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ERCIYES UNIVERSITY
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PHYSIOLOGY DEPARTMENT

INVESTIGATION OF NMDA RECEPTOR-MEDIATED DEPOTENTIATION RESPONSES IN RATS WITH INTRA-HIPPOCAMPAL INFUSION OF THYROID HORMONE: THE ROLE FOR MAPK SIGNALING PATHWAY

Prepared by

Marwa Wael Yousef

Supervisor

Prof. Dr. Cem Süer

Ph.D. Thesis

December 2018
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DECLARATION

I hereby declare that all information in this thesis has been composed and presented in accordance with the academic and ethical guidelines. I also declare that, as required by these rules and conduct, I have fully and accurately cited and referenced all materials and results that are not original to this work.

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COMPLIANCE WITH THE RESEARCH GUIDELINES

Ph.D.'s Thesis titled **" Investigation of NMDA Receptor-Mediated Depotentiation Responses in Rats with Intra-Hippocampal Infusion of Thyroid Hormone: The Role For MAPK Signaling Pathway"** has been composed in accordance with the Postgraduate Thesis Proposal and Thesis Writing Directions of Erciyes University.

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‘’Neuroplasticity can be defined as the ability of the nervous system to respond to intrinsic and extrinsic stimuli by reorganizing its structure, function and connections; can be described at many levels, from molecular to cellular to systems to behavior; and can occur during development, in response to the environment, in support of learning, in response to disease, or in relation to therapy. Such plasticity can be viewed as adaptive when associated with a gain in function or as maladaptive when associated with negative consequences such as loss of function or increased injury, points illustrated by animal models and some human studies.” (1)

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ABSTRACT

Synaptic plasticity is regarded as the dominant candidate mechanism for synaptic information storage and memory formation in the hippocampus. It comprises multiple forms; long-term potentiation (LTP), long-term depression (LTD) and depotentiation have been subjected to the greatest degree of scrutiny as putative long term memory mechanisms. Long-term potentiation (LTP), once it has been induced, can decay slowly over time, which is prevented by inhibition of α-amino-3- hydroxy-5- methylisoxazole-4- propionic acid AMPA receptor endocytosis and degradation.

In recent years, the contribution of non-genomic effects of thyroid hormones to the normal learning process and memory impairment has been increasingly studied. In this study, it is investigated that the dose-dependent effect of T4 hormone on the reversal of induced LTP at synapses in dentate gyrus as well as that in mitogen-activated kinase (MAPK) pathway. In addition, the amount and activity of Tau protein at the end of the reversal process were evaluated. Therefore, synaptic plasticity was enhanced by using the prior high-frequency stimulation pattern to the perforant pathway, and then the reversal of enhanced synaptic activity was induced by low-frequency stimulation pattern given after 5 min. The hippocampal tissues were removed at the end of the experiment, proteins of MAPK pathway and Tau protein were evaluated by using Western-blot method.

The main findings of this study are that T4 increases the reversal of enhanced synaptic activity at a concentration of 100 nM; this shows that it is associated with a decrease in Erk1 / 2 phosphorylation. Furthermore, at low doses, which do not affect reversal ability of the amplified synapse, T4 increases phosphorylation of JNK while reducing Tau phosphorylation. It was observed that the ability to reversal of the amplified synapses

increased with specific inhibition of Erk1 / 2 and decreased with the application of the JNK inhibitor.

This study demonstrates that the non-genomic effects of T4 hormone may affect the synaptic procedure that underlie physiological learning processes and that the MAPK pathway can mediate these effects.

These non-genomic effects of the T4 hormone can also interact with changes in Tau protein phosphorylation, which can trigger a pathological process leading to Alzheimer's disease.

Key words: T4; depotentiation; hippocampus; MAPK inhibitors; Alzheimer's disease

NMDA RESEPTÖR ARACILIKLI DEPOTANSİYASYON YANITLARINA RATLARDA İNTRA-HİPOKAMPAL TİROİD HORMON İNFÜZYONUNUN ETKİSİNİN ARAŞTIRILMASI: MAPK SİNYAL YOLAĞININ ROLÜ

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Danışman: Prof. Dr. Cem Süer

ÖZET

Sinaptik plastisite, bilgi depolama ve bellek oluşumu için önemli bir mekanizma olarak kabul edilmektedir. Başlıca formları uzun dönemli güçlenme (UDG), uzun dönemli baskılanma (UDB) ve depotansiyasyon olarak olarak bilinen formlardır. UDG, bir kez indüklendikten sonra, kalıcı hale gelebildiği gibi, düşük frekanslı uyarımlar verilerek, sinaptik güç artırılmadan önceki haline de geri çekilebilir. Bilginin öğrenme sürecinin altında yatan mekanizmalardan biri olan bu geri-çekilme, α-amino-3-hidroksi-5-metilizoksazol-4-propionik asit AMPA reseptör endositozunun ve degradasyonunun inhibe edilmesi ile önlenebilir.

Tiroit hormonlarının genomik olmayan etkilerinin normal öğrenme sürecine ve bellek bozukluğu ile karakterli hastalıkların oluşumuna katkısı, son yıllarda artan bir şekilde çalışılmaktadır. Bu çalışmada, T4 hormonun UDG oluşturulmuş Dentat girus sinapslarının geri-çekilme yeteneğini doz bağımlı olarak nasıl etkileyeceği ve bu sürece Mitojen ile Aktive Olan Kinaz (MAPK) yolağının katkısı araştırılmıştır. Ayrıca, geri çekilme sürecinin sonunda oluşan Tau protein miktar ve aktivitesi değerlendirilmiştir. Bunun için, dentat girus hücrelerinin başlıca afferenti olan perforan yol önce yüksek frekanslı uyarım kalıbı kullanılarak sinaptik plastisitenin güçlenmesi sağlanmış; 5 dk sonra verilen düşük frekanslı uyarım kalıbı ile de güçlenen sinaptik etkinliğin geri-çekilmesi indüklenmiştir. Deney sonunda çıkartılan hipokampal dokularında MAPK yolağının proteinleri ve Tau proteini Western-blot yöntemi kullanılarak değerlendirilmiştir.

Bu çalışmanın başlıca bulguları T4'ün, 100 nM konsantrasyonda, güçlenen sinaptik etkinliğin geri-çekilmesini artırdığını; bunun Erk1/2 fosforilasyonundaki azalma ile birlikte olduğunu göstermektedir. Ayrıca, Güçlenmiş sinapsın geri-çekilme yeteneğini

etkilemeyen düşük dozlarında, T4 JNK fosforlasyonunu artırırken Tau fosforilasyonunu azaltmaktadır. Güçlenmiş sinapslarda geri çekilme yeteneği özgül Erk1/2 inhibisyonu ile artarken, JNK inhibitörünün uygulanması ile azalmakta olduğu gözlenmiştir.

Bu çalışma, T4 hormonunun genomik olmayan etkilerinin, fizyolojik öğrenme süreçlerinin altında yatan temel sinaptik süreçleri etkileyebileceğini ve bu etkilerin oluşumuna MAPK yolağının aracılık edebileceğini göstermektedir. T4 hormonunun bu genomik olmayan etkileri, Tau protein fosforilasyonundaki değişmeler ile de etkileşebilir ve bu etkileşim Alzheimer hastalığına kadar giden patolojik bir süreci tetikleyebilir.

