A Survey of the Effect of Omega-3 Fatty Acids on the Level of Passive Avoidance Learning in Adult Male Wistar Rats

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Abstract: The reduction in the level of learning and memory over time due to various factors, especially brain aging in humans has had a significant influence on the process of human life. On the other hand, the deficiency and/or the absence of mechanisms for the prevention and treatment of this disease have made it important to do a research on the disease. With regard to the importance of omega-3 fats and the role of omega-3 fish oil as one of the most important foods to improve memory and learning, empirical studies have examined this issue in the present research. For this purpose, adult male Wistar rats were randomly divided into 5 groups of 8, sample group and control group and experimental groups of 1, 2 and 3, respectively. The sample group was only given food and the control group was given only distilled water but the experimental groups were fed omega-3 fish oil for 4 weeks at doses of 2/0, 5/0 and 1 mg/kg in the form of gavage. The rats of group of five (5) entered into learning tests with the method of shuttle box for surveying passive avoidance behavior and the data were recorded. The data showed that the delay while crossing rats indicated a significant difference in the groups gavaged with the fish oil compared to the sample group and the control group. Generally, the results of this survey indicated that consumption of Omega 3 fish oil at a dose of 0/5 mg/kg is able to increase the amount of recalling the information.

Key words: Omega-3 Fish Oil • Memory • Passive Avoidance Learning • Rat • Shuttle Box

INTRODUCTION

Learning and memory are considered the highest functional levels of the central nervous system. Learning is a neurological phenomenon in which an organism alters its behavior through practice. While memory refers to the process of storing the acquired things [1].

Learning and memory involve large changes in the structure and function of the nervous system which are mainly limited to synapses involved in the guidance route of messages and sensory information on the nervous system. Structural changes include changes in the number of synapses and changes in the expansion of the postsynaptic membrane at the site of the contact. And physiological changes include changes in the ion conduction of presynaptic and postsynaptic membranes [2].

It has been observed that short-term memory is associated with the cortex and long term memory is associated with the limbic system. However, a special place for the storage of memory has not still been specified because with the removal of the different parts of the brain, memory does not generally disappear [3].

Researchers investigating the short-term memory in the animals under study make them show a particular behavior. Recalling this particular behavior after a period of time is a test for reminding that task (conditioned), that is, the long-term memory. It is proven that in long-term
memory long-lasting and structural changes are created in
the structure of the nervous system [4]. While learning
process and short-term memory have a key role in the
physiological changes, especially in neurotransmitter
release [5].

In long-term strengthening, more structural changes
are created in the creation sector of synapses and
receptors which were observed in the siphon neurons of
the Plazya snail’s gill for the first time [6].

Following this report, it was found that this
phenomenon also occurred in neurons of the spinal gray
[7], in grainy neurons with jagged ridges in the temporal
lobe [8] and in the neocortex. With regard to the
importance of omega 3 fish oil in improving memory and
learning and also its low side effects, this study examines
the effect of this oil as a memory booster [9]. The main
components of this oil like eicosapentaenoic acid (EPA),
docosahexenoic acid (DHA) can boost the level of
acetylcholine in the brain as an important factor in
reinforcing memory. They can also have an effective role
in increasing the level of the memory by creating Long
Term Potential (LTP) through imitating glutamate
receptors [10] and also inhibiting lipid peroxidation.

Omega-3 Fatty Acids: These fatty acids contain
hydrocarbon chains which methyl factor is located at one
end and carboxyl group is located at the other end. These
molecules play different roles. Moreover, they are the
major source of energy. They also enter into the
membrane structure and set the biophysical properties
and are involved in the function of receptors.

