BAŞKENT UNIVERSITY

HEALTH SCIENCE INSTITUTE

PHYSIOLOGY DEPARTMENT

PHYSIOLOGY MASTER'S PROGRAM

THE EFFECT OF A HIGH DOSE OF VITAMIN D ON SPATIAL LEARNING AND MEMORY IN RATS

BY

TAHA HUSSEIN ALI ELSHAHOUBI

MASTER'S THESIS

ANKARA- 2021

BAŞKENT UNIVERSITY
HEALTH SCIENCE INSTITUTE
PHYSIOLOGY DEPARTMENT
PHYSIOLOGY MASTER'S PROGRAM

THE EFFECT OF A HIGH DOSE OF VITAMIN D ON SPATIAL LEARNING AND MEMORY IN RATS

BY

TAHA HUSSEIN ALI ELSHAHOUBI

MASTER'S THESIS

THESIS SUPERVISOR

PROF. DR. NAZAN DOLU

ANKARA- 2021

BAŞKENT UNIVERSITY

INSTITUTE OF HEATH SCIENCE

This study, which was prepared by Taha Hussein Ali Elshahoubi within the framework of the Department of Physiology Master's Program, was accepted as the Master's Thesis by the following jury.

Thesis Defense Date: … / … / …..…..

Title of the Thesis: The Effect of a High Dose of Vitamin D on Spatial Learning and Memory in Rats

Thesis Jury Members (Title, Name - Surname, Institution) Signature

……..…… ..……...

……..…… ..……...

……..…… ..……...

……..…… ..……...

…… …… If Required

……..... …… If Required

APPROVAL

……..

…… Institute

Director Date: … / … / …..…..

BAŞKENT UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
MASTER'S THESIS STUDY ORIGINALITY REPORT

Date: 18 / June / 2021

Student Name Surname: Taha Hussein Ali Elshahoubi

Student Number: 21910605

Department: Physiology

Program: Master thesis

Supervisor's: Prof. Dr. Nazan Dolu

Title of Thesis: The Effect of A High Dose of Vitamin D on Spatial Learning and Memory in Rats

According to the originality report, which was taken by the plagiarism detection program named Turnitin by the personal / thesis supervisor on 17/June/ 2021on the 40-page part of my Master's thesis study mentioned above, which consists of Introduction, Main Sections and Conclusion Section, the similarity rate of my thesis is 13 %. Applied filters:

1. Excluding bibliography
2. Excluding quotations
3. Excluding text parts that contain less than five (5) words of overlap

I have examined the Başkent University Institute Thesis Study Originality Report Obtaining and Using Procedures and Principles. I hereby declare that my thesis work does not include any plagiarism, that I accept all kinds of legal responsibilities that may arise in case the contrary is determined and that the information given above is correct.

Student Signature

APPROVAL

Date: 18 / June / 2021

Prof. Dr. Nazan Dolu

ACKNOWLEDGEMENT

To everyone helping me in preparing my thesis especially Prof. Dr. Nazan Dolu for helping me and pushing me towards success and progress in this thesis.

Great thanks for my big and small family

ABSTRACT

TAHA HUSSEIN ALI ELSHAHOUBI, THE EFFECT OF A HIGH DOSE OF VITAMIN D ON SPATIAL LEARNING AND MEMORY IN RATS, BASKENT UNIVERSITY, INSTITUTE OF HEATH SCIENCE, PHYSIOLOGY THESIS MASTER'S PROGRAM, 2021

"Vitamin D" is a prohormone steroid and has a place with the fat-soluble vitamins. It's liable for endocrine, paracrine and autocrine functions. Vitamin D is likewise basic for calcium absorption, bone mineralization, calcium and phosphorus homeostasis, nerve conduction, hormonal release, and neuromuscular function, acts as a natural antioxidant. Its impact on learning has not been widely reported. In the literature, there are several of discrepancies among age groups when it comes to this condition. This study aimed to see how vitamin D supplementation affected the cognitive performance of young male rats in the Morris water maze. Serving this purpose, 36 young male Wistar rats aged 8 weeks old age was divided into three groups with the control group (oral gavage normal saline), low dose vitamin D group (400 IU/ day) and high dose vit D group (1000 IU/ day. Rats were tested for their capacity to memorize the location of a platform after 8 weeks of daily supplementation in two phases: acquisition (next 3 days, fixed platform location), and retention (next 3 days, variable platform location) (forth day, removed platform). There were four trials per day (interval between trials 20-25 minutes). In spatial learning and working memory, the time spent finding the platform and duration of time spent in the quarter area of the maze including the platform were compared statistically in a number indicating the percentage of total time. There were no significant inter-group differences ($p>0,05$). From the first to the third day of training, all groups of animals improved their learning performance while decreasing the time spent searching for the platform ($p<0,05$). In this study, it was shown that 8 weeks of 400 and 1000 IU/day vit D application did not have any effect on learning to locate, but it did not cause impairment in the learning process either.

Keywords: Vitamin D. Learning. Memory. Morris water maze (MWM)

This work was supported by Baskent University Research Council with DA20/18 research number.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBRIVATIONS

AAP	American Academy of Pediatric
ACTH	Adrenocorticotropic Hormone
ADH	Antidiuretic Hormones
BG	Basal Ganglia
BM	Barnes Maze test
CNS	Central Nervous System
CR	Conditioned Response
DLPF	Dorsolateral Prefrontal Cortex
FMRI	Functional Magnetic Resonance Imaging
FPI	Four Plate Test
HDVD	High Dose Vitamin D
ITC	Inferior Temporal Cortex
IU	International Unit
LDVD	Low Dose Vitamin D
MTL	Medical Temporal Lobe
MWM	Morris Water Maze
PFC	Prefrontal Cortex
PMC	Premotor Cortex
SD rodent	South Dakota rodents
UR	Unconditioned Response
US	Unconditioned Stimulus
UV	Ultraviolet

1. INTRODUCTION

Vitamin D2 was isolated in 1932 by Askew et al. Windaus and Linsert had discovered that vitamin D1 was an artifact of an adduct between vitamin D2 and lumisterol. Windaus et al. discovered 7-dehydrocholesterol in 1935, and Windaus and Bock found vitamin D3 in 1937. Vitamin D3 is the form that occurs in the skin from 7-dehydrocholesterol with sunlight (Figure 1.1) [{1}].

Vitamin D is a prohormone steroid that is in the same category as fat-soluble vitamins. Endocrine, paracrine, and autocrine functions are all performed by it [{2}]. Vitamin D is also essential for calcium absorption, bone mineralization, calcium and phosphorus homeostasis. In addition, it plays a role in the hormonal balance nerve conduction, release, and neuromuscular function [{3}].

Because sunshine encourages adequate vitamin D synthesis in the skin, no food supplementation is required under normal conditions of sunshine exposure. Inadequacy can cause bone distortion (rickets) in children and bone weakness (osteomalacia) in adults [{4]].

Fish (not greasy fish), egg yolk, and offal, such as the liver, are excellent sources of vitamin D (3). In many countries, dietary vitamin D intake is poor, especially because dietary sources are limited. Supplement use is significant and, by all accounts, particularly where dietary sources are limited [{5}].

Vitamin D deficiency is common in every society, especially in the elderly. If left untreated, it can cause osteopenia, osteoporosis, and fatal falls. Vitamin D measurements should be made in high-risk patients, especially in patients with minor trauma fractures, and the change in blood 25-hydroxyvitamin D concentration should be checked 3-4 months after starting supportive therapy. For women the age of ≤ 50 years and males the age of ≤ 70 years, all patients should take calcium intake of nearly 1,000 mg. And 1,300 mg for women > 50 and men > 70 [{6}].

According to The American Academy of Pediatrics (AAP) issued a recommendation in 2003, all children older than two months can take 200 IU of vitamin D daily.

Infants who received 100 or 200 IU of vitamin D daily were more averse to create rickets [{7}].

Vitamin D synthesis begins in the skin. Irradiation of human skin with UV B (280–320 nm) pre-Vit D3 to Vit D3 initiates the photochemical conversion of 7dehydrocholesterol to Vit D3. Vitamin D3 two hydroxylation steps in two organs, the liver and kidney, are required for its activation. The completed product, 1α,25-dihydroxyvitamin D3 (calcitriol) is a hormonally active vitamin D3 (calcitriol) [{8}].

Vitamin D is considered potentially harmful in humans if taken above 100 ng/ml (250 nmol/l). Signs of toxicity are observed at high blood levels resulting from excess intake. Taking too much vitamin D can cause excessive calcium absorption, leading to dangerous symptoms. High doses of vitamin D therapy can cause nausea, vomiting and loss of appetite. High vitamin D doses that lead to elevated calcium levels can cause constipation or diarrhea. Although vitamin D is necessary for calcium absorption, excessive amounts can induce bone loss by interfering with the function of vitamin K2. In persons with healthy kidneys and those with established renal disease, too much vitamin D can cause renal harm [{9}].

Lifelong dietary supplementation with high doses of regular vitamin D3 (800 IU/day), prevents diabetes in mice safely way, and is accompanied by the induction of Tregs. This preclinical study confirms the potential of exploiting the vitamin D system in the prevention of type 1 diabetes in humans [(10)].

It would take 100,000 IU of vitamin D3 to have a 50% probability of killing 0.5Ib rat [(11)].

The Morris water maze (MWM) is a behavioral test that evaluates hippocampal-dependent memory and learning. It's been frequently utilized in mouse models to investigate neurobiology, neuropharmacology, and neurocognitive problems [{12}].

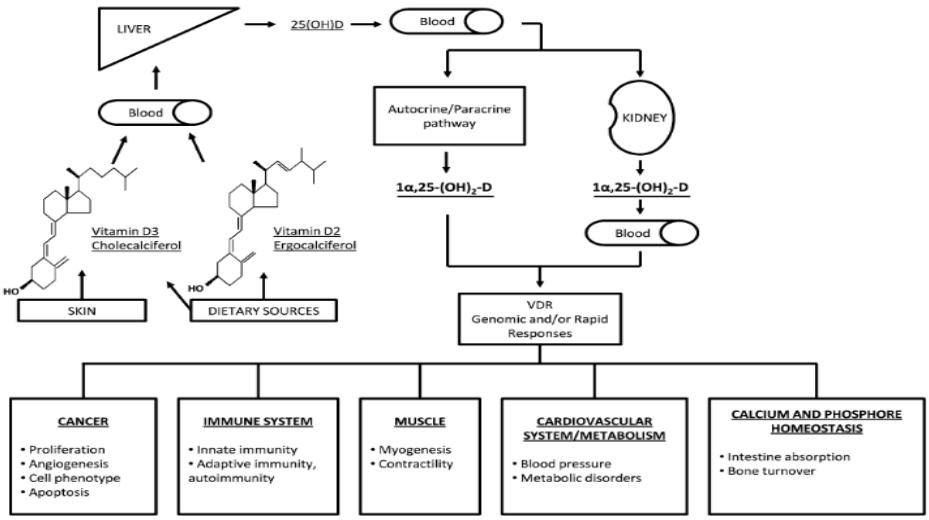

Figure 1.1. Metabolism and physiological effects of vitamin D [13]

There are limited studies investigating the effect of Vitamin D on learning in the Morris water tank. According to Khan study, he showed that employing scopolamine to cause learning impairment in rats, followed by therapy with curcumin and vitamin D3, resulted in neuroprotection and mitigation of cognitive deficits, as seen by reduced brain tissue damage in histoanalysis [14].