Anahtar kelimeler: T4; depotansiyasyon; hipokampus; MAPK inhibitörleri; Alzheimer hastalığı

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FIGURE LEGENDS

ABBREVIATIONS

Aβ: Amyloid-β

AC: adenylyl cyclase

AD: Alzheimer's disease

AMPA: amino-3- hydroxy-5-methyl-4-isoxazole propionic acid

Ant: anterior thalamic nuclei

ARE: AU-rich-element-

ATP-c: direct ATP-competitive inhibitors

BBB: brain–blood barrier

BDNF: Brain-derived neurotrophic factor

CA: cornu ammonis

CaMKII: calmodulin dependent protein kinase II

CaMKIV: calmodulin dependent protein kinase IV

CaMKs: Ca2+/calmodulin-dependent protein kinases

CNS: Central nervous system

COX-2: cyclooxygenase-2

CPPi: cell-permeable peptide inhibitors

CREB: cyclic adenosine monophosphate response element–binding protein

CRE = cyclic adenosine monophosphate response element

D1 and D2: deiodinases types 1 and 2

D3: deiodinase type 3

DAG: diacylglycerol

DG: dentate gyrus

DP: depotentiation

DMSO: Dimethyl sulfoxide

EC: entorhinal cortex

EC: endocannabinoids

EGF: epidermal growth factor

ERAC: Experimental Research and Application Center of Erciyes University

ERK1/2: extracellular signal-regulated kinase 1 and 2

fEPSP: field excitatory postsynaptic potential

FP: field potential

GIRK: G-protein-activated inwardly rectifying K+

Glu: glutamate

HF: hippocampal formation

HFS: High-frequency stimulation

IL-1β: interleukin-1β

iNOS: inducible nitric oxide synthase

IP: infrapyramidal

JNK: c-Jun N-terminal kinase

KA: kainic acid

LFS: low-frequency stimulation

LTD: long term potentiation

LTP: long term depression

MAGUK: membrane-associated guanylate kinase

MB: mamillary bodies

Mct8: monocarboxylate transporter 8

Med: medial thalamic nuclei

MEK = MAPK kinase

MGluR: metabotropic glutamate receptor

MKP: MAPK phosphatase

MLK: mixed lineage kinase

MP: Metaplasticity

MTL: medial temporal lobe

MTT: mamillothalamic tract

Na+ /H+ exchanger: sodium–proton exchanger

NaCl: Sodium chloride

NaOH: Sodium hydroxide

NcoR: nuclear co-repressor

NMDA: N-methyl-D-aspartate

NMDAR: NMDA receptor NMDAR

NO: nitric oxide

NOS: nitric oxide synthase

Oatp1c1: organic anion transporter polypeptide 1c1

PHFs: paired-helical filaments

PI3K: phosphatidylinositol 3-kinase

pJNK: phosphorylated JNK

PKA: cAMP-dependent protein kinase, Protein Kinase A

PKC: Protein kinase C

PLC: phospholipase C

PSM: postsynaptic membrane

PP: perforant pathway

PP1: protein phosphatase 1

PP2A: protein phosphatase 2A

PP2B: protein phosphatase 2B

PS: population spike

SMRT: silencing mediator of retinoid and thyroid hormone receptors

SN, septal nuclei

SRE = serum response element.

SRF- : serum-response-factor-

T3: Tri-iodothyronine

T4: Thyroxin

THs: thyroid hormones

TNF-α: tumor necrosis factor-α

TREs: Thyroid hormone-responsive elements

TR: thyroid hormone receptor

TRα and TRβ: Thyroid hormone receptors

TSH: thyroid stimulating hormone

TRH: Thyroid hormone releasing hormone

TTP: tristetraprolin

1. INTRODUCTION AND PURPOSE

Neuroplasticity isn't formed by learning and memory alone but is also a broker of responses to neuron exhaustion and damage and it is called compensatory plasticity. As a continuous process it interacts to neuronal cell activity and damage, death, and genesis, which includes the modification of structural and functional processes of synapses, dendrites, and axons. The plasticity is found in the following structural parts as long-term potentiation (which can be defined as a cellular correlate of learning and memory), remodeling and synaptic efficacy, axonal sprouting, dendritic remodeling, synaptogenesis, neurogenesis and recruitment (2). The synaptic plasticity's role defects have been emphasized in many disorders characterized with dementia. Generally, the cognitive impairment reasons are not known till now and resulting pathology can lead to death. Hippocampus can be considered as one of the most important brain regions which affected in these diseases (3). Therefore, the factor that cause or trigger dementia is the subject of extensive research. The findings of our previous studies showed that normal thyroid hormone levels may have an important role in the formation of the balance between long term depression LTD and long term potentiation LTP, which both are the indicators of synaptic plasticity.

T4 infusion into the dentate gyrus leads attenuation in LTP and a prolongation in LTD via a non-genomic mechanism. Thus, a role of thyroid hormone for the formation of three important indicators of synaptic plasticity (LTP, LTD and DP), which are the cellular mechanism of learning and memory, and mitogen-activated protein kinases (MAPK) responsible for these effects can be demonstrated. Considering that the underlying mechanisms of learning process of new information and forgetting can be LTP and LTD/DP, respectively (4).

In addition, these findings were confirmed by applying intra-hippocampal thyroxin. It has been also concluded that changes in the activation of MAPK pathway could be responsible from these effect. For confirming these pathways which involved in the T4 responses, MAPKs inhibitors were also infused in hippocampus. However, depotentiation (DP), the third form of synaptic plasticity, has not been studied so far is in this context.

The cascade of MAPK is mediated the extracellular stimulations to intracellular responses (5). It is approved that extracellular signal-regulated kinase 1/2 (ERK1/2), p38-MAPK and c-Jun NH2-terminal protein kinase (JNK), are playing an important role in LTP, LTD, and DP. Nevertheless, the role of p38-MAPK in synaptic plasticity in the dentate gyrus (DG) has been not well characterized yet.

The aim of this study is to investigate that non-genomic effect of thyroid hormones will affect which direction and the relation between Tau protein phosphorylation and electrophysiological activated MAPK pathway which is mimicked by learning and memory. By exploring whether the selective MAPK inhibitors (SB203580 for P38 MAPK, SP600125 for JNK, and PD98059 for MEK) may be implicated in enhancing cognition in the treatment of some dementia disorders. Furthermore, it is thought to be important in the explanation of pathogenesis of the disease characterized by cognitive impairment, such as Alzheimer's disease, and in the discovery of new therapeutic targets.

2. BACKGROUND

2.1. PHYSIOLOGICAL EFFECTS OF THYROID HORMONES

2.1.1. Metabolism of thyroid hormones.

Thyroid follicular cells produce the thyroid hormones (THs) which consist of Tri-iodothyronine (T3) and Thyroxin (T4). The biological active hormone T3, is formed in peripheral tissues after deiodination of T4 by 5`-iodothyronine deiodinases types 1 and 2 (D1 and D2) (figure 1). T4 deiodination and degradation by type 3 deiodinase (D3) and transport through the brain–blood barrier (BBB) have an important effect on bioavailability of T3 in the brain (6-8).

