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The Effect of Palmitic Acid on Spatial Learning and Extinction in Adult Male Rat

¹Zohreh Hosseinzadeh, ¹Ahmad Ali Moazedi and ²Rahim Chinipardaz

¹Department of Biology,

²Department of Statistics and Mathematics, Shahid Chamran University, Ahwaz, Iran

Abstract: The aim of the present study is for evaluating the effect of different doses of palmitic acid on spatial learning in T-maze. So, Wister adult male rats were used in five groups and considered 10 rats for each group. The first group as a control group was fed with ordinary diet. Four groups were fed with a diet containing palmitic acid (10%) for 1, 2, 3 and 4 weeks. then rats were trained for spatial learning task by using T-maze at subsequently 9 days based on standard method. The result showed that, spatial learning increased by diet containing palmitic acid 10% for 1, 2 and 3 weeks were significant ($p < 0.05$). However, dietary palmitic acid effect on learning because palmitic acid via palmitoylation modifies numerous important neuronal protein including GAP-43, NMDA receptor, AMPA receptor and these receptors are critical role in learning.

Key words: Palmitic acid, spatial learning, T-maze, rat

INTRODUCTION

The fatty acid composition of brain was measured to determine the relationship between the resulting range in brain fatty acid composition and performance on the working memory task in morris water maze (Wainwright *et al.*, 1998). The relation between a biological factor (fatty acids, FA) and a cognitive processing speed factor (temporal processing acuity, TPA) that are both suggested to relate to neuronal and cognitive functioning (Laasonen *et al.*, 2006).

n-3polyunsaturated fatty acids (linoleate and linolenate) and saturated fatty acids (palmitate, stearate) and monounsaturated fatty acids (oleate) were supplied in the diet to artificially reared rat pups over the period of the brain growth spurt and the onset of myelinogenesis (Edmond *et al.*, 1998).

The promising relationships between epilepsy and seizure on one hand and fatty acids on the other hand complex and not obvious (Mostosky *et al.*, 2004).

Few topic in nutrition cause as much controversy and concern and are as frequently misunderstood, as fat (Taubes, 2001). The reported cautionary warning from the medical profession has been to dramatically reduce the amount of fat one eats, so as to minimize the risks associated with cardiovascular diseases, diabetes and other chronic disorders (Kodas *et al.*, 2004). Fatty acids deficiency may impair normal neurological development (Yehuda *et al.*, 2006).

However deficiencies in fat intake are equally likely to contribute to health hazards, including increased risk of infection, dysregulation of cyclic and rhythmic

activity and impairment cognitive and sensory function (especially infants) (Ruthrich *et al.*, 1999).

A consensus has emerged from recent research that it is not so much amount of fat we eat as it is the balance of the different type of fats (Yehuda, 2003, 2002).

The type of dietary fat we consume affects the biology of each cell and determines how well it can perform its vital function and its ability to resist diseases (Ruthrich *et al.*, 1999). Indeed among the significant component of cell membranes are the phospholipids, which contain fatty acids. The type of fatty acids in the diet determines the type of fatty acids that is available to the composition of cell membranes. A phospholipids made from a saturated fat has a different structure and is less fluid, than incorporates an unsaturated and essential fatty acid (Yehuda, 2003; McCann and Ames, 2007). Based on different reports about the effects of saturated fatty acids spatially palmitic acid on nerve system and learning and memory and in past studies our laboratory showed that compare between groups fed dietary contain butter (10%) for 1, 2 and 3 weeks indicated enhanced learning performance that may be cause presence of saturated fatty acids in butter and also the most amount of saturated fatty acids in the butter is palmitic acid. So, in this study the effects of palmitic acid on spatial learning of rats by using T-maze task have been investigated.

MATERIALS AND METHODS

Subjects: This study was done in lob of learning and memory, Shahid Chamran university for 4 months in 2006.

So, 50 adult male Wistar rats weighing 275±25 g were used. The rats were housed in individual cages with free access to water and Pellets food. Their ages were 1.5-2 months at the training test.