The other importance of these molecules is that they
play the role of message transmitting molecules to cells
and also regulate the basis of some genes [11]. Omega - 3
and Omega-6 fatty acids are metabolized by the same
enzymes and thus they compete with each other. Since in today’s diet of people they receive much more
LA (5 to 20 times more) than ALA, LA generally enters
this route and produces AA. Converting DHA, EPA ALA
to EPA in the body is low and only 8% of EPA is
produced through this way and thus the diet is also the
major source of DHA, EPA [12]. Unsaturated fatty acids
with a few double bonds are abundant in the brain and
constitute 20% of the total brain mass. DHA and AA
constitute 90% of unsaturated fatty acids in the brain.
There is a small amount of EPA in the brain tissue
which has an effective role in transmitting cell messages.
In lymphocytes and T cells that are part of the immune
system, depending on the amount received in the diet
EPA, DHA and AA competitively enter in the cell
membrane. Affected by Oxidizing enzymes, they produce
significant amounts of DHA and EPA acids. These acids
also affect the immune system activity and are involved in
the production of inflammatory cytokines. They also
regulate the transmission of serotonergic, dopaminergic
and glutaminergic messages in the brain tissue and they
are involved in various processes, such as depression
and sleep [13].

MATERIALS AND METHODS

Grouping of Animals:
Control Group: Without being gavage, the rats (Wistar,
Male, 80-90 days) of this group were trained in the
passive avoidance method (n = 8).

Sample Group: The rats of this group were given a
solution of distilled water with a daily rate of 1 cc by
gavage and then training and testing stages were carried
out on them (n = 8).

Experimental Groups: The rats of this group were treated
with omega-3 fish oil with the oral gavage style eating for
28 days and then training and testing procedures were
applied on them.

Experimental Groups Are as Follows:

- Group 1 receiving omega-3 fish oil with a dose of
  2mg/kg (n=8).
- Group 2 receiving omega-3 fish oil with a dose of
  5mg/kg (n=8).
- Group 3 receiving omega-3 fish oil with a dose of
  1mg/kg (n=8).

Materials and Equipment: Materials and equipment used
in this survey were classified as follow:

Shuttle Box: This equipment was used to train rats by the
passive avoidance learning method. The mentioned
equipment was made by Noortab Company. The kit
consists of two parts; a training box and a controller
section. The training box has actual dimensions of
20.21.63 cm and is made of clear Plexiglas sheets. The
training box has two equal smaller chambers which are
separated from each other by the lateral walls.
For darkening one of the chambers, all its exterior surfaces were painted black. A rectangular opening with the dimensions of 9.7 cm is considered for letting the animal pass to the lateral wall. The valve is opened and closed by the guillotine door. The chambers’ floors are of metal rods with a diameter of 2/5mm and with a distance of one centimeter from each other. The bars in the dark chamber are connected to every other positive and negative poles. On the internal end of the wall in the light chamber with a little away from the ceiling of the chamber, an AA lamp is installed. The controller unit is equipped with screws regulating the period of time the lamp is on, the period of time to establish the shock and the level of shock in terms of Frequency, Voltage and Ampere.

Materials and Equipment Needed to Do the Job:

- Insulin Syringes 1 and 0/5 and 0/2 ml manufactured by Supa factory
- Saline solution
- Distilled water solution
- Sensitive scale with an accuracy of 10g for weighing rats made in Nikita factory
- Cotton and alcohol for sterilization
- Savlon and Betadine disinfectant solutions to wash and sterilize hands and the environment after finishing the job.
- Capsules containing omega - 3 fish oil which are the product of ISN company containing DHA, EPA
- A refrigerator to keep materials
- A special syringe for gavage
- Disposable and thick gloves.
- Appropriate amounts of special feed for animals provided from the institute for animal feed
- Sawdust in order to use in shelves that were provided from Shiraz carpenters.

Methods

Gavage Method: In this method with the help of syringe we first pulled oils from the capsule and placed it in a special container and then transferred it to insulin syringes at considered doses and finally attached it to the special needle of gavage syringe and then to considered groups as seen in the figure, by holding two sides of the rat and putting the tail of the rat in the right hand with the help of left hand we injected the oils into the oral cavity of the rats. In this case we were careful with oils not to enter into the trachea instead of entering into the esophagus because in this case it leads to the death of the rats. At the time of injecting oil food into the rats they were in complete consciousness.