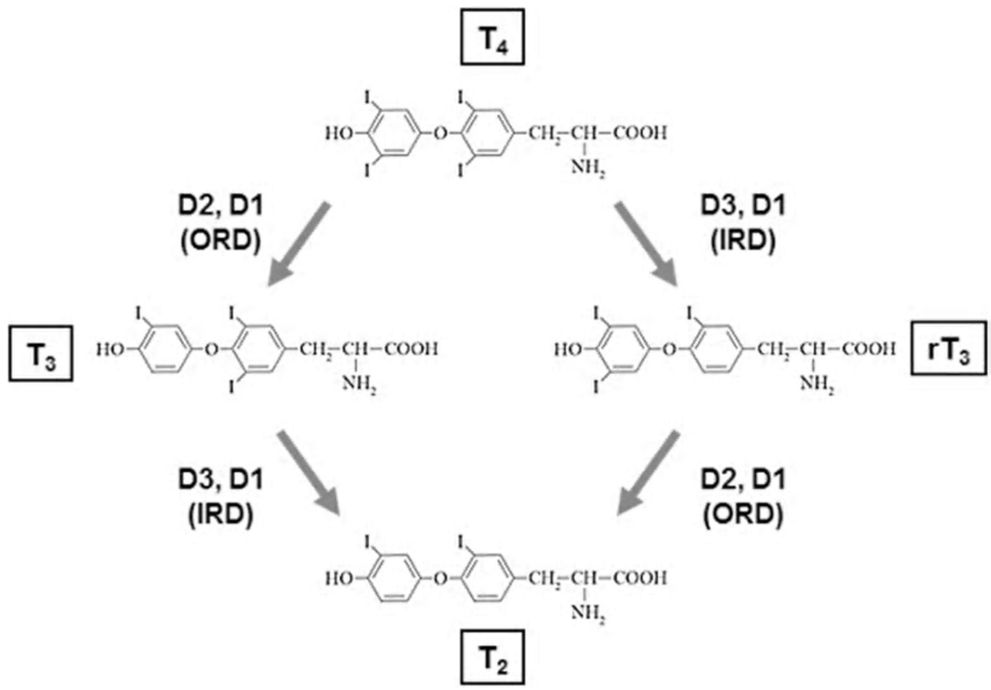

Figure 1. Synthesis of T3 and rT3 through deiodinases.

2.1.2. Genomic and Non genomic effects of Thyroid Hormones

Thyroid hormone has two different signaling pathways:

(i) The nongenomic transcription-independent pathway that tacks place in the cytoplasm.

(ii) (ii) The genomic transcription-dependent pathway that tacks place inside the nucleus.

The genomic actions of thyroid hormone's mechanism occur by binding of nuclear thyroid hormone receptor (TR) with T3 to activate several intracellular responses (9, 10). Thyroid hormone-responsive elements (TREs) in the promoter of different genes bind with 2 types of receptors, TRα and TRβ, and four different isoform, TRα1, TRβ1, TRβ2 and TRβ3.

Nongenomic, or transcription-independent, actions of TH are started at a specific receptor on a cell surface receptor (integrin αvβ3) (11) or at truncated isoforms of nuclear receptor TRα at the plasma membrane or in cytoplasm (12, 13). Through another TR isoform, TH also acts transcription-independently on mitochondria (14). The affinity of the thyroid hormone on αvβ3 is significantly higher for T4 than for T3 (15) in contrast to hormone affinities at TR (16). Mainly, without the need of normal TRs, mediated effects of T4 are started at the membrane and then transduced into nuclear transcriptions as shown in Figure 2.

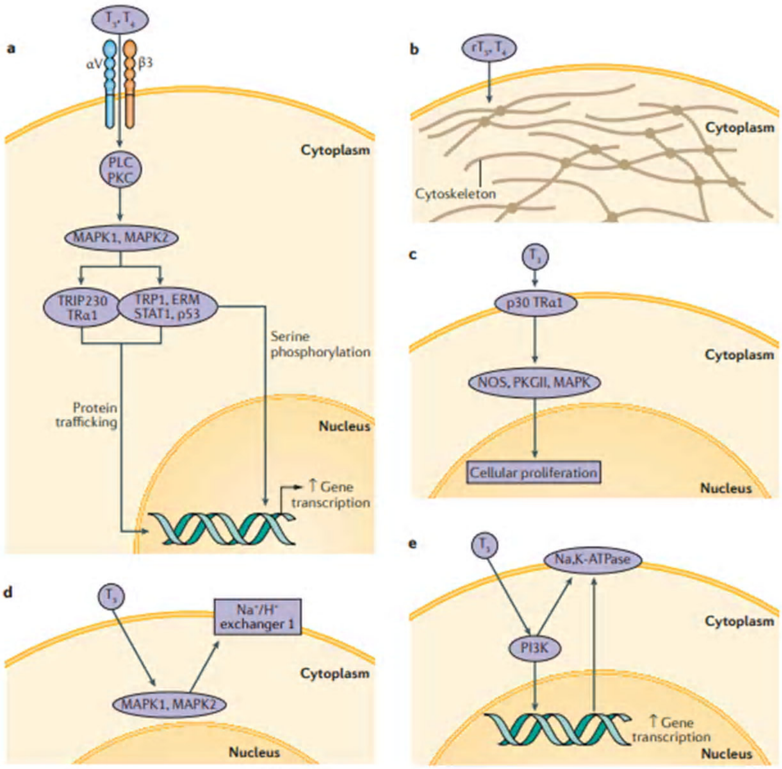

Figure 2. Non-genomic actions of thyroid hormones.

a) TH's nongenomic actions initiate at integrin αvβ3 receptor. T4 mediated effects comprise regulation of intracellular trafficking of specific proteins to the nucleus and serine phosphorylation of some of these.

b) T4 and rT3 regulate the cytoskeleton in cytoplasm.

c) The binding of T3 to a truncated TRα1 at the plasma membrane started cell proliferation. Increasing of cell proliferation occurred by activates nitric oxide synthase (NOS) and a chain of proteins (PKGII and ERK) by TR–T3 complex.

d) T3 through MAPK1 and MAPK2 activate the sodium–proton exchanger (Na^+ /H^+ exchanger) in the plasma membrane.

e) Through phosphatidylinositol 3-kinase (PI3K)/AKT by T3 can make local activation of the plasma membrane sodium pump (Na, K-ATPase).

2.1.3. Effect of Thyroid Hormones on Brain

Thyroid hormones (THs), tri-iodothyronine (T3) and thyroxin (T4) have major effects on the brain developing and mediate remarkable events in the central nervous system (CNS) throughout life. Diseases as cretinism and mental retardation are caused by insufficient maternal iodine intake through pregnancy and TH decrease during development of human. Throughout brain maturation a lack, or deprivation, of THs causes molecular, morphological and functional changes in the hippocampus, cerebral cortex and cerebellum. Untreated hypothyroidism in newborn humans leads to a notable phenotype change as mental retardation, deafness, and dwarfed stature which is called cretinism (17).