Group treatment and diets: Palmitic acid (10 g) and standard food (90 g) were mixed then rats were fed freely all the time. Briefly, the diets contained the following: 5% soyabean protein isolate, 0.3% DL-methioninm, 32.7% corn starch, 25% sucrose, 2% cellulose powder, 5% mineral mixture, 1% vitamin mixture and 10% palmitic acid. Rats were categorized as 5 groups (N = 10) as follow:

- Group 1: Fed palmitic acid 10% for 1 week
- Group 2: Fed palmitic acid 10% for 2 weeks
- Group 3: Fed palmitic acid 10% for 3 weeks
- Group 4: Fed palmitic acid 10% for 4 weeks
- Group 5: As control group was fed ordinary food (Hoseinzadeh *et al.*, 2006)

Behavioral testing: Palmitic acid (C₁₆H₃₂O₂) were obtained from Merck chemical company. All rats were trained in T-maze during 9 days based on standard method (Annet *et al.*, 1989).

Three days before the behavioral test, rats were fasted for 23 h day⁻¹ to maintain 80% of normal body weight. Water was available ad libitum throughout all experiments (Cocco *et al.*, 2002).

Apparatus: A wooden T-maze with 15 cm high walls and start and goal boxes 15Å-20 cm was used. The 48 cm long stem led to two L-shaped arms. The first part of each arm was 39 cm long and the second part, which led to the goal box, 28 cm long. Guillotine doors separated the start box from the stem and the stem from the L-shaped arms.

Procedure: Preliminary training took place on days 1 to 3. On day 1 food pellets (4 mg) were left in the stem, arms and goal boxes with all the guillotine doors open. The rats were placed individually in the start box and left for 5 min to eat the pellets and explore the maze.

On days 2 and 3 each rat was confined to the start box for 10 sec and then allowed to find 4 pellets in either of the goal boxes, where it was confined for a further 40 sec.

On days 4 and 5, two pellets were available in both of the goal boxes on every trial. Each rat was confined to the start box for 10 sec before being allowed to choose one of the goal boxes, where it was confined for a further 20 s. Eleven consecutive trials were given per day and the choice of right or left goal box was recorded, as was the latency between leaving the start box and entering the chosen arm.

On days 6, 7 and 8 were considered for spatial learning and reversal. Only one of the goal boxes was now rewarded and the rats had to learn which was correct. For half the rats the reward was to be found in the right goal box and for the other half the left goal box was rewarded. Trials continued until the criterion of 5 consecutive correct responses had been achieved. The choice of arm and latency between leaving the start box and entering the chosen arm were recorded on every trial. Immediately after reaching criterion the contingencies were reversed so that the previously unreinforced goal box was now correct. Training continued until the new response had been learnt, again to a criterion of 5 consecutive correct responses. This procedure commenced on day 6 and was repeated on days 7 and 8. Each day the rat was always required to start by re-learning the spatial discrimination which had been correct last during the previous day's training.

On day 9 as a extinction, after a spatial learning had been completed to 5 consecutive Correct responses, an extinction stage were introduced. The food pellets were removed from the T-maze and goal box choices and start box to arm latencies were recorded over a further 10 trials.

Statistical analysis: For comparison between each dietary group and control were made using student t-test and for compare all groups, then data elevated by two way ANOVA. For comparison two by two dietary groups were assessed by post hot the tukey test.

RESULTS

The result showed that a decrease in trail to criterion simple learning between palmitic acid (10%) fed group for one week were significant (p<0.05) but not another groups. The number of trail to criterion on revers learning, in palmitic acid(10%) fed group (1, 2 weeks) decreased (p<0.01) (Fig. 1). But did not differ in number of error on simple learning compare with control group. A decrease in number of error on revers learning in palmitic acid(10%) fed group for 1 week were significant (p<0.001) but not about the other groups (2, 3 and 4 weeks) (Fig. 2). Mean latencies on simple learning in all of groups were not significant but mean latencies on revers learning and extinction trails in palmitic acid (10%) fed groups for 1, 2, 3 weeks were decreased (p<0.05, p<0.001) (Fig. 3 and 4). Generally, learning parameters in groups fed palmitic acid for 1, 2 and 3 weeks were significantly increased.

