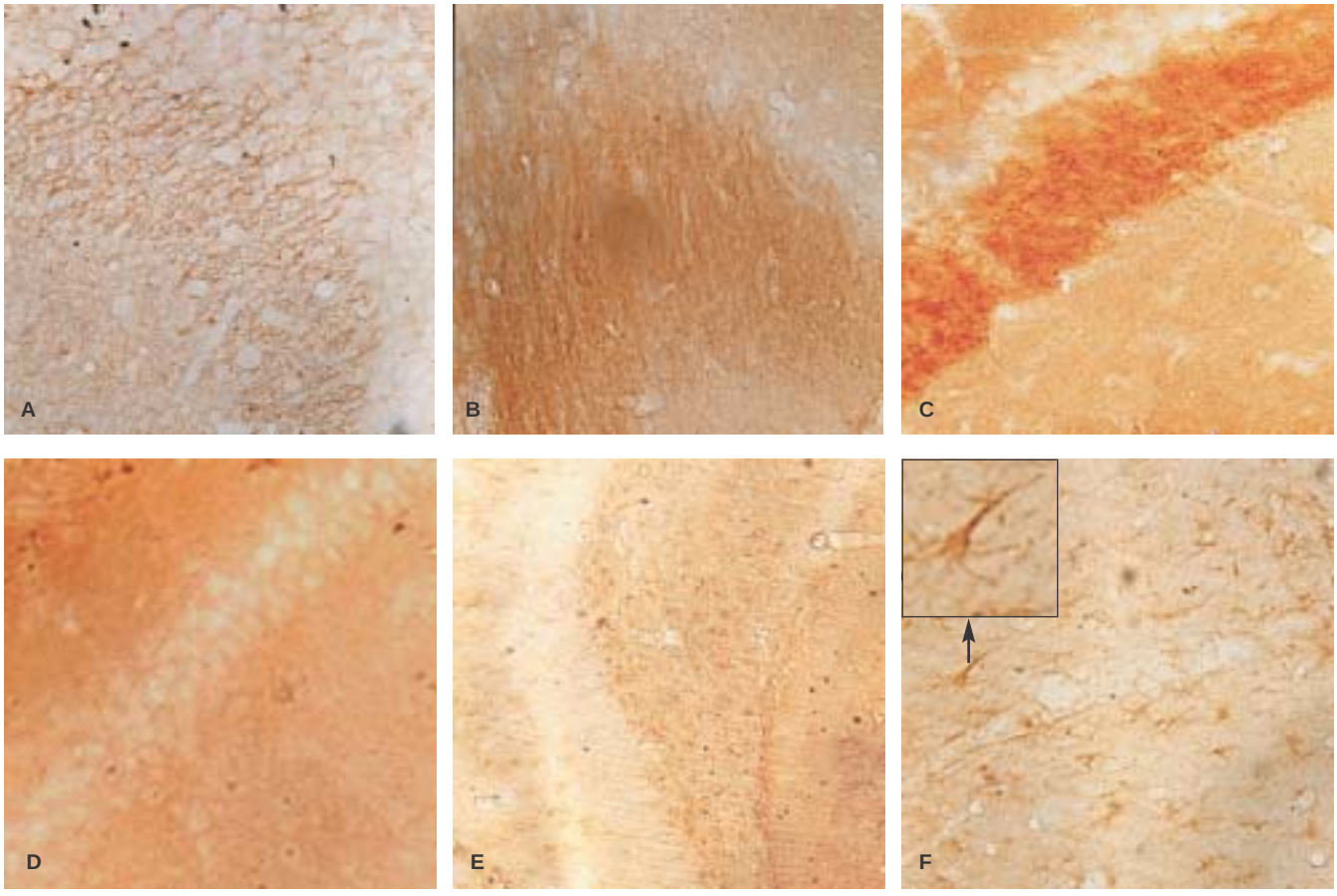

Figure 3.
Immunocytochemical staining of tau in control (A, C and E) and trained rats (B, D and F). The hippocampus sections were stained with Tau-1 (A and B), PHF-1 (C and D) and M4 (E and F). Amplification for panels A, B, C, D and F is 200, for E is 100. Insert in panel F is amplified for 400 times.