In puberty, thyroid dysfunction leads to memory impairment by causing neurological and behavioral abnormalities. The cognitive impairment which is caused by dysfunction of thyroid relies on hippocampal modifications. Lack of THs throughout puberty cause changing in the function of hippocampal synapses and damage the hippocampus-dependent memory and learning behaviors (18). Several tasks which depend on hippocampal spatial memory like Morris water maze, radial arm maze, object recognition tasks and contextual fear conditioning showed negative results with hypothyroidism patients. These abnormal changes in behaviors and cognition come correlated with biochemical and biophysical changes in the hippocampus (19, 20).

Generally, here we are going to investigate some important functions of THs (i) acceleration of myelination, (ii) effect cell migration, (iii) control of cell differentiation and maturation. On the other hand, THs regulate the expression of many genes and protein transcription as well, like neuromodulin/GAP-43 and Ca^{2+} /calmodulin-dependent protein kinase II, which regulate different forms of synaptic plasticity and formation of memory (21-23).

2.1.4. Relationship between Thyroid Hormones and the Hippocampus

Mainly, the organic anion transporter polypeptide 1c1 (Oatp1c1) and monocarboxylate transporter 8 (Mct8) help the entrance of T4 and T3 through BBB (figure 3). The transportation of T4 through the BBB and the astrocytes and also accelerating T4 deiodenation to produce T3 in them is occurred by Oatp1c1 (24). The BBB of primates has low level of OATP1C1 protein, for that reason they depend on MCT8 for transporting of thyroid hormone. So, mutations of MCT8 in humans cannot be recompensed as in rodents for Transportation of T4. As we mentioned before the thyroid hormone receptors (TRα and TRβ) are expressed during the developing of brain, and activate the gene expression. Also there are TR-subfamily isoforms which have specific actions. Specifically, TRα1-T3 complex activates for example. Throughout the development from fetal stages till maturation, gene expression is regulated by T3. It can be considered that thyroid hormone regulates directly and/or indirectly most of genes.

T3 regulates around 5% of all expressed genes in CNS, and around one third of them are controlled directly at the transcriptional level (25).

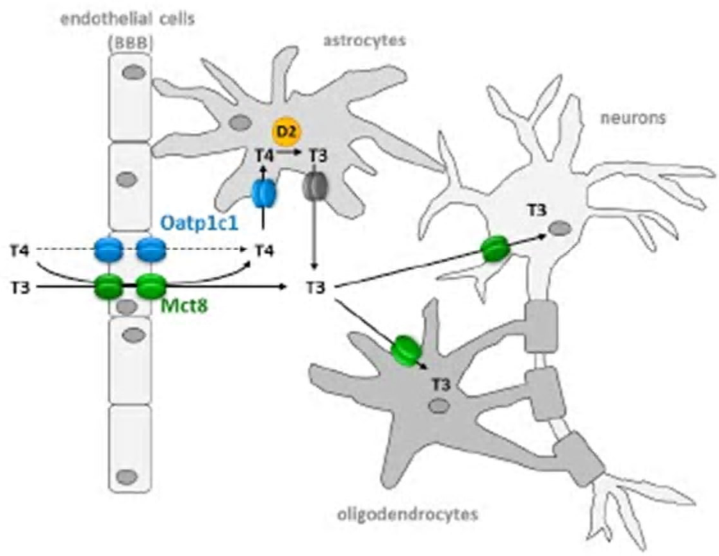

Figure 3. Transportation of THs is facilitated by Oatp1c1 and Mct8 through BBB

The hippocampus, which is an important part of the brain which responsible for memory consolidation and learning as well (3), is considered to be an important target of TH (26). The mature brain neurons contain large amounts of TRα1 protein levels. Therefore, these proteins can be found in hippocampus especially (the dentate gyrus and CA1–3 regions) (27). Also, there is found many integrin proteins such as (a3, a5, a8, av, b1, b3, and b8) especially located at synapses of the hippocampus and/or cortex (28-30). Previous findings showed that integrin in CNS have a major role for the cohesion receptors during cell migration (31) development (32), and cellular positioning (33). It is approved that in the pubescent, synaptic transmission is modulated by integrin as well.

It has been reported that induction of hypothyroidism reduces the number of granulocytes in dentate gyrus (34) and density of pyramidal cell spine (35) and also cause kainate-induced gene expression alterations in the hippocampus (36, 37).

Hyperthyroidism, initiated at birth, produces an infrapyramidal (IP) bundle of mossy fibers in stratum oriens of field CA3a-b of Ammon's horn.

2.1.5. Thyroid hormone and the N-methyl-D-aspartate receptors (NMDA) receptor

The amino acid glutamate (38) has important effects in normal and impaired CNS. Metabotropic and ionotropic are considered the main types of Glu receptors. The ionotropic receptor group has 3 subtypes (i) amino-3- hydroxy-5-methyl-4-isoxazole propionic acid (39), (ii) N-methyl-D-aspartate (NMDA), and (iii) kainic acid (KA) where Glu initiate the cell response via them.

NR1, NR2 (A, B, C, D), and NR3 (A and B) are the subunits of NMDA receptors (40).

In the hippocampus of rodents, THs control the NR1, and mRNA expression of NR2B subunit. It is suggested that in the NR1 and NR2B gene expression regulatory apparati possess a hormone response element (41), or a TH responsive protein. A hypothyroid cause decrease in the level of the NMDA receptors in the hippocampus would lead to significant physiological alternations in hippocampus like LTP or LTD and also cause impairing cognitive tasks which depend on hippocampus as Morris water maze (42). In addition to that the previous finding approved also the decreasing or dimension in the expression of NR2B mRNA is occurred because of hyperthyroidism. Therefore, a change in the expression of glutamatergic receptor subunit mRNA following adult-onset hypothyroidism could provide a hypothetical explanation for the decline in cognitive performance noted in animal and human research.

2.1.6. Thyroid hormone and MAPK

As reported previously, thyroid hormone binds with the plasma membrane via the integrin receptor then the intracellular signal transduction is started (43). This means that many processes are initiated like regulate the movement of solutes as ions and small molecules across the cell membranes) (44) figure 4.