Moghadamnia AA, et al (2015)[15] assumed that, there was a two-way interaction between vitamin D and learning, suggesting that the tors (VDR) gene may influence the risk of type 1 diabetes. However, it is involved in the growth and function of the brain. The production and derivative mechanisms of 1 α, 25-(OH) 2D3 have been discovered in the hippocampus and other parts of the brain. Several pieces of evidence, on the other hand, indicated the extensive existence of vitamin D target controlled-gene pathways in important cognition and behavior functions. As a result, vitamin D plays an important role in memory and learning planning, processing, and formation.

Vitamin D also play a role in the cerebral detoxification processes by interacting with reactive oxygen and nitrogen species in the rat brain and influencing the activity of glutamyl transpeptidase, a crucial enzyme in glutathione metabolism [16].

In the literature, there is no study investigating the effect of varying doses of Vitamin D on learning. In this study, we investigate the effect of vitamin D on spatial learning and memory at low and high doses of vitamin D using Morris Water Maze (MWM) in rats.

2. GENERAL KNOWLEDGE

2.1. VITAMIN D

2.1.1. Metabolism of vitamin D

The exposure of skin with ultraviolet B (280–320 nm) starts the photochemical change of 7-dehydrocholesterol through provitamin D3 to nutrient D3. Vitamin D3 is formed by hydroxylation in the liver and kidney. Its hormonally active form, 1α,25-dihydroxy vitamin D3 (calcitriol), reaches its target tissues through the blood [8].

2.2. General Effects of Vitamin D

Vitamin D (vit D) is important in many organs apart from calcium-phosphorus balance and bone mineralization. Vit D is an indispensable neuroactive steroid in the neural system, in brain development. Vitamin D deficiency during development and in adults may be associated with schizophrenia, depression, Parkinson's and Alzheimer's disease. [17] [18].

The possible function of vitamin D as an antioxidant could not be affirmed. There are controversial impacts of the capacity of vitamin D to prevent or ameliorate the irregularity between oxidant and antioxidants species [19].

Cholecalciferol (Vitamin D3) (active metabolite 1,25-dihydroxycholecalciferol) and ergocalciferol (Vitamin D2) are considered membrane antioxidants with their membrane stabilizing properties against lipid peroxidation [20].

2.3. The effect of vitamin D on Learning

Cognitive functions incorporate memory, perception, thought, and imagination and the damage to at least one of these functions can actuate dysfunction in learning and memory. Vitamin-D can apply a significant regulatory impact on different physiological measures. Numerous investigations have affirmed that Vitamin-D can ensure the nerves in the brain tissue. Likewise, Vitamin-D can improve learning and memory capacity [21].

Vitamın D is portrayed by the capacity to control the immune framework and ensure the central nervous system, and the deficiency of vitamin D is related to the event and advancement of

cognitive dysfunction, and the applicable studies have uncovered that vitamin D can ameliorate the intellectual dysfunction. {22}

The hippocampus is the primary area influenced by nutrient vitamin D deficiency since it is very dynamic. This movement makes it more vulnerable to nutrient D deficiency than different zones of the brain {23}.

Adult vitamin D deficiency, in any case, solid adult mice may assume a vital part in hippocampal-subordinate learning and memory arrangement. Spatial learning shortfalls could be from disruptions inside the privilege hippocampal structural availability {23}.

2.4. Learning and Memory

Learning and memory are cognitive abilities that include a number of different subcomponents. These segments can be organized in various ways. We can concentrate on their transient nature or distinguish between distinct types of memory based on the quality of their substance or security processes. The psychological structure of learning and memory appears to be complex, as it should be.

Declarative memory joins semantic and verbose memory and alludes to regular memory capacities, which are ordinarily disabled in amnesic patients. Declarative memory is considered to depend fundamentally on average temporal projections, hippocampus projections. Non-declarative memory incorporates different subcomponents, of which procedural memory or the development of motor memories is the most conspicuous. Non-declarative memory is considered t to rely upon the striatum, cerebellum, and cortical affiliation zones. Nonetheless, procedural memory likewise incorporates cooperative learning structures, such as old-style and operant conditioning, and non-associative learning structures, for example, preparing, adjustment, and learning of perceptual and cognitive events. Prominently, motor learning has been viewed as a less intellectual type of memory capacity, and most exploration makes an unmistakable qualification among motor and nonmotor memory functions. Subsequently, it appears to be evident that declarative and nondeclarative memory measures are intelligent and mostly covering domains {24}{25}.

2.4.1. Learning Types

2.4.1.1. Associative Learning

We "learn" that two boosts are related to one another in cooperative learning, or that a response is associated with a specific occasion or has a specific outcome. Maybe significant in clinical contemplation, an individual can likewise learn that a result is not related to a response. Thus, the person can learn that the situation he encounters does not match what he has done. It is the process by which a person learns to associate two events or a behavior with a stimulus. If an event that depends on another stimulus reminds another event, it is called associative learning. {26}

2.4.1.2. Classical Conditioning

Classical conditioning is an example of Pavlov's experiment. Here, the dog identifies the bell with food and salivate when it hears the bell, even if no food is given. The chime must be rung prior to the delivery of the meat powder for classical conditioning to occur. On a regular basis by a set fundamental season interval (of the request for half second). Imagine the bell as a signal that the meat powder will be introduced, which really is one way to think about classical conditioning.

The meat powder is characterized as the unconditioned stimuli in Pavlov's paradigm because it normally stimulates salivation without the assistance of the experimenter (it is inborn or may have been previously "unconditioned stimulus" US). The unconditioned response which is the name given to this reaction (UR). The chime begins to evoking salivation when it is consistently coupled with meat powder; it is referred to as the (conditioned stimulus) in this way (CS). The reaction to it (again salivation) is known as the "conditioned response" (CR). The UR and CR are frequently comparable; they are not always separable in type or quality {27}.

2.4.1.3. Operant Conditioning

B.F. Skinner, a psychologist, developed the concept of operant conditioning. Skinner's investigations include the following: until the behavior is learned using a schedule of fortifications, prizes, and Punishments-Positive punishment would have the opposite effect on behavior. Negative punishment, on the other hand, is to take something away. As a result, we learn to engage in actions that produce favorable outcomes while avoiding those that create unfavorable outcomes. We might think of operant shaping as a cycle in which creatures figure out how to repeat activities that produce favorable outcomes while avoiding or avoiding unfavorable outcomes {28}.

2.4.1.4. Non-Associative Learning

Non-associative learning is a type of learning that is procedural rather than declarative. Reduces (habituates) or strengthens (sensitizes) a creature's sensory perception with time when a sensory signal is rehashed or introduced repeatedly, the behavioral reaction changes. It is not similar to associative learning in that it does not require feedback in response to the stimulus. Repeated exposure to a stimulus results in a permanent change in the strength of the response [29].

2.4.1.5. Habituation

After repeated stimulus, the term "habituation" refers to a type of learning that responsiveness to a stimulus gradually decreases or ceases. Basically., the body learns to cease responding to stimuli not intrinsically significant at this time [30].

2.4.1.6. Sensitization

“Sensitization” is a “non-associative” learning in which the repeated arrangement of an event or stimulus. The response results in the gradual improvement. “Sensitization” is termed by an improvement in reaction to a broad range of stimuli. In “sensitization”, Increasing the strength of one pathway improves the reflex strength of another [31].

2.4.2. Types of memory

2.4.2.1. Sensory Memory

Sensory memory means sensory information short of what one second after a thing is taken. The ability to recall what it seen like with only a glance a case of sensory memory is a brief moment of observation or memorization. It is due to a lack of cognitive ability control and is a natural reaction. Participants were given extremely brief introductions.

There are three types of sensory recollections. Iconic memory is a short-term storage of visual knowledge; it's a type of sensory memory that remembers a picture seen for a short time. Another type of sensory memory, echoic memory, is a rapid decaying store of auditory knowledge.

Haptic memory is a type of sensory memory that communicates with a touch stimulus [28].

2.4.2.2. Short Term Memory

Working memory is another name for short-term memory. Short-term memory is partly responsible for recall. Its skills are equally limited without practice for a few seconds to a moment. For storing information, short-term memory is thought to rely on an acoustic coding for storing information and, to a lesser extent, a visual code reviewing acoustically comparable letters rather than outwardly comparable letters leads to the conclusion that the letters were encoded acoustically. Manages the encoding of prepared content; as a result, while acoustic segments may be used in written language memory, generalizations to other types of memory are impossible {32}.

2.4.2.3. Long- Term Memory

Long-Term memory can store a lot bigger quantities of information for the possibly limitless duration (some of the time an entire life span). Its ability is immeasurable. Given an irregular seven-digit number we may recall it for a just moment prior to failing to remember. Then; we can recollect numbers for a long time because to redundancy; these data are designed to be stored in a long-term storage facility remembering. While short-term memory stores data in an acoustic format, long-term memory stores it in a digital format. In terms of semantics, long- term memory is kept up by more perpetual changes in neural ways project all through the brain. The hippocampus is critical for the solidification of information from short term to long term memory {24}{33}.

2.4.2.4. Prospective Memory

Prospective memory is associated with near term behaviors and includes the goal of completing a psychological or physical action. It is critical to hold intentions and activate them at the appropriate moment and in the appropriate place to understand an objective later on. Planned memory processes involve working and long-term memory processes, just as attentional cycles do, depending on how much time occurs between the intention and the action, and whether the activity is started outside (setting feature) or internally (internal pacemaker) {34}.

Inside this unique situation, it has been recommended that, during encoding, prospective memory substances acquire a special status, where they are labeled as not being accomplished at this point. During the presentation of prospective memory cues, temporal regions are dynamic, potentially speaking to stimulus-driven attentional measures. The deferral period between encoding the expectation and the real demonstration is loaded up with a cognitive activity that forestall dynamic and cognizant rehearsal, which separates prospective memory from WM or watchfulness.