According these result and in comparison palmitic acid (10%) fed group for 1, 2, 3 and 4 weeks were assessed by tukey test, in conclusion that if usage of palmitic acid increases, spatial learning will decrease, so the low

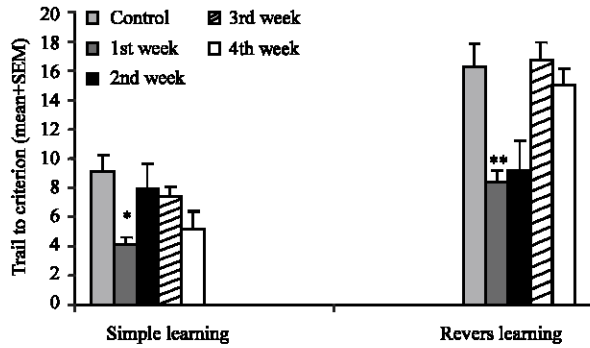


Fig. 1: Mean of trail to criterion on simple and revers learning in rat which were fed with a diet containing palmitic acid (10%) 1, 2, 3 and 4 week groups and control, $p < 0.05^*$, $p < 0.01^{**}$

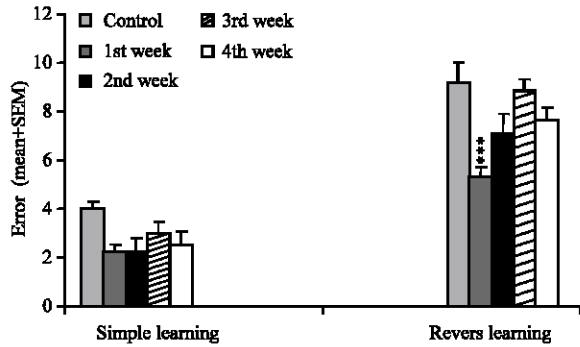


Fig. 2: Number of error on simple and revers learning in rat which were fed with a diet containing palmitic acid (10%) 1, 2, 3 and 4 weeks groups and control, $p < 0.001^{***}$

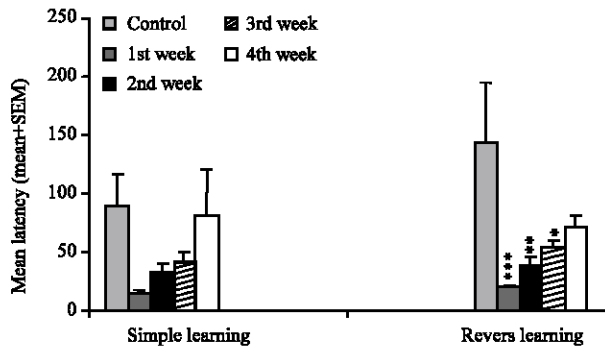


Fig. 3: Mean latencies on simple and revers learning in rat which were fed with a diet containing palmitic acid (10%) 1, 2, 3 and 4 weeks groups and control, $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$

dosage of palmitic acid has positive effect on spatial learning and high dosage of that probably has negative effect.

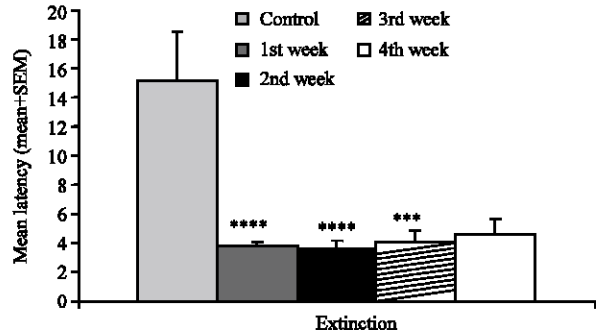


Fig. 4: Mean latencies on extinction in rat which were fed with a diet containing palmitic acid (10%) 1, 2, 3 and 4 weeks groups and control, $p < 0.001^{***}$, $p < 0.000^{****}$

DISCUSSION

The present results point to dietary of palmitic acid (10%) in 1, 2, 3 weeks were significantly increased in spatial learning.