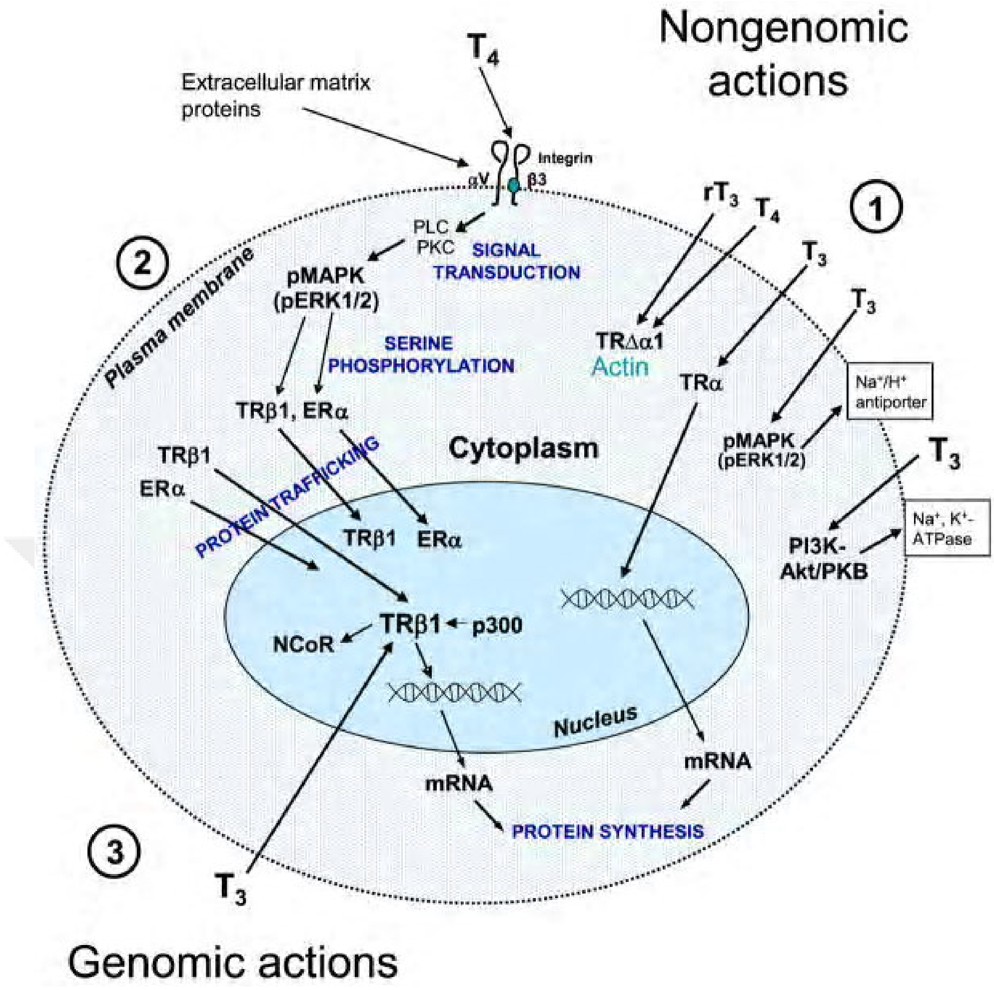

Figure 4. The nongenomic and genomic actions of thyroid hormones.

At the top of the image nongenomic mechanism of T4 and T3 are started by binding with integrin αvβ3 receptor. Number 1 illustrates that through the process including TRα1 T3 controls the actin cytoskeleton in cytoplasm. However, T3 acts locally in the plasma membrane to regulate the activity of Na+/H+ exchanger in the plasma membrane, the hormone may act genomically to make gene expression in the nucleus. In the same time, T3 initiate a local activation for Na^+, K^+- ATPase which are found in the plasma membrane through phosphatidylinositol 3-kinase (PI3K/Akt/PKB), but on the other hand the sodium pump gene transcription is genomically affected by the THs. Number 2 thyroid hormone nongenomically drives cytoplasmic TRβ1 and ERα into the cell nucleus, each in a complex with activated MAPK (pMAPK; pERK1/2). Nuclear TR is partially readied for transcriptional activity i.e., prepared to act genomically when, in response to nongenomic signaling from hormone at αvβ3, it sheds co-repressors and attracts co-activator proteins and is able to bind nuclear T3. Number 3 is normal genomic mechanism of T3, where the hormone goes into the nucleus and combines with TR to accelerate and express many genes.

The trafficking of distinct proteins from cytoplasm into the nucleus process is initiated and regulated from integrin αvβ3 (45). Also, the activation of integrin αvβ3 leads to signal transducing kinases stimulation such as MAPK, or ERK1/2 and Akt (which have downstream targets in nuclear compartment) (46) and transcription factors activation which control process of genes expression.

The binding of L-thyroxin (T4) (47) with the plasma membrane integrin $\alpha v\beta 3$ receptor stimulate nongenomically intracellular targets proteins for example MAPKs (47) after that, MAPKs proteins phosphorylate serine which can activate many nucleoproteins for transducing several genes. G protein-coupled receptor in the plasma membrane (47) starts the influence of T4 (47) and that has been evaluated in different cells which have TRs in their nucleus.

In contrast, the binding of T3 with specific nuclear receptors activates genomically the transcription of specific genes. T3-TR complex binds with retinoid X receptor to TREs as a monomer, a homo- or heterodimer for regulating upstream parts of certain hormone-responsive genes (10, 48, 49). In contrast, the binding of unliganded TR with the co-repressor proteins, silencing mediator of retinoid and thyroid hormone receptors (SMRT) which binds with TRs between 211 till 240 amino acids (50) and nuclear co-repressor (NCoR) requires the absence of T3 to bring TR in the inactive (repressed) state (51). In the presence of T3, TR can bind with it and that causes the separation of co-repressor proteins from TR as well as the inductions of activator proteins which initiate the transcriptional activity (52).

Previous findings explained the TR isoforms serine phosphorylation (53-57). In these researches, the activity of cellular cAMP-dependent protein kinase (PKA), serine kinase inhibition or phosphatase inhibition in treated cells or a serine/threonine kinase indicated the presence of phosphorylation process (53, 54). Also, the reduction of TR monomer binding to specific gene areas presented the serine phosphorylation of TRa1 (53). TRb1 is selectively stabilized against protease degradation by serine phosphorylation and transcriptional activity of the receptor is significantly increased (56, 57). Previous studies (54) were resulted that the phosphorylation process increased TRb1 transcriptional activity. Katz et al. (58) compared the phosphorylatable and nonphosphorylatable forms of TRa2 (58) and summarized that serine phosphorylation of the receptor isoform reduced its ability to heterodimerize with retinoid X receptor at TRE site. Using a serine/threonine kinase inhibitor, H7, Jones et al. (59) showed a reduction in T3-induced transcriptional activity of both TRa1 and TRb1.

2.1.7. Thyroid hormone and degenerative disease

Alzheimer's disease (AD) is an advanced neurodegenerative disease with complex pathophysiology and multifactorial etiology. The "cholinergic hypothesis" of AD disease is characterized by distinctive reduction of acetylcholine and presynaptic cholinergic markers in hippocampus and cortical neurons of AD brain (60, 61). Previous researching by utilizing magnetic resonance spectroscopy or single photon emission computed tomography had detected that there is identity between the results of AD and hyperthyroidism patients with cognitive dysfunction (62-64). Likewise in AD, hyperthyroid brain showed decreasing in choline levels. After administering anti-thyroid therapy, choline levels showed piecemeal recovery (62).

It has been approved that, there is association between AD and other dementias and changings in the endocrine system. Several studies in the progressing of dementia included resistance of Insulin (65), increasing cortisol levels (66) and decreasing estrogen levels (67) and testosterone (68) levels. The serum thyroid stimulating hormone (TSH) level is the most important test for evaluating dementia patients (69) where the hyperthyroidism and hypothyroidism patient always associated with cognitive impairment (70, 71). Otherwise, many studies reported that increase or decrease in the levels of TSH is associated with cognitive dysfunction, however some other researches failed to approve this relation (72, 73). Recently there is strong evidence that any dysfunctions in thyroid like hypo- (72, 73) and hyperthyroidism (74) causes a high risk for the dementia development.

In the contrast, giving T4 gave rise to an improvement of learning potency in rodents that associated with rising in cholinergic performance in the hippocampal and cortical cells (75). Curiously, the cholinergic pathways of the cortex and hippocampus are strongly affected by TRH (Thyroid hormone releasing hormone) (76). Intraseptal and intracerebroventricular infusion of TSH propagated the change of acetylcholine in rodent hippocampal and parietal cortıcal cells, respectively (77, 78). Hence, acetylcholine reduction could be caused by decreased TRH levels, along with the cognitive alterations in AD.