Prospective memory and WM assume a unique position inside the memory space as they firmly depend on chief cycles. Nonetheless, forthcoming memory and WM connect with various brain regions. While WM demands the dorsolateral prefrontal cortex (DLPFC) action, prospective memory have been related primarily to the enactment in the rostral PFC, which is ensnared in "future thinking". Such, to a great extent hypothetical, contemplation got from cautious errand investigation and psychological and intellectual model formation can be tried tentatively utilizing NBS {35}{24}.

2.4.2.5. Declarative Memory

Declarative memory also known as unequivocal memory, the non-associative hypothesis of long-term memory proposes that the psyche creates a permanent record of experience (follow column). Declarative memory, by and large, includes some exertion and intention and we can utilize memory strategies. For example, review and acknowledgment, in which a person is fully aware that they are being tested. It tends to lessen with age. Recalling the list of goods to get up at the store, a name of an old friend, recollecting knowledge for learning a phone number and test are all examples of models. {28};{36}.

2.5. Factors that play a role in learning

Numerous variables have been examined to address gives what may confront learners in various learning environments. These covers, for example, foundation, demographic factors, earlier information, working memory capacity, information preparing speed, goals, interests, and learning styles {37};{38}.

2.5.1. Brain regions involved in learning

Several brain systems are likely involved in category learning, including most of the hippocampus, the neocortex and the basal ganglia.

2.5.1.1. Visual Cortex

By focusing on the visual system, the most thoroughly researched modality. In any event, comparison cycles will almost certainly be implemented in other sensory modalities, such as the auditory, somatosensory, and olfactory systems. {39}

The inferior temporal cortex (ITC) is one example, with neurons that show complicated shape selectivity. Since the seminal chip away at "face cells," researchers have been thinking about neurons having category-like tuning properties. During learning of new face categories, the human fusiform face region, an ITC region with most face cells, is used.

2.5.1.2. Prefrontal Cortex

The prefrontal cortex appears to be more promptly modifiable by experience than the sensory cortex. PFC neurons additionally reflect unique standard-based unmitigated distinctions not connected to the behavioral response [{40}].

2.5.1.3. Parietal Cortex

The parietal cortex appears to concentrate visuospatial processes and connect perceptual cortex inputs with potential responses. Numerous direction-selective neurons are in the extra-striate region V5/MT and task to the lateral inferior parietal projection and insula, that incorporate speaking movement patterns [{40}].

2.5.1.4. Premotor and Motor Cortex

Categorical decision additionally includes the choice and execution of proper behavior. This enlisted people's premotor cortex (PMC) and essential motor cortex inside the frontal flap.

PFC harm especially newly influences learning. When persons conducted recognizable versus novel classifications, there are important signals in the dorsal PMC than in the PFC. Following that, the PFC may obtain new categories, although different areas, include PMC, may carry them out once they become natural [{40}].

2.5.1.5. Hippocampus and the Medial Temporal Lobe

The cortex contains physical and functional connections with the medial temporal lobe (MTL), which appears to be specialized for fast learning of individual occurrences. The circuitry of the MTL and cortex structures a circuity: knowledge from neocortical regions over the parietal, frontal, and temporal cortices tasks to the entorhinal district of the para-hippocampal region. From the entorhinal cortex, the projections firstly go to the dentate gyrus, the CA3 of the hippocampus, the CA1, and go back to the entorhinal cortex. The CA3 contains auto-associative intermittent links, which permit association arrangement during encoding and example finishing during recall.

Categorization can utilize the MTL's capacity to study singular occurrences. One errand that requires occurrence learning is the discretionary categorization task. fMRI considers finding that MTL (among different systems, including corticostriatal systems) is regularly enlisted during these tasks. Similarly, neurons in the hippocampal region and the cortex of the temporal region show category- specific activity in the wake of preparing monkeys to assemble discretionary stimuli. The MTL's instance- learning capacity may likewise be summoned to store special cases for rules and other categorical consistencies. Some level of occurrence memory might be needed in all categorization assignments that utilize novel stimuli; the MTL might be needed to set up a memory representation of every boost that would then be able to be got to by different frameworks [{41}].

Another significant expected commitment of the MTL follows from perceptions that information procured through the MTL can be moved to new situations. One model is procured comparability. For instance, if a subject discovers that stimulus an is in classifications 1 and 2, and improvement B are in classification 1, they can sensibly gather that stimulus B may likewise be in class 2. The MTL is associated with these errands [{40}].

2.5.1.6. The Basal Ganglia and Corticostriatal Loops

The basal ganglia are a group of subcortical nuclei that form corticostriatal loops with the cortex. Cortical inputs are normally received through the striatum and then coordinated back into the cortex via the thalamus. In various loops, the basal ganglia maintain a level of topographical detachment, ensuring that the yield to the corresponding cortical regions that provided ascent to the underlying contributions to the basal ganglia is enormous. The frontal cortex gets the biggest part of BG yields, recommending some type of close collaboration between these structures. Be that as it may, practically all cortical regions participate in corticostriatal loops. Despite the fact that there is a cover between the loops at their boundaries, it is helpful to discuss four loops: executive, motivational, visual, and motor. The basal ganglia apply a tonic restraint on the cortex; they physically and specifically discharge the cortex to consider the development or cognitive strategy of decision. This capability could be used in order errands to aid in the selection of both an acceptable category portrayal and related systems or behaviors [{42}; {43}].

2.5.2. Neurochemical and hormonal factors associated with learning

2.5.2.1. Hormones and Learning

It is conceivable that the catecholaminergic impacts on learning reflect the physiological function of adrenal medullary epinephrine. It is fascinating that different hormones of the pituitary-adrenal pivot likewise impact learning. Shockingly, not exclusively do ACTH (Adrenocorticotropic hormone) and glucocorticoids have significant impacts, yet vasopressin (antidiuretic hormone) likewise does. Since all these hormones are typically emitted during pressure this may emphasize the physiological part of pressure in learning. The significant impacts of ACTH and ADH are the rebuilding of learning shortfalls brought about by hypophysectomy, the improvement of retention for pitifully learned errands, and the inversion of amnesia prompted by an assortment of specialists.

There's also evidence that ACTH plays a role in mediating some of the neurochemical effects of foot shock or other stresses. As a result, ACTH may legally intervene in learning's effects. The complication is that hormones are typically simply facilitators of the learning cycle, rather than being essential to it. The challenge therefore becomes separated these ancillary effects from the learning appropriate [44].

2.6. Learning Tests Used in Experimental Animals

2.6.1. T-maze

A T-maze test is an experimental approach that is utilized in congenital research [45]. T-mazes can be utilized in an assortment of approaches to survey the cognitive capacity of a creature. The side (goal) arms can contain discriminative stimuli (cues, for example, examples or items that the creature must react to, by and large, to get a prize. A cohort of rodents may be prepared to choose the white arm of a T-maze, regardless of whether it is on the left or the correct side, staying away from the opposite dark arm (which they would intuitively pick, as they incline toward dull spots). This is a basic discrimination, commonly learned by rodents in around 40 trials. The arrangement is consistently the equivalent: pick white. This is named a 'reference memory' task.

Animals can by and large tackle troublesome reference memory issues, as long as there is no spatial part [46]. Reference memory assignments in the T-maze are consequently of restricted an incentive in surveying creature AD models. (The conspicuous special case is the place where there is a spatial

component.) The natural tendency of rodents and mice in a T-maze, be that as it may, is to substitute their decision of goal arm. They are utilizing 'working memory', i.e., the reaction on every preliminary change as indicated by what they have already quite recently done. The variation reflects the motivation of the creature to investigate its current circumstances and find the presence of assets, for example, food, water, mates, or sanctuary. Animals do not should be denied of such assets to show rotation behavior; for this situation, it is called 'spontaneous alternation'. This undertaking has been utilized for quite a long time in academia and industry yet has gained notoriety for inconstancy and irregularity. Regular rotation rates are around 75% [47].

T-form maze's is depicted as follows: T-maze with many parts of 12 cm broad, 10–20 cm long, and 11 cm high. The prize (a few food pellets) was kept in one of the arms (the objective compartment), and the right track measured 185 cm from the start to the goal compartment (figure 2.1). The rodents were trained to complete the labyrinth in three seconds without accidentally entering the wrong arm [48].

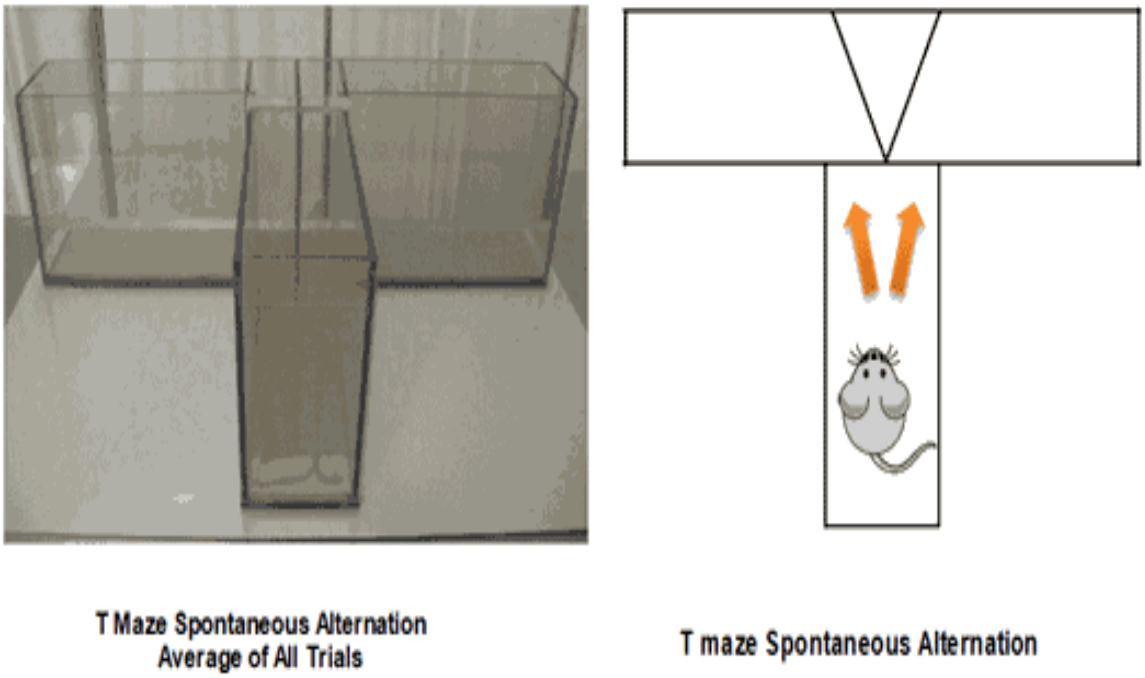

Figure 2.1. T- maze [49].