In past studies results showed that the group were dosed lovstatin for 9 days after 2 weeks administration of butter (10%) enhanced learning performance compared to rats that dosed only with lovstatin and historical results studies exhibited that have not had obvious signs of cell injury in rats hippocampus. However increases learning performance that may be due to presence of saturated fatty acids in butter and also the most amount of saturated fatty acids in the butter is palmitic acid. Indeed the developing brain produces all required palmitic acid (Marbios *et al.*, 1992).

Recently investigation show that palmitic modification of neuronal proteins is critical. Protein palmitoylation represents a common lipid modification of neuronal proteins (El-Husseini Ael *et al.*, 2002a; Greaves and Chamberlain, 2007). This posttranslational change involves addition of the saturated 16 carbon palmitic lipid in a thioester linkage to specific cystein residues. Palmitoylation modifies numerous important neuronal protein GAP-43, G protein and etc. Importantly, palmitoylation occurs in reversible fashion which allows palmitoylation to dynamically regulation protein function and to participate in diverse aspects of neuronal signaling (Fukata *et al.*, 2004). Moreover, Palmitate modifies both peripheral and integral membrane proteins and its addition can be permanent or transient, which makes it unique among the lipid modifications of proteins. The presence of palmitate on a protein affects how the protein interacts with lipids and proteins in a membrane compartment and the reversibility of palmitoylation allows different modes of trafficking between membrane

compartments (Linder and Descheres, 2007). It seems palmitic acid via some mechanisms effects on nerve system and cognition function which here indicated them.

At first, Glutamate receptor channels mediate most of the fast excitatory synaptic transmission in the central nervous system. At excitatory synapses, ionotropic glutamate receptors such as α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kinate type and N-methyl-D-aspartate (NMDA) type are highly concentrated in the postsynaptic density (PSD) and these receptors are important in learning mechanism. It has recently been proposed that protein-protein interaction of some cytosolic proteins with the C-termini of glutamate receptor subunits are involved in anchoring/clustering receptors at the PSD and coupling the receptors to cytoplasmic signaling molecules (Yamazaki *et al.*, 2001). The postsynaptic density protein, PSD, represents a major palmitoylated protein in brain (Topinka and Brecht, 1998). This lipid modification is critical for PSD-95 clustering of AMPA receptors at excitatory synapses. Furthermore, the palmitoylation of PSD-95 is dynamically regulated by synaptic, such that cycling of palmitate on PSD-95 can contribute to aspects of synaptic plasticity and palmitate cycling on PSD-95 is augmented by calcium influx through NMDA receptors (El-Husseini Ael *et al.*, 2002b). According these study and present results dietary of palmitic acid may be by effects on NMDA receptor induced learning.

On the other hand, Changes in glutamate receptor number at the PSD provide a critical mechanism for rapidly altering synaptic strength (Malawian *et al.*, 2000). Interestingly, the two principle classes of ionotropic glutamate receptors are differentially anchored at the PSD. NMDA receptors are firmly tethered to the PSD, whereas the AMPA receptors are more loosely attached (Malawian *et al.*, 2000; Malenka and Nicoll, 1999). Dynamic cycling of AMPA receptors on and off the synaptic membrane occurs in an activity-dependent fashion and appears to synaptic plasticity (Beattie *et al.*, 2000) furthermore, the PDZ domains from the PSD-95 were first shown to bind the c-terminal tails of NMDA receptor typ-2 subunit and shaker-type k⁺channels (Kim *et al.*, 1995). However, the PSD-95 family of membrane-associated guanylate kinase (MAGUK) can also interact with AMPA receptors. Furthermore, the AMPA receptor trafficking protein, stargazing, associates with PDZ domains from several neuronal MAGUKS (Chen *et al.*, 2000) and MAGUKS proteins regulate synapse development and plasticity. In cultured hippocampal neurons, overexpression of PSD-95 accelerates development of excitatory synapses and selectively