Discussion

Alzheimer's dementia is a major cause of disability and mortality in contemporary human populations. Memory impairment followed by more global cognitive deficits is its clinic feature. Since the deposition of protein aggregates or plaques and the formation of neurofibrillary tangles are typical neuropathological hallmarks of the disease, they were believed to be the reason for memory impairment in AD patients. Recently, many studies have shown that Aâ can induce spatial learning and memory deficit in animal models. However, the relationship between memory and tau phosphorylation is not reported.

It is known that phosphorylation of tau in rat is developmental regulated[8]. Our recent study also demonstrated that PHF-1 epitope was highly phosphorylated in fetal rats, dephosphorylated at one month old, then partially rephosphorylated when rats were grown to two to three months old. And heavier phosphorylation at the same epitope was seen when rats were raised to 24 months. Similar results were observed with Tau-1 antibody (manuscript in preparation). As hyperphosphorylation is one of the most recognized pathological processes towards neurofibrillary tangle formation, we studied in the present study the correlation of tau phosphorylation with learning and memory. We found for the first time that water maze training could decrease the phosphorylation of tau. Since water maze has been proved to be a selected apparatus to investigate spatial learning and memory in rodent models, the above finding indicated that the phosphorylation of tau is correlated with spatial learning and memory. The fact that low education and Down syndrome are major risk factors for Alzheimer disease[9] further support the speculation that tau phosphorylation is closely related to learning. The mechanism that training deceases phosphorylation of tau deserves further investigation. Some phosphatases and kinases may be involved in the processes[10-13].

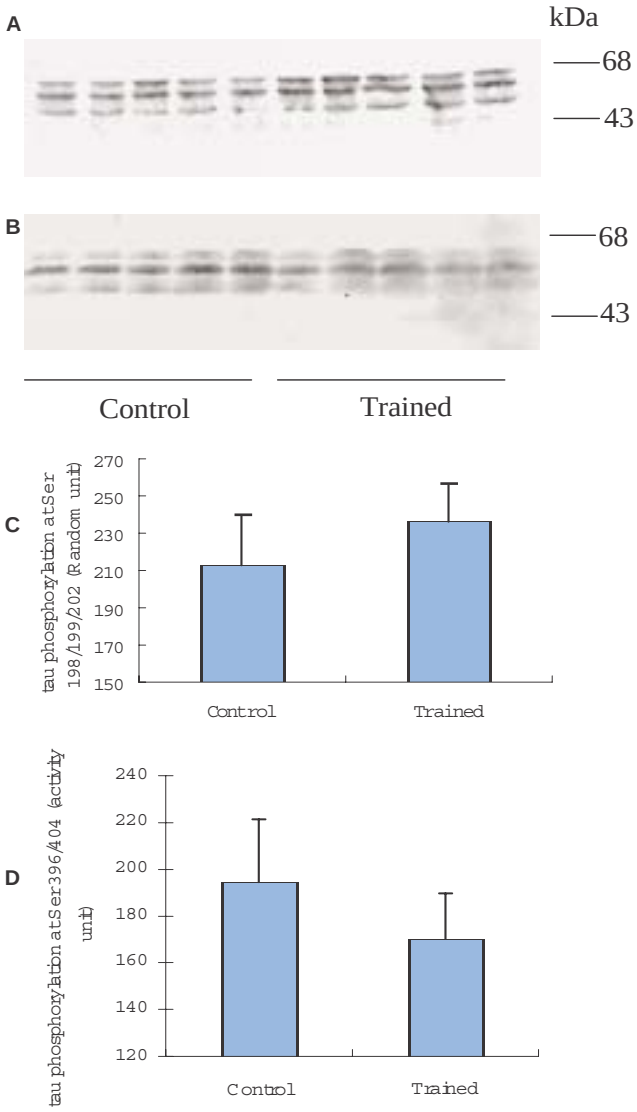

Figure 4.

Western blot of tau determined with Tau-1 (A) and PHF-1 (B). And the blots were scanned and quantitatively analyzed by Kodak Digital Science 1D system (C and D). *$P < 0.05$

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