2.6.2. Complex enclosed maze

Another type of complicated encased labyrinth is the tunnel maze, which does not rely on any special fortification and instead rely on rodents' natural need to study their surroundings efficiently, as shown in spontaneous rotation projects. There are six arms from a center to the periphery and each arm has a rear entrance door. Passages into obscured back streets are viewed as reference memory errors [50].

2.6.3. Hebb–Williams maze

The Hebb Williams maze is made up of 12 types of obstructions encased in a field. The creature is usually given multiple preliminaries in each of a few or all of the variants, and they approach intra- and extra-maze obvious prompts. Mistakes are considered turns made that don't speak to the most immediate pathway to the goal. Normally, the experimenter is in charge of scoring. This maze has been widely used to investigate the effects of explicit mind lesions on learning [50].

2.6.4. Stone 14-unit T-maze

The Stone maze is enclosed, and the rodent must perform a sequence of left right position discrimination to find the prize at the end of the maze [50].

2.6.5. Symmetrical Y maze

A maze with three indistinguishable arms summons dynamic investigations, and the example of arm passages, just as all outnumber of sections, might be informative. The mouse must re-visitation the center point of the maze after each arm passage, and it would then be able to re-visitation the arm it visited, the arm it visited on the last entry, or the one least as of late visited. The spontaneous alternation file, communicated as percentage alternations, possibly consider those instances when it moves to another arm. A "prior" passage happens when it goes to the arm that was the most as of late another arm visited, and "alternation" happens when it goes to the third arm, the least as of late visited. The short-term working memory understanding of rotation attests that inclination for the least as of late visited arm shows memory for the historical backdrop of arm entries. The legitimacy of this understanding is tested by the way that a basic outspread strategy (123123123123) will yield 100% rotations, although the fact that it will happen if the mouse recalls only consistently makes a left turn when leaving an arm [51].

2.6.6. Four plate tests

It (FPT) is an anxiety beast model that relies on spontaneous reactivity. Animals are put to a new environment. The delivery of modest electric foot shock unanticipated to quadrant crossing suffocates the investigation of this novel's surroundings. The creature can escape from this aversive circumstance by staying motionless (latent shirking). This model of adapted dread presents a few preferences (figure 2.2). It is a straightforward and speedy method and there is no requirement for earlier preparation of animals [52].

Figure 2.2. Mice in the Four-Plate Test [52]

2.6.7. Barnes maze

The Barnes maze (BM) test is based on the premise that a mouse or rat placed outside a platform must learn and remember the safe zone outside the platform. The testing phases include the familiarization period and the acquisition period. It is used in the evaluation of spatial memory. [53]; [54]

2.6.8. Passive avoidance test in rats

The Passive Avoidance Task is used to assess learning and memory. They learn to avoid the environment they are in a deterrent stimulus (such as a foot shock).

There are two areas, light and dark. There is a door between the two areas. First the animals are allowed to inspect the two compartments. The next day, a mild foot shock is given in one of the areas. To test their learning and memory, the mice are then placed back in the chamber where no shock was given. Mice with normal learning and memory will avoid entering the room where they were recently shocked (Figure 2.3).

This test is useful in testing the effects of new drugs on learning and memory [55][56].

Figure 2.3. Passive Test Avoidance [49].

2.6.9. Skinner's box

To study the learning cycle in rats, he constructed an experimental chamber (dubbed the Skinner box) (Figure 2.4). A switch was mounted on the front wall of the chamber. The response to being scholarly is to press the switch. The hungry rodent is placed in the chamber and immediately begins to act erratically. Sometime later, the rodent accidentally presses the lever, and a pellet of food automatically drops on the plate and rat eats it. In the wake of eating the pallet, the rodent again begins activity in the chamber. After some activity, it again presses the switch and gets a pellet (a reward). Continuously the random action changes to a more explicit action around the switch. At long last, the rodent discovers that pressing the switch brings about the dropping of the food, a wonderful result. At the end of the day, the squeezing of the switch by the rodent is instrumental in giving food (reinforcement). The reaction (pressing the lever) is reinforced and the conduct is

obtained or learned. The squeezing of the switch by the rodent is instrumental in getting food, a fantastic outcome (positive reinforcement) and that is the reason this sort of learning is additionally called instrumental learning. It is additionally called operant molding on the grounds that the behavior of a rodent or any organism is a sort of activity on the climate [28]; [57].

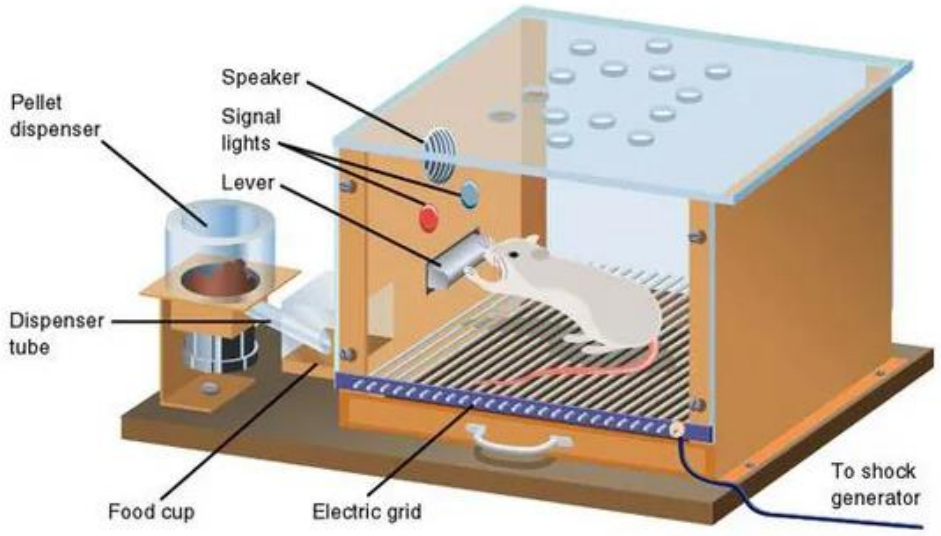

Figure 2.4. skinner's box [58]

2.6.10. Morris water tank

The Morris water maze (MWM) is a behavioral errand to test hippocampal-dependent learning and memory. It has been utilized in the investigation of neurobiology, neuropharmacology, and neurocognitive problems in rat models [12].

The tank's shape changes from time to time, but it's usually round and is 80 to 180 cm across and 30 to 60 cm tall. Tanks with a smaller width are better for testing mice. To complete the task, you'll need an escape platform. The plat-structure can be clear or opaque (white or dark, depending on the colour of the water) and ranges in width from 5 to 12 cm. Before the preliminaries start, the tank is loaded up with enough water so that the animal can't arrive at the base with either its limbs or tail. To darken the platform, the water might be delivered opaque with milk powder or white paint (for pigmented animals) or ink (for albino animals), or the tank might be colored black and

the water left untreated. Since the way taken by the animals to the platform is regularly recorded by a camcorder, the differentiation between the rat and the water is important for imaging.

The room in which the testing mechanical assembly is located contains environmental signs, which help the rat in spatial learning. These prompts may be high-contrast geometric designs on the room partitions, flags, or anything else visible from the water to the animal and platform, for example, room dividers, hardware, furniture, and entryway outlines. Thus, the extraordinary consideration should be taken that such prompts remain fixed all through the term of the experiment [{59}]; [{60}]; [{61}].

3. MATERIALS AND METHOD

3.1. Experimental Animal:

A total of 36 male Wister rats 8 weeks old age ware used. Animal ware obtained from Başkent University Experimental Research Center. They were housed in standard cages; unlimited amounts of water and feed were allowed. All animal experiments carried out in Başkent University, Faculty of Medicine, Department of Physiology, Animal Experiment Laboratory. The experiments were conducted after the approval of the Animal Experiments Ethics Committee of Başkent University (20/16).

3.2. Experimental Groups:

Rats were randomly divided into 3 groups (n = 12) in equal numbers.

- **Control group (group 1) [n=12]:** Each rat was administered oral gavage of normal drinking water
- **Low dose Vitamin D (group 2) [n=12]:** Each rat was administered Vitamin D by oral gavage at dose 400 International Unit [IU]/ day
- **High dose Vitamin D (HDVD) (group 3) [n=12]:** Each rat was administered Vitamin D by oral gavage at dose of 1000 IU / day

The substances were administered to the groups once a day for 8 weeks. At the end of this period Morris water maze was done.

3.3. Morris water maze

The Morris water maze test was applied to evaluate the spatial learning and memory of the rats. The water maze model was used in a circular black tank (diameter 140 cm, height 50cm) filled with water (temperature 25 $C^o \pm 2$). Divide the water maze tank equally into 4 equal regions using two mutually perpendicular lines.

In the center of the fourth quadrant, a platform was sunken 1cm below the water's surface. The platform is invisible to rats since it is submerged. To figure out where the platform is, it must rely on particular visual clues scattered throughout the tank (figure 2.5).

The rats' swimming movements were observed by a video camera mounted overhead, which was automatically recorded by a video- tracking system. At the outset of each trial, the rats were placed

in the water facing the pool wall in one of the four quadrants. Each rat has 120 seconds to find and mount the platform. The rat was kept on the platform for 10 seconds after it discovered it.

Figure 2.5. Ours MWM picture

3.4. Procedure

1. To allow the rats to acclimate to the experimental conditions, we placed them in a maze for 30-60 seconds.

2. The animal was then placed in the maze's desired starting position, facing the tank wall. At water level, the animal is released into the water (not dropped). When the animal is freed, a timer or computer-tracking software is initiated.

3. When the animal reached the platform, we stopped the timer (A trial limit of 2 min per trial). The animal was left on the platform for ten seconds.

4. The animal was moved to a new starting point (second quadrant) and the experiment was repeated from there (third and fourth quadrant). The rat was rested in a tiny heated cage for 20-25 minutes between each session, four times a day.

5-The three groups of rats were subjected to an identical procedure for three days. 6. The platform was removed from the tank on the fourth day of the experiment, and each rat was given a 2-minute swimming research period from where they began swimming in the pool.

3.5. Statistical Analysis

The data are analyzed through descriptive statistics and mean comparisons between the three study groups: control, LDVD, and HDVD. The differences between the groups are tested through a one-way ANOVA, followed by a post-hoc test (Tukey HSD) to understand the specific mean differences and statistical significance ($p < 0.05$).

All statistical analysis was considered statistically significant when $p \leq 0.05$ and highly significant when $p < 0.005$.