Alzheimer disease is remarked by the β amyloid plaque, and the neurotoxicity which caused by β amyloid characterized with inactivation of acetylcholine in the cortex and hippocampus, oxidative stress which caused by free radical damage, abnormalities on mitochondria, and finally apoptotic cell death (79-82). Parallel to that, thyroid hormones had been shown to affect the systemic oxidative stress. Experimental and clinical researches had been reported that in hyperthyroidism, there is increasing in reactive oxygen species and lipid peroxidases and that affects the anti-oxidative enzymes (38, 83). Otherwise, T3 influences the formation of certain β-amyloid precursor protein isoforms, which are formed especially in the brain of AD patient (84). So, thyroid hormones have an effect on AD by playing a critical role in regulating the intracellular and extracellular component of β-amyloid precursor protein isoforms (84, 85). In hypothyroid rats, falling of LTP induction illustrates the role of TH.

2.2. KEY STRUCTURE OF MEMORY: THE HIPPOCAMPUS

2.2.1. Basic Anatomy

The large reciprocal network of areas that contain neocortical association areas, the medial temporal lobe (MTL), subcortical nuclei, parahippocampal areas, and the hippocampal formation play an important role in encoding, consolidation and retrieval of mnemonic information (memory) (Figure 5). The research model is used by rat hippocampal area for exploring both ordinary and pathological cases, including the processes involved in memory and learning and neurodegenerative diseases. The C-shaped structure of the rat hippocampal formation is located in the caudal part of the brain. The cortex that forms the hippocampal formation has a three-layered appearance, typical of the so-called allocortex.

The hippocampal region is subdivided into two major parts:

(i) Hippocampus proper and dentate gyrus: Hippocampus proper includes a series of Cornu Ammonis (CA) areas: CA1, CA2, CA3 and CA4. The CA areas are all filled with densely packed Pyramidal cells similar to those found in the neocortex.

(ii) The parahippocampal formation:

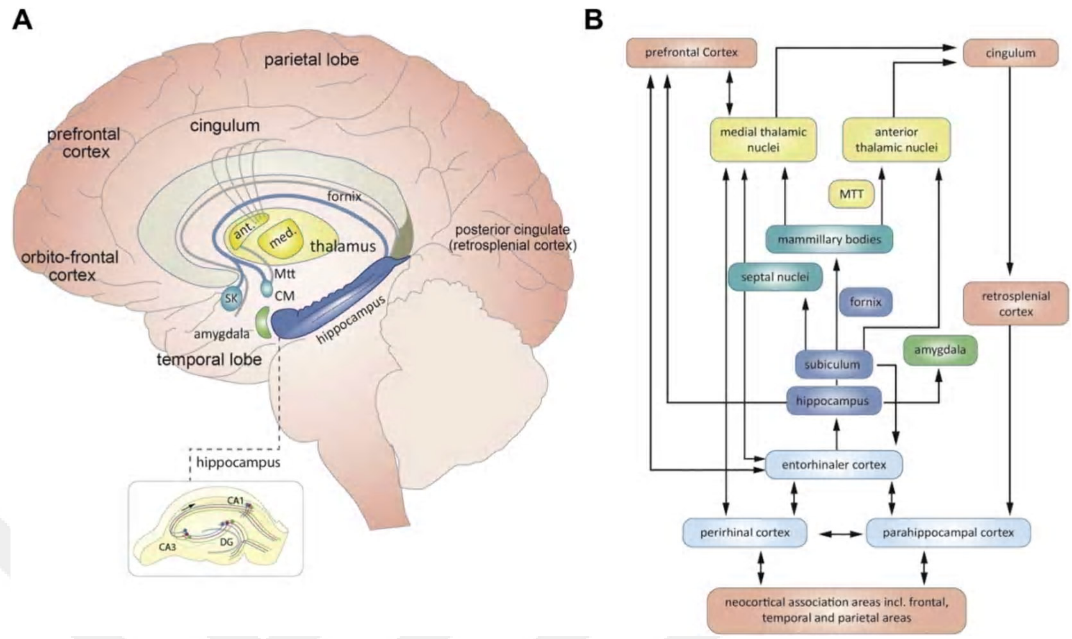

Figure 5. (A) Illustrate the hippocampus structure and other brain regions which responsible for learning and memory. (B) Scheme illustrate the important structures of memory ın different brain areas

2.2.2. Basic Hippocampal Circuit

Three-layered allocortical temple of the hippocampus is alternately linked to other regions like cortical and subcortical (Figure 5, 6). From all neocortical association areas, it can receive input throughout perirhinal and parahippocampal cortices and eventually through the entorhinal cortex (EC) (86).Through the hippocampus, the main paths of the signal can flow and merge to form a loop. Through the axons, which form a structure called perforant path, the outer input comes from the neighboring entorhinal cortex. These axons get up from second layer of layer 2 of EC and terminate in the DG and CA3. There is a special path between the third layer of the CA1 and EC, point to the temporoammonic or TA-CA1 pathway. Axons of granule cells in the DG (known as mossy fibers) were sent to CA3. Pyramidal cells of CA3 send to CA1 their axons (Schaffer collaterals). Pyramidal cells of CA1 were sent to the subiculum and deep layers of the EC their axons. Subicular neurons were sent to the EC their axons. Perforant makes a connection path to-DE to CA3-to-CA1 where Per Andersen nominated it the Trisynaptic circuit (Figure. 6) (87, 88).

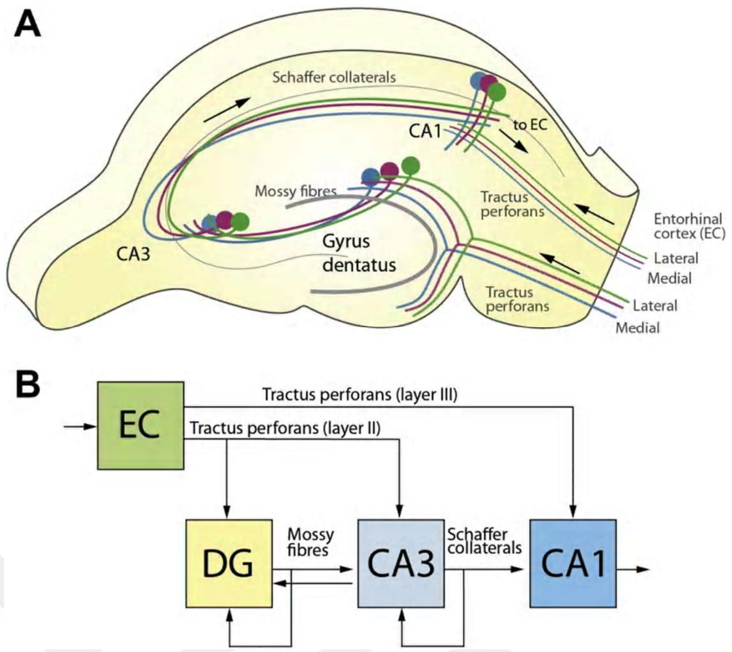

Figure 6. (A) shows the anatomy of the hippocampus with descrying the tri-synaptic pathways which response of processing the information through the hippocampus. (B) shows a diagram which describe how the three different pathways connect the different hippocampal compartments.