enhances clustering of AMPA receptors (El-Husseini Ale *et al.*, 2000). On the other hand, targeted disruption of PSD-95 causes sever abnormalities of synaptic plasticity, such as long-term potentiation (LTP) enhanced and long-term depression(LTD) is eliminated. These abnormalities in synaptic plasticity presumably explain why PSD-95 mutant mice are impaired in spatial learning (Magaud *et al.*, 1998). It verifies these results about positive effect of palmitic acid dietary in 1, 2 and 3 weeks.

Also, Nerve Growth Factor (NGF) is critical for neuronal survival and for the establishment and maintenance of synaptic connection (Chao, 2003). NGF binds the tropomyosin-related kinase receptor A (TrkA) and P75 receptor with comparable affinities. Indeed Ncam1 is the best-characterized member of the calcium independent adhesion molecules (Bartsch, 2003).

It is present throughout the developing nervous system and its expression persist in the adult (Montag-Sallaz *et al.*, 2003). During brain development, Ncam1 is heavily polysialylated, while the developing brain has a high level of Ncam1, the adult brain has appreciable levels only in the areas that continually undergo structural reorganization and demonstrate synaptic plasticity, such as the olfactory bulb, the hypothalamus and hippocampus (Bukalo *et al.*, 2004). Ncam1 is transiently upregulated at the synapse during learning and memory (Sytnyk *et al.*, 2004). So, it may be dietary of palmitic acid via regulation this molecule effect on spatial learning because palmitic acid can cross from blood barrier brian.

Moreover, P75 receptor is required for both resting and NGF-induced Ncam1 expression. NGF-induced Ncam1 expression is dependent on the presence of an intact palmitoylation site within P75 receptor (Mirnics *et al.*, 2005). It may be palmitic acid by regulate this factors effects on learning function. The gamma 2 subunit of neuronal and recombinant GABA receptors is palmitoylation. In cultured hippocampal neurons, inhibitors of protein palmitoylation reduced the synaptic clustering of GABA receptors and steady-state cell surface receptor number (Ruthenberg *et al.*, 2004), so palmitoylation is critical for regulation GABA receptor. Interestingly an increased impulse flow in the septohippocampal GABAergic pathway facilitated enhanced cognition initiated by a muscarinic agonist (Li *et al.*, 2002).

GABAergic neurons sense the excitatory transmission and regulate synaptic strength by sending feed forward and/or feedback inhibitory inputs to the principal neurons. Regulation of synaptic efficacy by integrated excitatory and inhibitory transmission within specific neuronal network is thought to underlie memory encoding

and retrieval in hippocampus (Zhao *et al.*, 2004). Present of palmitic acid is necessary for regulation molecular mechanism that influence in learning and memory which prove these result about positive effect of dietary palmitic acid at 1, 2 and 3 weeks on spatial learning (Fig. 1-4).

On the other hands, Epidemiological studies suggest that high fat diets significantly increase the risk of Alzheimer's Disease (AD). In addition, the AD brain is characterized by high fatty acid content compared to that of healthy subjects. Nevertheless, the basic mechanism relating elevated fatty acids and the pathogenesis of AD remains unclear. The present study examines the role of fatty acids in causing hyperphosphorylation of the tau protein, one of the characteristic signatures of AD pathology. Hyperphosphorylation of tau disrupts the cell cytoskeleton and leads to neuronal degeneration (Patil *et al.*, 2005). Present result indicted spatial learning in a group fed palmitic acid for 4 week was not only increased but also it is decreased.

CONCLUSION

According these studies regulate many protein in nerve system by palmitoylation is very important in cognition process such as, learning and memory.

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