4. RESULTS

4.1. Learning ability

The MWM test was done to determine whether vitamin D influence on spatial learning. As a measure of learning, escape latency ((the time required to arrive at the platform)) was used. Table 4.1 shows the mean escape latencies of three groups of rats undergoing training in the concealed platform water maze tasks for four days. All 3 groups gradually reduced the time necessary to locate the platform throughout the course of the training sessions [F (2,33) = 0.798, p-value =0.459]. Because one rat died on the first day (Day 1, p-value =0.019), the post-hoc analysis revealed statistical differences in the escape latencies between the low dosage group and the high dosage group (n=11), but (Day 2, p-value =0.947), and (Day 3, p-value =0.514) was not significant.

It's also worth noting that in Figure 4.1, there was no difference in learning speed across the groups. The escape latency of the low dose group (400 IU/day) was not significantly different from the control group. Throughout the acquisition courses, the learning curve of the low dose group was partly parallel to that of the control group., and The learning curve of the low dose group was always higher on the first day and lower on subsequent days than that of the control groups.

Meanwhile, on the first two days, the escape latencies of the high dose (1000 IU/day) groups were parallel to those of the control group, but on the third day, they were the escape latencies semi symmetric. During the first three learning sessions, the rats given high and low doses seemed to take longer to locate the platform than the control rats (significant).

Table 4.1. Comparison of escape latencies of days 1, 2, and 3 for the control group, high dose Vit D (HDVD) and low Dose Vit D groups (LDVD)

Days	control group (n=12) Mean ± SE	LDVD (n=12) Mean ± SE	HDVD (n=11) Mean ± SE	F	P-Value	F value and P-value for all days
1	52.5 ± 25.6	69.0 ± 31.4	37.3 ± 16.5	3.344	< 0.05*	0.798; 0.05
2	25.8 ± 20.0	20.9 ± 10.4	22.1 ± 32.2	0.223	> 0.05	
3	14.0 ± 7.6	10.3 ± 12.1	14.9 ± 20.5	0.244	> 0.05	
P	<0.01	<0.01	<0.01			

* The group has a significant difference from day 1.

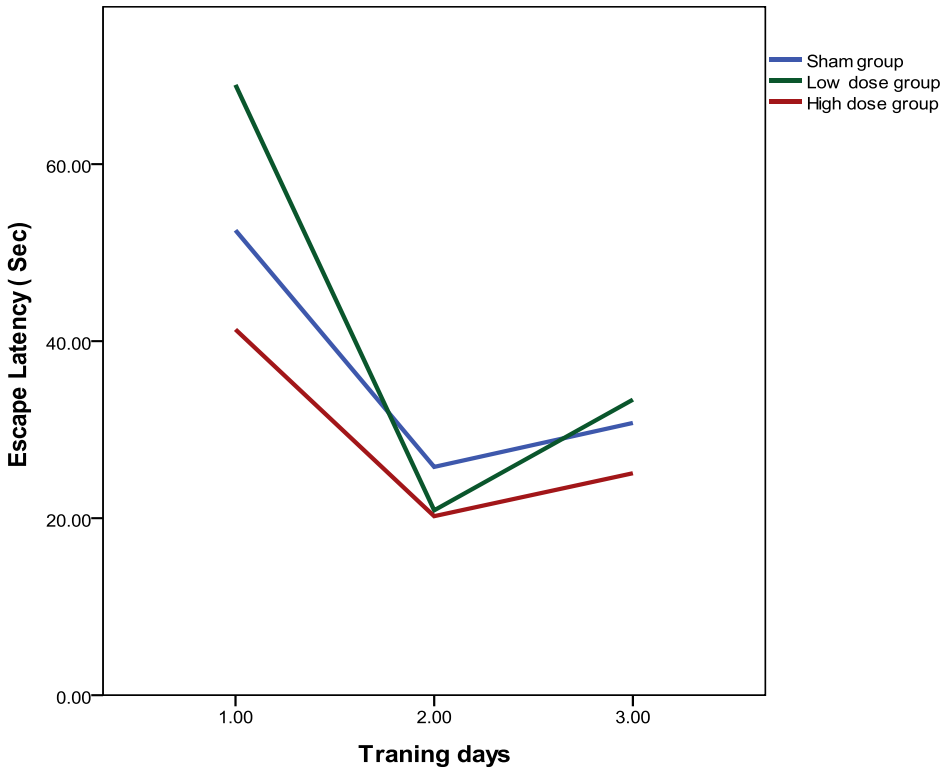

Figure 4.1. Effects of vitamin D on escape latency during the acquisition- training phase of the Morris water maze tasks on an invisible platform.

There were no treatment-related change in the percentage of swimming time in the platform, as Shown in Table 4.2, [F (2,105) = 1.029, P- value = 0.361]. This result showed that the dose treatment utilized in this investigation had no influence on the animals' mobility or motivation in the Morris water maze challenge. Figure 4.2 exhibited control group and low dose group were semi parallel progressively reducing the time swimming in the outer zone during training sessions and little reduction in day 3 compared to day 2 for low group [% time in outer zone of three groups: (show: Day 1 34.08 ± 2.63, Day 2 31.63 ± 3.68, Day 3 43.76 ± 6.27); (low dose: Day 1 21.41 ± 0.66, Day 2 21.57 ± 2.07, Day 3 45.65 ± 8.98); (high dose: Day 1 32.05 ± 3.40, Day 2 42.32 ± 6.86, Day 3 39.50 ± 6.00,)]

Table 4. 2. Means and standard error of percentages of swimming time on the platform area in days 1, 2, 3, and 4, and significant differences at the 0.05 level between control, HDVD and LDVD groups

Days	Trial	Percentage of swimming time in platform			P
		control group (n=12) Mean ± SE	LDVD (n=12) Mean ± SE	HDVD (n=11) Mean ± SE	
1	1	34.1 ± 9.1	21.4 ± 2.3 *	32.2 ± 12.4	**0.003**
	2	31.6 ± 12.7	21.6 ± 7.2 **	44.8 ± 23.3	**0.005**
	3	43.8 ± 21.7	45.7 ± 31.1	40.7 ± 21.4	0.893
	4	69.7 ± 33.0	49.6 ± 28.8	71.1 ± 32.2	0.191
2	1	33.3 ± 16.9	36.8 ± 18.6	29.3 ± 22.2	0.648
	2	31.1 ± 14.4	38.6 ± 15.6	32.5 ± 11.5	0.391
	3	36.0 ± 17.9	34.8 ± 14.3	26.6 ± 12.2	0.288
	4	45.5 ± 34.6	65.5 ± 31.1	69.2 ± 37.5	0.214
3	1	43.1 ± 21.5	46.2 ± 27.0	40.9 ± 15.0	0.847
	2	39.1 ± 13.2	46.0 ± 17.5	33.8 ± 13.0	0.155
	3	48.6 ± 15.5	51.3 ± 21.2	42.4 ± 10.8	0.430
	4	41.1 ± 24.4 **	63.6 ± 31.2	90.1 ± 22.3	**0.000**
4	1	35.5 ± 11.1	34.9 ± 14.2	34.4 ± 9.5	0.975

*Significant differences with both control and high dose groups

** Significant differences with high dose groups

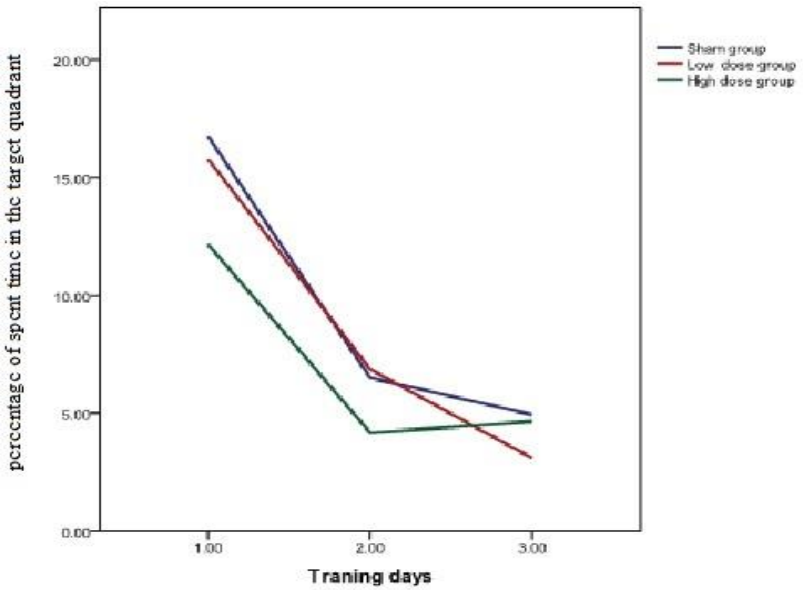

Figure 4.2. Comparison of the percentage of time spent in the target quadrant with invisible platform of groups

4.2. Memory retention

To evaluate the rats' spatial reference memory, probe trials were conducted 24 hours after the last acquisition trial (target quadrant without platform). Figure 4.3 shows that the percentage of time spent in the target quadrant without platform did not differ statistically significantly between the three groups [F (2,32) = 0.25, P-value =0.975]. The high dosage vitamin D group spent no more time in the target quadrant than the control and low dosage vitamin D group rats, according to the post-hoc analysis as shown in table 4.2.

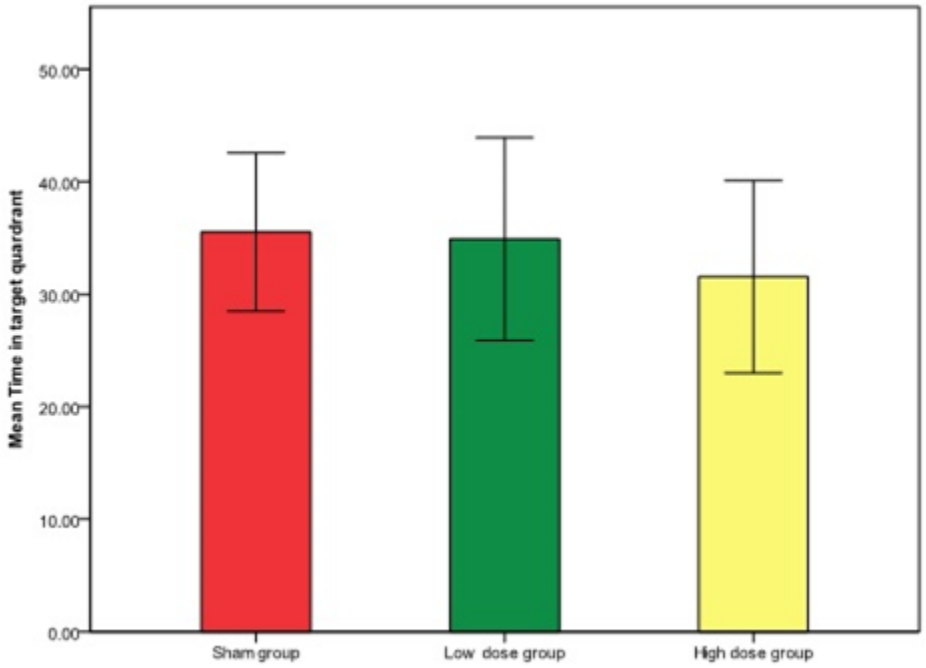

Figure 4.3. Comparison of probe trials in the Morris water maze without platform

5. DISCUSSION

The effect on spatial navigation tasks in the Morris water maze was explored in this study, as well as the role of vitamins D in such outcomes. The results reveal that different Vitamin D doses had no effect on acquisition performance, the time spent in the target quadrant and platform position, or the time it took to cross the platform location in a reference memory task session. However, the MWM acquisition- training outcome shows a consistent decrease in swimming latency over time, suggesting that both groups of could learn.