2.2.3. The role of the dentate gyrus in the circuit of hippocampus:

Dentate gyrus is considered as an entrance of the hippocampus, where it lays in the center of Trisynaptic circuitry and exerts filtering incoming excitation from the entorhinal cortex. Although, the function of DG is still unclear (89), any damage in the DG affect associative and spatial learning (90-93). Early reports illustrated by modeling work of hippocampal circuitry (94) that the dentate gyrus is one of the most important compartments for pattern separation, the capacity to differentiate between identical events. Pattern separation is a necessary cognitive characteristic and is considered to outcome from non-overlapping performance of provisional and spatial signs of comparable experiences. Pattern separation is occurred by encoding of the memories with increases their independency and reduces reciprocal involvements. The characteristics of granule cells (as showing low rate of firing) prepared them for the pattern separation feature (95, 96). Theoretically, various types of granule cells will encode identical inputs, and they outnumber both their presynaptic entorhinal partners and their postsynaptic CA3 pyramidal targets by an order of magnitude (in rats there are around 112,000 principal cells in layer II of the entorhinal cortex, 250,000 CA3 pyramidal cells and 1,200,000 granule cells).

Efficiently, the DG is implicated in spatial pattern separation feature (97), and Pattern completion is another information-processing function of the hippocampal network, and both are fundamental features of episodic memory (98). The increasing of the performance was easily erased at the synapses between granule cells. the encoding of new data leads to the replacement of identical previously stored memory, that leads to specific pattern separation. In addition, normal physiological processes as LTP and LTD involve in the pattern separation and pattern completion influences. So cognitive impairments can be resulted from unbalance between LTP and LTD (99), (100). Taken together, we concluded that difficulty to express LTD / DP makes the DG possible to discriminate between overlapping memory engrams.

2.2.4. Field Potentials in the Hippocampus

Both a positive-going synaptic component (field excitatory postsynaptic potential, fEPSP), and a superimposed non-synaptic component (population spike, PS) is formed the hippocampal field potential (FP). The somatic response is measured by PS and PS amplitude is affected by the number of granule cells that discharge in synchrony. Moreover, the EPSP is a measure of postsynaptic depolarization which makes the post synaptic neuron more likely to fire an action potential. Neuroplasticity (which consists of both synaptic plasticity and non-synaptic plasticity) means the changes in neural activities and synapses. In fact, the generation of an action potential is the essential output of neuronal activity. The capacity to fire action potentials depends on the excitatory synaptic dynamics, inhibitory control, and intrinsic membrane properties. The plasticity of excitability in the DG has been neglected. In addition, in the CA1 area of the hippocampus (101) and mature granule cells of the DG (102) are observed PS potentiation in the absence of any potentiation of the synaptic response. Adding to that, without LTD (103), sometimes low frequency stimulation (LFS) leads to a strong PS potentiation along with a reduction in fEPSP slope in the DG (104, 105). This case referred to that in the DG is established a particular form of neuroplasticity where the output of neurons can be potentiated by a process independent of changes in synaptic strength (102, 106, 107). This form of plasticity which called metaplasticity in the DG especially is not well studied.

The perforant pathway (PP) is the main afferent connection to the hippocampus, and connects the entorhinal cortex with the granule cells in the DG. Electrical stimulation that is relatively confined to the PP produces synchronized activity of many granule cells which can be extracellularly recorded as a field potential (FP) by a glass microelectrode figure 7. As it mentioned before, The FP includes both of fEPSP with PS.

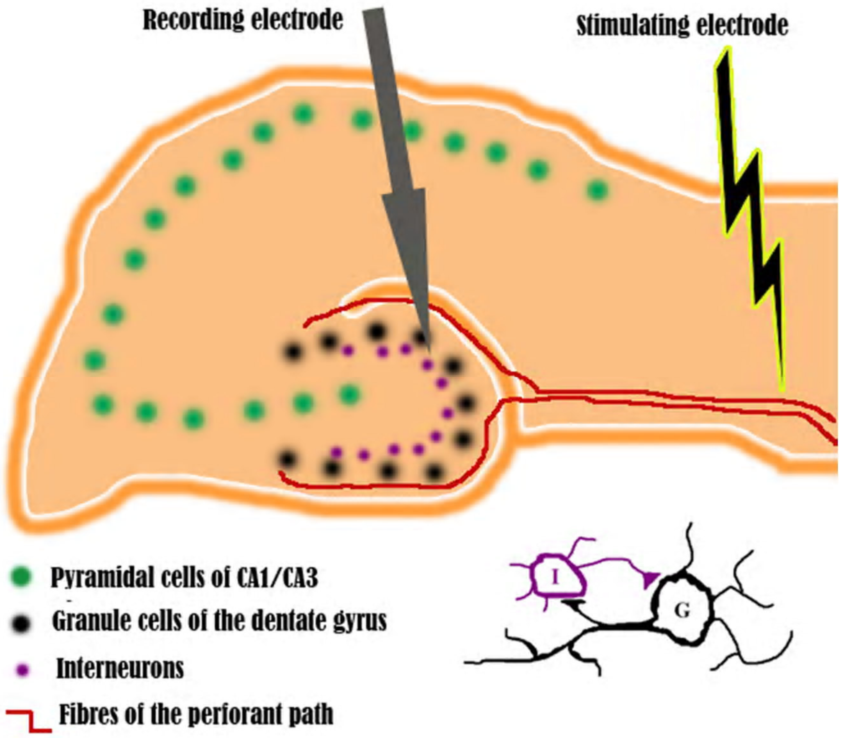

Figure 7. The hippocampal areas where electrodes can be inserted for recording (108).

Although both PS and EPSP can be potentiated LTP, PS potentiation in the absence of any potentiation of the synaptic response can be observed in both the CAI area of the hippocampus (101) and mature granule cells of the dentate gyrus (102). Moreover, LFS may in some circumstances result in a robust and durable PS potentiation with a depression of fEPSP slope in the DG (104, 105), indicating neuronal output can be potentiated by a process independent of changes in synaptic strength. This form of non-synaptic plasticity, which is known as E-S potentiation is often induced concurrently with synaptic LTP as shown by greater PS potentiation relative to fEPSP slope potentiation (109).

Comparing with many areas in Hippocampus of mature rats, creating synaptic plasticity in granule cells of DG is found to be relatively difficult (110, 111), and potentiation of the PS in the DG depends largely on a change in the EPSP/spike relationship (106) (102). Notwithstanding, according to the level of potentiation which induced by EPSP, CA1 pyramidal cells stimulation has been regulated (39). So, E-S potentiation in the DG might play an exclusive role in the formation of hippocampal function (107).

2.3. CELLULAR AND MOLECULAR BASES OF MEMORY: SYNAPTIC AND NEURONAL PLASTICITY.

The strength of brain synapses can be altered or adapted according to neuronal firing with forming neuroplasticity. The formation of memory is strongly dependent on this neuronal plasticity as well as the brain development.