Passive Avoidance Learning Method: Passive avoidance learning involves three steps of habit, training and remembering. The basis of this kind of learning is establishing a relation between the two conditioned (darkness) and unconditional stimuli (electric shock).

Habit Session: First, in order to habituate the animal, it is placed in the light place of the device and after 5 seconds we slowly open the guillotine door. When the animal enters the dark part we close the guillotine door and after about Thirty seconds we re-open it so that it can go to the light chamber and if the animal does not go to the light chamber, we guide it with the hand and then let the animal out of the equipment. We repeat this step after 30 minutes. After 100 seconds at each of the habit meetings, if the animal does not enter the dark part we consider it inappropriate learner and it will be excluded from further testing.

Training Session: Thirty minutes after the second habit meeting, we saturate the animal feet to physiological serum to drive easily an electric shock to its feet. 30 seconds later we open the Guillotine door. When the animal enters the dark part, we gently close the guillotine door and apply a mild electric shock at the rate of 2/5 mA for 5 seconds and with 50 Hz to the animal's feet and by opening guillotine door we wait until the animal comes out of the dark chamber, then put it in a cage. Again after a few minutes we put the animal in the light chamber and after 30 seconds open the guillotine door. If in two minutes the animal does not enter the dark compartment, we terminate the training session, otherwise, we repeat the shock giving process. It is noteworthy that habit training stages were carried out between 8 a.m. to 12 at noon.

Memory Retention Meeting: To determine the amount of memory retention, 24 hours after the training session, the animals are placed in the light part of the device and after 30 seconds slowly open the Guillotine door and we record the time it takes the animal to enter the dark part of the device as the delay in arrival at the dark part or (STL). The maximum time which we consider to enter the dark compartment is 600 seconds.
- Results about the changes of body weight
- Results about changes in memory and learning (STL)

**T-test with Paired Sample (Two Dependent Samples):**

Paired Sample T-test is used to analyze those tests in which each agent is observed in two different situations, that is, before and after stages. Accepting $H_0$ in paired data plan indicates that statistically there is no difference between the values of the means in the two paired samples. And in contrast to this hypothesis which says that there is a significant difference between the means, there is $H_1$. It should be noted that paired sample t-test is the same as single-sample t-test which performs zero-mean test and its hypotheses are as follow:

- $H_0 : \mu_1 - \mu_2 = 0$  
- $H_1 : \mu_1 - \mu_2 \neq 0$

**Analysis of Variance:** Analysis of variance examines the relationship between a dependent variable and an independent variable (qualitative) and it gives us the opportunity to examine the relationship among more than two statistics community. In the analysis of variance (ANOVA), the dependent variable is a quantitative variable and factors which are qualitative variables or grouped variables have two forms as follow:

- Factors among the subjects or between the groups
- Factors within the subjects or within the groups which are also called the underlying errors. In the one-way analysis of variance (ANOVA), there are only two variables, i.e. an operating variable and a dependent variable (the factor divides the observations into several groups or levels) and this is the reason why it is called one-way analysis of variance (ANOVA). In this analysis of variance (ANOVA), the presence of a significant difference between groups of variables is surveyed by F-test and its (formula/hypothesis/practice) are as follows:

  - $H_0 : \mu_1 = \mu_2 = \ldots = \mu_k$
  - $H_1 : \mu_i \neq \mu_j$

**Findings of the Research:** Reviewing the results obtained from the effect of fish oil on body weight:

- Reviewing the results obtained from the comparison of the control group and the sample group before the experiment by paired-sample T-test

Statistical results obtained from the comparison of the sample group and the control group by paired-sample T-test show that there is no significant difference between the groups. Also, the comparison of the mean value of sample group with the control group shows that there is no significant difference between the groups. Here the control group is selected for comparing it with the experimental groups.