During the probe trial, the animals show better learning ability. The trend was a preference for north-west (NW) and south-west (SW) quadrants, which had the escape platform during the acquisition training days. However, there was no significant difference in the values for the test group compared to the control.

MWM results may differ depending on the length of vitamin D exposure, different dosages of vitamin D administered, dietary vitamin D levels, rodent age, rodent strain, behavioral tests employed, and the effects of these tests on different brain areas.

In comparison, few studies in rodents have shown that Vitamin D supplementation improves learning and memory. (Choobdar S, et al. 2019) {62} examined all the rats were randomly divided into two groups; stress and control groups (each had 3 subgroups). Stress-group animals were exposed to restraint stress for 28 days, 3 hours/day. During 28 days, vitamin D (5 or 10 mg/kg), or vehicle was injected (IP) twice weekly to both groups. On day 29, blood sample collected for serum corticosterone assay. Morris water maze (MWM) test was performed in the order of 4 days training, one-day probe testing and finally working memory test. The passive avoidance test was performed after MWM. In MWM test showed that, all groups learned the location of the platform during training. Latency in reaching the platform was reduced by the training days ($p = 0.0001$) for all groups. Stress groups arrived at the platform faster than control groups, according to a probe test. Vitamin D supplementation reduced delay ($p = 0.0002$). Stress groups, on the other hand, spent less time in the darker than the control group in the passive avoidance test ($P = 0.0997$). The addition of vitamin D (10 g/kg) had no effect on the results. {62}

(Latimer CS, et al. 2014) {63} tested for 5–6 months, middle-aged F344 rats were fed diets containing low, medium (typical amount), or high (100, 1,000, or 10,000 international units'/kg

diet, respectively) vitamin D3, and hippocampal-dependent learning and memory were then tested in the Morris water maze. On maze reversal, a particularly difficult activity that detects subtler changes in memory, rats with high vitamin D levels had the highest blood levels (in the adequate range) and considerably outperformed low and medium groups.

Eighty female Wistar rats were studied by (Babaei B, et al. 2017) [{64}], who were separated into two groups: sham operated (n = 8) and ovariectomized (n = 72). OVX was then separated into nine groups: low dosage Vit D [OVX (ovariectomized) + EXE (exercise) + LD (low dosage)], high dosage Vit D [OVX + EXE + HD (high dose)], and no Vit D [OVX + EXE + HD (high dose)], (OVX + EXE + Veh) deficiency in vitamin D (OVX + EXE + Veh) deficiency in vitamin D (OVX + EXE + Veh) deficiency in vitamin D (OVX + EXE + Ve (sesame oil,). Non-exercised OVX groups receiving a high dosage of vitamin D (OVX + HD), a low dosage of vitamin D (OVX + LD), vitamin D deficiency (OVX D), and Veh (OVX + Veh) were also included.
After two months of relevant medications, MWM was used to measure spatial memory, and then metabolic syndrome components were assessed. Whether or not the patients exercised, consuming a high dose of vitamin D lowered weight ($p = 0.001$), lipid profiles ($p = 0.001$), visceral fat ($p = 0.001$), and waist circumference ($p = 0.001$). There was no difference in cognitive function. The Vit D deficient group had considerably greater serum BDNF levels ($p = 0.001$), while the OVX + HD group had lower levels. Irisin, on the other hand, had no significant association with Vit D serum concentration, although being much higher in exercised groups compared to non-exercised counterparts.

Female mice (C57BL/6) were randomly separated into three groups receiving diets supplemented with three levels of vitamin D3T (AIN93G rodent diet: Trophic, China; deficiency: 0 IU, standard: 1,000 IU, overdose: 10,000 IU), according to (Liang Q, et al investigations 2018) [{65}]. Male puppies continued to eat the same diet as their mothers after weaning (at 21 days). Each group consisted of pups from three to four litters. The animals were kept in groups of five (5 per cage) and given free access to water and food on a 12-hour light/dark cycle. The Morris water maze was used in this investigation, and according to a spatial reference memory test related to hippocampal dependent memory, postnatal VD deficit impeded spatial learning, while overdose had a lower effect. Nonetheless, all three groups learned the task after 12 days of learning, and the spatial reference

memory test on day 13 revealed no significant differences. Importantly, a third spatial memory test at day 28 revealed that postnatal VD deficit, and to a lesser extent, overdose, was largely damaging to long-term memory and increased memory loss. To fine-tune hippocampus function, the correct postnatal VD intake may be required. The impacts of VD intake status may be context and task dependent, and they may not be seen until the job becomes difficult.

Wistar rat pups born to vitamin D–deficient moms were studied by (Al-Harbi, et al. 2017) {66}. Control (C), deficient during gestation (dG), and deficient during lactation (dL) were the four groups (dL), as well as being inadequate during pregnancy and lactation (dGL).
The Morris water maze test was used to assess spatial learning and memory at PND 24 and PND 45. At PND 63,
On PND 32 and PND 63, cortical thickness was assessed at the hippocampal level, and synapses were counted in specific hippocampal sites. The dGL group's learning (escape latency) was shown to be impaired (by 42%) at PND 24, while both the dL and dGL groups showed learning impairment (by 47 and 45 percent, respectively) at PND 45, compared to their respective C groups. The developmental vitamin D deficiency (DVDD) had no influence on short-term or long-term memory at PND 24 and PND 45. All of the DVDD groups showed less connection in the molecular layer of the hippocampus than the C group. These findings show that a 6-week prenatal and postnatal DVDD impact learning but not memory in rat pups.

(Taghizadeh, et al.2013) {67} randomly assigned adult male Wistar rats weighing 250-300 g to one of three experimental groups (2013). A typical diet with 15-20 g vitamin D per kg of body weight per day was administered to the control group (CON, n = 14). The other two groups were provided a diet comparable to the control group, with the exception that one group (CON-D, n = 9) was given a vitamin D-free regimen and the other group (CON+D, n = 11) was given a vitamin D supplement (1,000 ng/100 g dry food). For ten weeks, each group received treatment.
Spatial learning performance was assessed in the Morris water maze. They discovered that the children's behavior had improved.

The difference between CON+D rats and CON rats was not statistically significant. The amount of time it took to find. The hidden platform revealed that there was no discernible difference in the performance of the two groups CON and CON+D. During the training stage of the experiment,

they measured the distance traveled to discover the hidden platform and rated the path taken by the subjects. The three groups examined for spatial learning behaved differently statistically. The CON-D rats had to swim a longer distance to reach the labyrinth target than the CON or CON+D rats. There was no statistically significant difference between the vehicle-treated rats and the 1,25(OH)2D3-treated animals. {67}

The positive effects of vitamin D (vitamin D) on the central nervous system illnesses have been suggested by (Mansouri F, et al.2021) {68} The protective effects of vitamin D on scopolamine-induced learning and memory deficits, oxidative stress criteria, brain-derived neurotrophic factor (BDNF), and nitric oxide (NO) in the brain were studied in this study.

Rats were divided into five groups, including (1) Control, (2) Scopolamine (2 mg/kg), (3–5) Scopolamine + Vit D (100, 1000, and 10,000 IU/kg) groups were used to split rats into five groups. Behavioral tests, including the Morris water maze (MWM) and passive avoidance (PA) tests, were conducted after two weeks of vitamin D administration and three weeks of scopolamine administration The activities of BDNF, catalase (CAT), and superoxide dismutase (SOD), as well as thiol content, NO metabolites, and malondialdehyde (MDA) concentration, were measured in cortical and hippocampal tissues. The rats' performance on the MWM and PA tests was severely harmed when they were given scopolamine. It increased MDA and nitrite levels in the brain while decreasing thiol content, BDNF levels, and SOD and CAT activity. Both 1000 and 10,000 IU/kg vitamin D administration enhanced cognitive outcomes in MWM and PA tests. Vit D also increased thiol content, SOD and CAT activity, and BDNF levels, while lowering the nitrite and MDA levels. Vit D also boosted vitamin D and calcium levels in the blood. The findings showed that vitamin D protects against scopolamine-induced learning and memory loss by increasing BDNF levels and reducing NO and brain tissue oxidative damage. This could be one of the reasons for the unfavorable results of our investigation.

In MWM and PA tests, both 1000 and 10,000 IU/kg vitamin D treatment enhanced cognitive outcomes. Vitamin D also raised thiol levels, SOD and CAT activity, and BDNF levels for reduced nitrite and MDA levels. Vit D also increased calcium and vitamin D levels in the blood. Vitamin D protects against scopolamine-induced learning and memory loss by raising BDNF levels and

decreasing NO and brain tissue oxidative damage, according to the findings. This could be one of the causes for our investigation's negative findings. {68}

6. CONCLUSION

Vitamin D is an important mineral for overall wellness. It aids in the absorption of calcium, which is one of the most important building blocks for strong bones. Vitamin D, when combined with calcium, helps to prevent osteoporosis, a disease that causes the bones to shrink and weaken, making them more likely to shatter. Vitamin D is essential for a number of different bodily functions. It is necessary for muscular movement and the passage of messages between your brain and your body via your nerves. Vitamin D is required by your immune system to fight against bacteria and viruses.

The effect of vit D on memory and learning in animals and humans has been a source of controversy in the research. The long-term aim of this study was to figure out how Vitamin D (control, low dose, and high dose) improved learning and memory, among other things.

In the current study, the MWM test did not show a significant increase in memory and spatial learning in rats.

It's worth noting that among the three groups, there was no difference in learning speed and no treatment-related change in swim speed. Memory retention also suggests that there was no statistically significant difference between the three groups in the percent of time spent in the target quadrant.