- Reviewing the results obtained from the comparison of the control group and the experimental groups before the experiment by analysis of variance (ANOVA) test.

The statistical results obtained from the comparison of the control group with the experimental groups by analysis of variance (ANOVA) test show that there is a significant difference between the studied groups.

- Reviewing the results obtained from the comparison of the control group and the sample group after the experiment by paired-sample T-test:

**T-Test:** Statistical results obtained from comparing the control group and the sample group by paired sample T-test show that there is no significant difference between groups at the 0.05 level.

- Reviewing the results obtained from the comparison of the weight of samples of the control group and experimental groups by ANOVA test after experiment

The statistical results obtained from comparing the weight of the control group with the experimental groups by ANOVA test show that the weight of the samples between the studied groups were not significant at 0.05 level.

- Reviewing the results obtained from the comparison of the means of body weight before and after testing

The statistical results obtained from the effect of fish oil on the weight of subjects (rats) before and after the experiment (end of the 30th day) in different groups show them in terms of gram. The table is set on the basis of statistics such as: mean ± standard error (Mean ± SE).

- The results obtained from the effect of fish oil on the memory and learning of the samples (STL)
Table 1: Comparison of the means of body weight before and after testing

<table>
<thead>
<tr>
<th>After of test (Mean±SE)</th>
<th>Before of test (Mean±SE)</th>
<th>Number</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>186.25±4.84</td>
<td>119.75±4.61</td>
<td>8</td>
<td>Control</td>
</tr>
<tr>
<td>179±5.14</td>
<td>118.875±3.94</td>
<td>8</td>
<td>Saline</td>
</tr>
<tr>
<td>187.75±3.81</td>
<td>152.625±2.99</td>
<td>8</td>
<td>Doze [0.2]</td>
</tr>
<tr>
<td>188.125±4.6</td>
<td>159.5±2.8</td>
<td>8</td>
<td>Doze [0.5]</td>
</tr>
<tr>
<td>186.25±3.75</td>
<td>156.75±3.01</td>
<td>8</td>
<td>Doze [1]</td>
</tr>
</tbody>
</table>

The results obtained from the comparison of the control group and the sample group by paired sample T-test imply the acceptance of the null hypothesis and the selection of the control group for the comparison with the experimental groups at the significance level of $P > 0.05$.

In the comparison of the mean in the control group with the mean of the sample group, there is no significant difference between the groups and the control group is selected for the comparison with the experimental groups.

- The results obtained from the comparison of the mean (SLT) between the control group and the experimental groups by ANOVA test.

The statistical results obtained from the comparison of the control group with the experimental groups show that in the comparison performed by ANOVA test between the control group and the experimental groups, the significance level is $P < 0.01$ with the minimum, average and maximum doses of $(0, 1/5, 2)$.

The comparison of the mean of the control group with the experimental groups in relation to the memory and learning of the samples (STL) show that in the comparison performed by ANOVA test between the control group and the experimental groups, the significance level is $P < 0.01$ with the minimum, average and maximum doses of $(0, 1/5, 2)$.

**DISCUSSION AND CONCLUSIONS**

In this study, the effects of omega-3 fish oil on learning and passive memory were assessed and the results showed that its application for adult male Wistar rats enhances learning and memory in the passive avoidance learning test. This reasoning is obtained on the basis of the increase in delay time for first entering the dark room (STL), of the increase in the total time spent in the light chamber (CTL) and of the reduction in the total time spent in the dark room (TDC) in the experimental group compared to the control group.