Thus, more similar studies with variations in species, age, dosage, length, and other variables are required.

REFERENCES

1. Deluca HF. History of the discovery of vitamin D and its active metabolites. Bonekey Rep. 2014 Jan 8; 3:479. doi: 10.1038/bonekey.2013.213. PMID: 24466410; PMCID: PMC3899558.

2. Mostafa WZ, Hegazy RA. Vitamin D and the skin: Focus on a complex relationship: A review. J Adv Res. 2015 Nov;6(6):793-804. doi: 10.1016/j.jare.2014.01.011. Epub 2014 Feb 8. PMID: 26644915; PMCID: PMC4642156.

3. Wimalawansa SJ. Associations of vitamin D with insulin resistance, obesity, type 2 diabetes, and metabolic syndrome. J Steroid Biochem Mol Biol. 2018 Jan; 175:177-189. doi: 10.1016/j.jsbmb.2016.09.017. Epub 2016 Sep 20. PMID: 27662816.

4. Nair R, Maseeh A. Vitamin D: The "sunshine" vitamin. J Pharmacol Pharmacother. 2012 Apr;3(2):118-26. doi: 10.4103/0976-500X.95506. PMID: 22629085; PMCID: PMC3356951.

5. Lamberg-Allardt C. Vitamin D in foods and as supplements. Prog Biophys Mol Biol.2006; volume 91(1) 33-38. https://doi.org/10.1016/j.pbiomolbio.2006.02.017.

6. Gani LU, How CH. PILL Series. Vitamin D deficiency. Singapore Med J. 2015 Aug;56(8):433-6; quiz 437. doi: 10.11622/smedj.2015119. PMID: 26311908; PMCID: PMC4545131.

7. Gartner LM, Greer FR; Section on Breastfeeding and Committee on Nutrition. American Academy of Pediatrics. Prevention of rickets and vitamin D deficiency: new guidelines for vitamin D intake. Pediatrics. 2003 Apr;111(4 Pt 1):908-10. doi: 10.1542/peds.111.4.908. PMID: 12671133.

8. Lehmann B, Meurer M. Vitamin D metabolism. Dermatologic therapy. January 21, 2010; 23(1): 2-12. https://doi.org/10.1111/j.1529-8019.2009.01286.x.

9. Araki T, Holick M F, Alfonso B D, Charlap E, Romero C M, Rizk D, Newman L G. Vitamin D intoxication with severe hypercalcemia due to manufacturing and labeling errors of two dietary supplements made in the United States. "The Journal of Clinical Endocrinology & Metabolism" 2011 Dec;96(12):3603-3608. https://doi.org/10.1210/jc.2011-1443.

10. Tatiana Takiishi, Lei Ding, Femke Baeke, Isabella Spagnuolo, Guido Sebastiani, Jos Laureys, Annemieke Verstuyf, Geert Carmeliet, Francesco Dotta, Tom L. Van Belle, Conny A. Gysemans and Chantal Mathieu. Dietary Supplementation with High Doses of Regular Vitamin D3 Safely Reduces Diabetes Incidence in NOD Mice When Given Early and Long Term. Immunology and Transplantation. Jun 2014, 63 (6) 2026-2036; DOI: 10.2337/db13-1559.

11. Petersen V. Dangers of Vitamin D3. Root cause. Medical clinic.

12. Bromley-Brits, K., Deng, Y., Song, W. Morris Water Maze Test for Learning and Memory Deficits in Alzheimer's Disease Model Mice.*J. Vis. Exp* 2011 Jul 20;(53):2920 doi:10.3791/2920.

13. S Battault , S J Whiting, S L Peltier, S Sadrin, G Gerber, J M Maixent. Vitamin D metabolism, functions, and needs: From science to health claims. European Journal of Nutrition. 2012 Aug 12; 52(2):429-441. https://doi.org/10.1007/s00394-012-0430-5.

14. Khan S and Shad K F. Neuroprotective Effects of Curcumin and Vitamin D3 on Scopolamine-Induced Learning-Impaired Rat Model of Alzheimer's Disease. May 12th 2020, DOI: 10.5772/intechopen.92407.

15. Moghadamnia AA, Hakiminia S, Baradaran M, Kazemi S, Ashrafpour M. Vitamin D Improves Learning and Memory Impairment in Streptozotocin-Induced Diabetic Mice. Arch Iran Med. 2015 Jun;18(6):362-6. PMID: 26058931.

16. Kumar A, Dora J, Singh, Anup. A review of spice of life Curcuma longa (turmeric). International Journal of Applied Biology and Pharmaceutical Technology. 2011;2(4):371-379.

17. Groves NJ, Kesby JP, Eyles DW, McGrath JJ, Mackay-Sim A, Burne TH. Adult vitamin D deficiency leads to behavioural and brain neurochemical alterations in C57BL/6J and BALB/c mice. Behav Brain Res. 2013 Mar 15; 241:120-31. doi: 10.1016/j.bbr.2012.12.001. Epub 2012 Dec 10. PMID: 23238039.

18. Liang Q, Cai C, Duan D, Xinyu Hu, Wanhao Hua, Peicheng Jiang, Liu Zhang, Jun Xu and Zhengliang Gao. Postnatal Vitamin D Intake Modulates Hippocampal Learning and Memory in Adult Mice. Front Neurosci 03 April 2018;12 (141): | https://doi.org/10.3389/fnins.2018.00141.

19. Tagliaferri S, Porri D, De Giuseppe R, Manuelli M, Alessio F, Cena H. The controversial role of vitamin D as an antioxidant: results from randomised controlled trials. Nutr Res Rev. 2019 Jun;32(1):99-105. doi: 10.1017/S0954422418000197. Epub 2018 Oct 17. PMID: 30326975.

20. Wiseman H. Vitamin D is a membrane antioxidant. Federation of European Biochemical Societies. July 01, 1993; 326 (1-3): 285-288. https://doi.org/10.1016/0014-5793(93)81809-E.

21. Salari M, Janghorbani M, Etemadifar M, Dehghani A, Razmjoo H, Naderian G. Effects of vitamin D on retinal nerve fiber layer in vitamin D deficient patients with optic neuritis: Preliminary findings of a randomized, placebo-controlled trial. J Res Med Sci. 2015 Apr;20(4):372-8. PMID: 26109993; PMCID: PMC4468453.

22. Etgen T, Sander D, Bickel H, Sander K, Först H. Vitamin D Deficiency, Cognitive Impairment and Dementia: A Systematic Review and Meta-Analysis. Dement Geriatr Cogn Disord. 2012;33 (5):297–305. https://doi.org/10.1159/000339702.

23. Al-Amin M, Bradford D, Sullivan RKP, Kurniawan ND, Moon Y, Han SH, Zalesky A, Burne THJ. Vitamin D deficiency is associated with reduced hippocampal volume and disrupted structural connectivity in patients with mild cognitive impairment. Hum Brain Mapp. 2019 Feb 1;40(2):394-406. doi: 10.1002/hbm.24380. Epub 2018 Sep 25. PMID: 30251770; PMCID: PMC6865549.

24. Brem AK, Ran K, Pascual-Leone A. Learning and memory. Handb Clin Neurol. 2013;116:693-737. doi: 10.1016/B978-0-444-53497-2.00055-3. PMID: 24112934; PMCID: PMC4248571.

25. Wagoner B. "Learning and Memory." Psychology for the Third Millennium: Integrating Cultural and Neuroscience Perspectives. Edited by Rom Harré and Fathali Moghaddam. London: SAGE Publications Ltd, 13 Jul 2012; pp. 116-38. doi: http://www.doi.org/10.4135/9781446288542.n7.

.

26. Kirkpatrick K, Balsam PD. Associative learning and timing. Curr Opin Behav Sci. 2016 Apr; 8:181-185. doi: 10.1016/j.cobeha.2016.02.023. Epub 2016 Feb 17. PMID: 27175441; PMCID: PMC4861159.

27. Michael T. The SAGE Encyclopedia of Abnormal and Clinical Psychology. Classical Conditioning. 2017; 660-663. DOI:10.4135/9781483365817.n256

28. Gupta A, Singh MP, Sisodia SS. A REVIEW ON LEARNING AND MEMORY. Journal of Drug Delivery & Therapeutics. 2018; 8(2):153-157. DOI: http://dx.doi.org/10.22270/jddt.v8i2.1671

29. Schmid S, Poon CS. Nonassociative Learning. In: Seel N.M. (eds) Encyclopedia of the Sciences of Learning. 2012. Springer, Boston, MA. https://doi.org/10.1007/978-1-4419-1428-6_1849

30. Gagliano M, Renton M, Depczynski M, Mancuso S. Experience teaches plants to learn faster and forget slower in environments where it matters. Oecologia. 2014 May;175(1):63-72. doi: 10.1007/s00442-013-2873-7. Epub 2014 Jan 5. PMID: 24390479.

31. D Fioravante, Antzoulatos E. Sensitization and Habituation: Invertebrate. In book: Molecular Mechanisms of Memory, "Learning and Memory: A Comprehensive Reference. January 2008; 31-50. DOI:10.13140/2.1.3071.4561.

32. Norris D. Short-term memory and long-term memory are still different. Psychol Bull. 2017 Sep;143(9):992-1009. doi: 10.1037/bul0000108. Epub 2017 May 22. PMID: 28530428; PMCID: PMC5578362.

33. Cowan N. What are the differences between long-term, short-term, and working memory? Prog Brain Res. 2008; 169:323-38. doi: 10.1016/S0079-6123(07)00020-9. PMID: 18394484; PMCID: PMC2657600.

34. Wittmann M. The inner experience of time. Philos Trans R Soc Lond B Biol Sci. 2009 Jul 12;364(1525):1955-67. doi: 10.1098/rstb.2009.0003. PMID: 19487197; PMCID: PMC2685813.

35. Reynolds JR, West R, Braver T. Distinct neural circuits support transient and sustained processes in prospective memory and working memory. Cereb Cortex. 2009 May;19(5):1208-21. doi: 10.1093/cercor/bhn164. Epub 2008 Oct 14. PMID: 18854581; PMCID: PMC2665160.

36. May CP, Einstein GO, Freedman S. A Five-Day Unit Lesson Plan for High School Psychology Teachers. Memory. 2013:6-7.

37. Al-Azawei A, Lundqvist K. Which factors affect learner achievement? Analysing the role of psychological, surface level, environmental and learner effort variables. International Journal of Education. 2015;7(2):215-31.

38. Anjum I, Jaffery SS, Fayyaz M, Samoo Z, Anjum S. The Role of Vitamin D in Brain Health: A Mini Literature Review. Cureus. 2018 Jul 10;10(7): e2960. doi: 10.7759/cureus.2960. PMID: 30214848; PMCID: PMC6132681.