Studies have shown that exposure to stress increases the activity of the HPA axis, resulting in the rise of blood glucocorticoids [14] and the high values of these hormones have adverse effects on the brain and in particular on hippocampus. It was found that the hippocampus has the highest number of glucocorticoids receptors than other regions [15] and it has been proved that the hippocampus is associated with cognitive functions such as learning [15]. The increase in glucocorticoids leads to damage to hippocampal neurons and some hormone receptors in the hippocampus [16]. Stress causes damaging effects related to hippocampus. This Learning degradation is due to structural changes in the synaptic terminals and to the concavity of dendrites in the hippocampus. Chronic stress causes damage to dendrites of CA1 region and has an effect on the HPA axis and releases and increases glucocorticoids which in turn can cause damage to spatial memory space. Changes in NMDA receptor also occur during chronic stress [17]. The release of amino acids in stress stimulates CA1 hippocampal atrophy. Previous studies have shown that stress causes high doses of ACTH [17]. As mentioned, in many cases the activation of NMDA receptors and the flow of extracellular calcium ions through these receptors into the post-synaptic terminal is the key element for the induction of long-term strengthening [17]. Parallel to these researches, many scholars have examined the role of these receptors in learning phenomena such that the results clearly state that the competitive antagonist of NMDA reduces the function of rats in water maze (spatial learning) [18].

By comparing this research with our research it can be concluded that the presence of Omega-3 in foods can increase neurons reduced during the disease and thus results in the increase of memory and learning.

T-sen and his colleagues generated a group of rats which their NMDA1 channels in the hippocampal CA1 region were destroyed. These rats showed impairment in spatial learning [19]. The importance of CAMKII enzyme is in establishing the relationship between increased calcium within the postsynaptic terminal and the synaptic plasticity. Researches have shown that deletion of this enzyme leads to impairment in spatial learning and also to synaptic plasticity [20]. According to this experiment and by comparing it with our study we can conclude that omega-3 with impact on glutamate receptors increases the reconstruction of these channels and facilitates ion transfer and increases hippocampal function and improves spatial learning. Mayanov and his colleagues (2004) stated that the lack of omega-3 reduces the two subunits of NMDA named NR2, NR1 and also the rate of...
CAMKII and its activity [21]. Glutamate neurotransmitter is a stimulating dominant in the mammalian brains and rapid transfer of messages is made possible with the help of ionotropic glutamate. The absence of these receptors cause a dysfunction in Glutamate, because both kynonotropic (iGluR) type receptors and metabotropic (mGluR) type receptors are differently accumulated in pre-synaptic and post-synaptic parts of neurons to participate in the transmission of nerve messages and to process the signals i.e. the actions that will lead to learning and to the creation of memory. In some studies, it has been found that metabotropic glutamate receptors are vitally involved in the synaptic plasticity in various regions of the brain and it seems that they have a fundamental role at some stages in learning and memory [22]. Considering this, it seems that omega-3 fatty acids help learning and memory by involving in maintaining glutamate receptors [23]. In surveying cortisol and synaptic dysfunctions, Kyraly and colleagues stated that high levels of cortisol can cause cognitive and psychological disorders [2], hippocampus may play a key role in memory and learning, thus damage to the hippocampus can lead to disruption in its settings [17]. Previous studies have shown that glucocorticoids increase the influx of calcium into neurons and thereby are able to increase synaptic plasticity. Also, due to increased density of blood glucocorticoids, an excessive increase of calcium in the cells can have disruptive influences on a series of behaviors, such as memory retrieval [24]. By comparing this research with that of ours it is concluded that since glucocorticoids increase calcium entry into neurons, thus they increase the ion current and increase the activity of neurons which they themselves enhance memory and learning performance.

Other possible mechanisms increasing passive avoidance memory and learning by omega-3 fish oil which can be referred to are the effects of this oil on neurotransmitters such as serotonin, glutamate and GABA and as well as the increase of some receptors in the hippocampus such as NMDA receptors and glucocorticoids receptors and the increase in brain growth factor. Thus, it can be concluded that omega-3 fish oil increases the function of brain neurotransmitters such as glutamate, GABA, serotonin, norepinephrine and epinephrine, thus, it increases the brain function and it affects memory and learning.

Memory formation is a complex process that needs numerous pre-synaptic and post-synaptic changes to occur. Several studies have been conducted to determine the molecular mechanisms involved in synaptic changes during memory formation.

Many proteins such as transcription factors have been identified [2]. Hippocampus is part of the limbic system, which is essential for composing various types of learning and memory in rats and other mammals. In humans, damage to the hippocampus causes deficits in learning people about places and so on [16]. Cognitive functions in animals are commonly evaluated by shuttle box and Morris water maze which are used to assess spatial learning [25]. Some researchers have evaluated the role of the hippocampus in relation to spatial memory by using destruction and damage method [17]. Studies have shown that damage to the hippocampus, particularly the damage to CA1 region causes an impairment in creating memory of the events, places and incidents. In rats, the memory for registering locations which is usually checked through Morris water maze and shuttle box, creating physical damages in the hippocampus causes a dysfunction in this kind of memory [25]. The study of different regions of the hippocampus shows that dentate gyrus and CA1 regions are more involved in spatial learning. Several findings suggest an important role for the activity of cholinergic system on septohippocampal pathway in learning and memory functions [24]. With regard to the materials mentioned above and their comparison with the recent research it can be said that fatty acids probably play an essential role in better functioning of these areas and these brain regions are also the major center of learning and memory in the brain and any disturbance in the function of these areas reduces memory and learning. The presence of protein kinases may be required for the survival of synaptic changes [1]. Many studies indicate the importance of protein kinases in learning and memory [26,27].

Some genetic and pharmacological studies focus on the important role of the signaling pathway of protein kinase A and CAMP in the synaptic plasticity associated with learning and memory [28].

There are hypotheses regarding the possible role of protein kinase A in the regulation of synaptic plasticity in pre-and post-synaptic sites [28].

Also, Camp-oka pathway is essential for the stability and the transformation of short term memory to long-term by increasing the synthesis of proteins involved in long-term strengthening (LTP) [22]. Protein kinase A which is activated by protein G, not only has a direct effect on channels and zhycols of the nerve terminal but also affects the cell’s genome. This effect is necessary to transfer the kinases into the nucleus. It seems that protein kinase A creates a chain of events inside the cell nucleus which produce proteins that can lead to long-term increase in the rate of neurotransmitters that are released.
in dysnaps by increasing the rate of PKA that are present on the cells. Thus, potassium channels remain closed for longer time and the potential action is prolonged. These proteins also increase the formation of synaptic connections [7]. It seems that transcription factors (CREB), which are phosphorylated by PKA in the nucleus of neurons, increase the basis of VACHT and CHAT enzymes which are the main markers of cholinergic system. Consequently, the levels of these proteins in the nerve cells increase [29-32]. It is expected that a receptor, as a PKA inhibitor, prevents the activation of CREB and the synthesis of VACHT, CHAT as a result.

By comparing this case with the present study, it is concluded that the lack of proteins causes a reduction in neurotransmitters function and this in turn causes a reduction in the potential action in the brain's neurons which also indirectly plays a role in memory and learning.

The synaptic function of the brain changes as a result of aging and senility and thus the activity of the brain neurons decreases and the brain function is reduced in general. Studies have shown that the reduction of fatty acids such as DHA that is due to the accumulation of byproducts from Lipids which is associated with the reduction of Omega 3 fatty acids accompanied by functional changes in the morphology of neurons including swollen astrocytes, deformation of the nucleuses of the neurons, decrease in the release of acetylcholine and by decrease in fluidity of cell membrane [30].

With regard to this point and its relevance to our study, it can be concluded that with the consumption of Omega-3 fish oil, you can prevent functional changes in the brain cells in old ages and prevent outbreak of side disorders such as aging manic and memory loss.

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