39. Howard JD, Plailly J, Grueschow M, Haynes JD, Gottfried JA. Odor quality coding and categorization in human posterior piriform cortex. Nat Neurosci. 2009 Jul;12(7):932-8. doi: 10.1038/nn.2324. Epub 2009 May 31. PMID: 19483688; PMCID: PMC2834563.

40. Seger CA, Miller EK. Category learning in the brain. Annu Rev Neurosci. 2010; 33:203-19. doi: 10.1146/annurev.neuro.051508.135546. PMID: 20572771; PMCID: PMC3709834.

41. Meeter M, Radics G, Myers CE, Gluck MA, Hopkins RO. Probabilistic categorization: how do normal participants and amnesic patients do it? Neurosci Biobehav Rev. 2008;32(2):237-48. doi: 10.1016/j.neubiorev.2007.11.001. Epub 2007 Nov 19. PMID: 18226722.

42. Seger CA. How do the basal ganglia contribute to categorization? Their roles in generalization, response selection, and learning via feedback. Neurosci Biobehav Rev. 2008;32(2):265-78. doi: 10.1016/j.neubiorev.2007.07.010. Epub 2007 Aug 12. PMID: 17919725; PMCID: PMC2376049.

43. Brain JG, Burt AD. Abrikosoff's Tumor and the Hepatobiliary System: A Curiosa Revisited. Gastroenterol Hepatol (N Y). 2010 May;6(5):337-8. PMID: 20567591; PMCID: PMC2886489.

44. Dunn AJ. The neurochemistry of learning and memory. Environ Health Perspect. 1978 Oct; 26:143-7. doi: 10.1289/ehp.7826143. PMID: 31280; PMCID: PMC1637250.

45. Ngoc Hieu BT, Ngoc Anh NT, Audira G, Juniardi S, Liman RAD, Villaflores OB, Lai YH, Chen JR, Liang ST, Huang JC, Hsiao CD. Development of a Modified Three-Day T-maze Protocol for Evaluating Learning and Memory Capacity of Adult Zebrafish. Int J Mol Sci. 2020 Feb 21;21(4):1464. doi: 10.3390/ijms21041464. PMID: 32098080; PMCID: PMC7073029.

46. Arakawa T, Niikura T, Arisaka F, Kita Y. Activity-dependent neurotrophic factor, ADNF, determines the structure characteristics of Colivelin, a fusion protein of ADNF9 and Humanin analog. Journal of peptide science: an official publication of the European Peptide Society. 2008 May;14(5):631-6.

47. Kunesová G, Hlavácek J, Patocka J, Evangelou A, Zikos C, Benaki D, Paravatou-Petsotas M, Pelecanou M, Livaniou E, Slaninova J. The multiple T-maze in vivo testing of the neuroprotective effect of humanin analogues. Peptides. 2008 Nov;29(11):1982-7. doi: 10.1016/j.peptides.2008.06.019. Epub 2008 Jul 5. PMID: 18647630

48. Deacon RM, Rawlins JN. T-maze alternation in the rodent. Nat Protoc. 2006;1(1):7-12. doi: 10.1038/nprot.2006.2. PMID: 17406205.

49. Heshmati A, Komaki A, Faraji N, Karami J. Effects of butter and cheese on memory and learning in rats. Nutrition and Food Sciences Research. 2020 Jan 10;7(1):17-24.

50. Ingram DK. Rodent models of age-related memory impairment. InFunctional neurobiology of aging 2001 Jan 1 (pp. 373-386). Academic Press.

51. Wahlsten D. Tests of Mouse Behavior. Mouse Behavioral Testing. Academic Press (2011); 39-51. https://doi.org/10.1016/B978-0-12-375674-9.10003-5

52. Hascoët M, Bourin M. The four-plate test in mice. InMood and Anxiety Related Phenotypes in Mice 2011 (pp. 123-141). Humana Press.

53. Chawla A, Cordner ZA, Boersma G, Moran TH. Cognitive impairment and gene expression alterations in a rodent model of binge eating disorder. Physiol Behav. 2017 Oct 15;180:78-90. doi: 10.1016/j.physbeh.2017.08.004. Epub 2017 Aug 15. PMID: 28821448; PMCID: PMC5597488.

54. Gawel K, Gibula E, Marszalek-Grabska M, Filarowska J, Kotlinska JH. Assessment of spatial learning and memory in the Barnes maze task in rodents-methodological consideration. Naunyn Schmiedebergs Arch Pharmacol. 2019 Jan;392(1):1-18. doi: 10.1007/s00210-018-1589-y. Epub 2018 Nov 23. PMID: 30470917; PMCID: PMC6311199.

55. Nassiri-Asl M, Zamansoltani F, Javadi A, Ganjvar M. The effects of rutin on a passive avoidance test in rats. Prog Neuropsychopharmacol Biol Psychiatry. 2010 Feb 1;34(1):204-7. doi: 10.1016/j.pnpbp.2009.11.006. Epub 2009 Nov 13. PMID: 19914327.

56. Ogren SO, Stiedl O. Passive avoidance. In Stolerman IP, Price LH, editors, Encyclopedia of Psychopharmacology. 2nd ed. Vol. 2. Berlin Heidelberg: Springer-Verlag. 2015. p. 1220-1228 https://doi.org/10.1007/978-3-642-36172-2_160

57. Manabe K. The Skinner box evolving to detect movement and vocalization. Revista Mexicana de Análisis de la Conducta. 2017;43(2):192-211.

58. McLeod S. (2018). Skinner - Operant Conditioning. Retrieved 11 21, 2020, from simply psychology: https://www.simplypsychology.org/operant-conditioning.html

59. Mulder GB, Pritchett K. The Morris water maze. Contemp Top Lab Anim Sci. 2003 Mar;42(2):49-50. PMID: 19757627.

60. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006;1(2):848-58. doi: 10.1038/nprot.2006.116. PMID: 17406317; PMCID: PMC2895266.

61. Nunez J. Morris Water Maze Experiment. J Vis Exp. 2008 Sep 24;(19):897. doi: 10.3791/897. PMID: 19066539; PMCID: PMC2872979.

62. Choobdar S, Vafaei AA, Rashidy-Pour A, Sedaghat K. Protective role of vitamin-D on chronic stress induced-learning and memory deficits in rats. Koomesh. 2019 Sep 10;21(4):708-715.

63. Latimer CS, Brewer LD, Searcy JL, Chen KC, Popović J, Kraner SD, Thibault O, Blalock EM, Landfield PW, Porter NM. Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats. Proc Natl Acad Sci U S A. 2014 Oct 14;111(41):E4359-66. doi: 10.1073/pnas.1404477111. Epub 2014 Sep 29. PMID: 25267625; PMCID: PMC4205629.

64. Babaei P, Shirkouhi SG, Hosseini R, Soltani Tehrani B. Vitamin D is associated with metabotropic but not neurotrophic effects of exercise in ovariectomized rats. Diabetol Metab Syndr. 2017 Nov 15; 9:91. doi: 10.1186/s13098-017-0288-z. PMID: 29177013; PMCID: PMC5688735.

65. Liang Q, Cai C, Duan D, Hu X, Hua W, Jiang P, Zhang L, Xu J, Gao Z. Postnatal Vitamin D Intake Modulates Hippocampal Learning and Memory in Adult Mice. Front Neurosci. 2018 Apr 3; 12:141. doi: 10.3389/fnins.2018.00141. PMID: 29666565; PMCID: PMC5891641.

66. Al-Harbi AN, Khan KM, Rahman A. Developmental vitamin D deficiency affects spatial learning in wistar rats. The Journal of nutrition. 2017 Sep 1;147(9):1795-1805.

67. Taghizadeh M, Talaei SA, Salami M. Vitamin D deficiency impairs spatial learning in adult rats. Iran Biomed J. 2013;17(1):42-8. doi: 10.6091/ibj.1061.2012. PMID: 23279834; PMCID: PMC3600968.

68. Mansouri F, Ghanbari H, Marefati N, Arab Z, Salmani H, Beheshti F, Hosseini M. Protective effects of vitamin D on learning and memory deficit induced by scopolamine in male rats: the roles of brain-derived neurotrophic factor and oxidative stress. Naunyn-Schmiedeberg's Archives of Pharmacology. 2021 Mar 2:1-6.

APPENDIX 1: PROJECT APPROVAL

1993

BAŞKENT ÜNİVERSİTESİ

Tıp ve Sağlık Bilimleri Araştırma Kurulu

TS-EN-ISO 9001
KALİTE SİSTEM BELGESİ

Sayı : 94603339-604.01.02/ 33350 18/11/2020
Konu : Proje Onayı

SAĞLIK BİLİMLERİ ENSTİTÜSÜ MÜDÜRLÜĞÜNE

Fizyoloji Anabilim Dalında görev yapmakta olan Prof. Dr. Nazan Dolu'nun danışmanlığında Sağlık Bilimleri Enstitüsü / Fizyoloji Tezli Yüksek Lisans Programı öğrencisi Taha Hussein Ali Elshahoubi'nin sorumluluğunda yürütülecek olan DA20/18 nolu "The effect of high dose vitamin D on the spatial learning and memory in rats" başlıklı araştırma projesi Kurulumuz ve Hayvan Deneyleri Yerel Etik Kurulunun 02/11/2020 tarih ve 20/16 sayılı kararı ile uygun görülmüştür. Projenin başlama tarihi ile çalışmanın sunulduğu kongre ve yayımlandığı dergi konusunda Kurulumuza bilgi verilmesini rica ederim.

Not: Çalışma bildiri ve/veya makale haline geldiğinde "Gereç ve Yöntem" bölümüne aşağıdaki ifadelerden uygun olanının eklenmesi gerekmektedir.

— Bu çalışma Başkent Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmış (Proje no:…) ve Başkent Üniversitesi Araştırma Fonunca desteklenmiştir.

— This study was approved by Baskent University Ethical Committee for Experimental Resarch on Animals (Project no:…) and supported by Baskent University Research Fund.

DAĞITIM
Sağlık Bilimleri Enstitüsü Müdürlüğüne
Fizyoloji Anabilim Dalına

APPENDIX 2: ETHICAL APPROVAL

1993
BAŞKENT UNIVERSITY

LOCAL ETHICS COMMITTEE FOR ANIMAL EXPERIMENTS DECISION		
SESSION NO	**DECISION NO**	**DATE OF DECISION**
09	20/16	02/11/2020

Project DA20/18 no entitled "The effect of high dose vitamin D on the spatial learning and memory in rats" pending to be conducted by Nazan Dolu with the Department of Physiology has been reviewed and unanimously approved by the Local Ethics Committee for Animal Experiments.