2.3.1. Synaptic and Neuronal Plasticity

Neuroplasticity is considered the strength of synapses to adapt and organize the structure or function in response to internal or external activations (1). One of the essential features of neurons and circuits in hippocampus is that can be undergone neuroplasticity mechanisms. Formation of new neurons, alternations all of dendritic tree size, spines and number of synapses are depending on the hippocampal neuroplasticity (2).

In the case of advanced pathology likewise as in AD in humans is contained neuroplasticity as well as in physiology (112). Neuroplasticity plays an important role in the hippocampal activities modifications, especially on the network level. So, all the changes which derived on behavior, structure, and connections between hippocampal neurons are resulted by it (113, 114).

This case can be occurred because of the stimulation of glutamatergic pathway which considered to be an essential feature for neuroplasticity can also cause metabolic injuries which leads to many psychiatric disorders (115). This view is reflected in the suggestion by Bruce McEwen that ‘the plasticity of the hippocampus is the reason for its vulnerability’ (116).

2.3.2. The forms of plasticity

Two well-known types of activity-dependent plasticity are the long-term potentiation (LTP) which represents persistent increase and the long-term depression (LTD) which represents a reduction in synaptic weight. In the dentate gyrus, LTP is induced by applying High-frequency stimulation (HFS) (100 Hz) to the perforant pathway; however, LTD is induced by applying low-frequency stimulation (LFS) (1 Hz). The reversal of synaptic strength from the potentiated state to pre-LTP levels has been termed "depotentiation". Induction of DP in hippocampal neurons occurred by applying a stimulus which cannot induce LTD to synapses which received priming stimulus. DP mechanism is so intrinsic to avoid synaptic potentiation overload and raise the ability of memory formation. So, in the case of receiving the rat hippocampal neurons multiple tetani, the spatial learning and memory are stopped with failing in inducing LTP (117). Consequently, the memory formation process gets affected with the saturation of the synaptic modification (118). One of the best brain areas for investigation of neuroplasticity with their different models like LTD, LTP, DP, and Metaplasticity (MP) is the hippocampus (119, 120). These modifications are therefore activity-induced enhancement or reduction of synaptic strength and thereby neuronal output.

Prolonged stimulation of synaptic transmission is built on the induction of LTP. LTP has been a source of great fascination to neuroscientists since its discovery in the early 1970's (121) because it satisfies criteria proposed by Donald Hebb for a synaptic memory mechanism in his influential book 'The Organization of Behavior' (122). Remarkably, the complementary process of LTD, in which the synaptic transmission ability is decreased, participates these features and has also received much attention as a candidate mnemonic process (123, 124). LTP is long-lasting and input-specific changes can be induced at one set of synapses on a cell without affecting other synapses (123, 124). Adding to these models, DP (which is induced by strong stimulation) also can be transformed into a further decreasing form if a LFS is applied after LTP induction via HFS. As mentioned before, DP or "reversal of LTP" avoid the saturation of synaptic potentiation and increase the efficiency and the capacity of memory formation (125) (126). At the molecular levels, there is similarities between DP, LTP and LTD, in calcium influxes, activation of specific enzymes, and glutamate receptor trafficking, but several studies showed differences between DP and LTD (127).

2.3.3. Physiology of NMDA and AMPA receptors

NMDA receptors are a voltage gating channel receptor. Their channels allows passage of different cations as Na^+ and K^+, and in some cases small amounts of Ca^{2+}. NMDA receptors contain many subunits as NR1 and NR2 subunits where the NR2 subunit controls the opening and closing of the channel gate. On the other hand, the AMPA receptors are includes of GluR1-4 subunits (128). NMDA and AMPA receptors are considered the most important glutamate receptors which localized in the post synaptic membrane. When glutamate is released from the presynaptic membrane binds with these receptors to initiate the neural response.

The formation of new neural networks is started by activation of NMDA receptor ‘coincidence detector’ when bind with glutamate to create synaptic plasticity. At -65 mV (resting membrane potential), magnesium ion (Mg^{2+}) obstructed the NMDAR then it can’t bind with glutamate. After the depolarization of the postsynaptic membrane by afferent stimuli, AMPA receptor activation occurs along with presynaptic glutamate releasing, NMDAR becomes responsive to glutamate and thereby calcium influx through the NMDA receptor triggers the active insertion (or removal) of AMPA-type glutamate receptors (Figure 8) (129).

During the induction of LTP, AMPA receptors are rapidly inserted into the post-synaptic membrane. Consequently, the amplitude of synaptic currents is increased as a result for rising in synaptic strength. In contrast, during induction of LTD, both AMPA and NMDA receptors are taken off from the post synaptic membrane by endocytosis. Therefore, the amplitude of synaptic currents is reduced as a result of reduction in synaptic strength. Receptors can be again reinserted to the post synaptic membrane or are digested by lysosomes. It is supposed that the neuroplasticity which known also as synaptic strength is dependent on the insertion or removing of AMPA receptors from PSM throughout LTP or LTD. So the formation of synaptic plasticity is dependent on AMPA receptors activities, however NMDA receptors just regulate it. Otherwise, the metabotropic glutamate receptor (mGluR) can also initiate LTD induction(130, 131).

2.3.4. Direction of Synaptic Plasticity

It has been proven that the magnitude of expressed neuroplasticity depends on the postsynaptic stimulation of NMDA receptor (NMDAR) (124, 132, 133). The LFS-dependent LTD causes modest NMDAR activation, while HFS- dependent LTP stimulates potent activation for NMDAR. As a consequence of NMDA activation, the Ca^{2+} permeability is initiated through its channel via PSM. There are several evidences indicate that the neuroplasticity is strongly affected by the trafficking of Ca^{2+} through the membrane (134-136). Although the applying of LFS causes modest Ca^{2+} dynamics through the post synaptic membrane (PSM), the applying HFS results in high calcium transportation. In agreement with Ca^{2+} hypothesis, chelating postsynaptic calcium with EGTA blocks both high-frequency induced LTP and low-frequency-induced LTD (132, 137), and releasing of calcium alone can stimulate the occurrence of LTP or LTD (138) according to its concentration, and both effects are blocked by electrically induced plasticity (133).

2.3.5. Temporal Phases of Synaptic Plasticity

The expression of LTP phases has sharing in behavioral memory formation (139). In addition, LTP is divided into 2 phases; each can be distinguished on the basis of their induction, expression time and molecular mechanisms. An early-phase LTP (which depends on modification of pre-existing proteins) decays over a few hours and the inhibition of de novo protein synthesis does not block it, while a late-phase LTP is strongly dependent on new protein synthesis and remains stable for many hours or days. The induction necessities for both of phases are different. A single train of HFS induces only decaying LTP, but three or four spaced trains of stimuli, are needed to recruit non-decaying LTP. De novo protein synthesis occurs between 15–45 min after HFS applying (140, 141).

2.3.6. Molecular Processes Responsible of both LTP and LTD

The occluded induction of LTP (without effect on its preservation) is occurred by using NMDAR antagonists as AP5 or CP (142). In addition, prevention of NMDAR expression in the CA1 pyramidal cell population stops LTP induction, as well as the expression of several forms of hippocampus-dependent memory (143). Interestingly, this antagonism also blocks the induction of hippocampal LTD (124, 132) demonstrating that the two forms of plasticity share a common NMDAR-dependent induction mechanism.

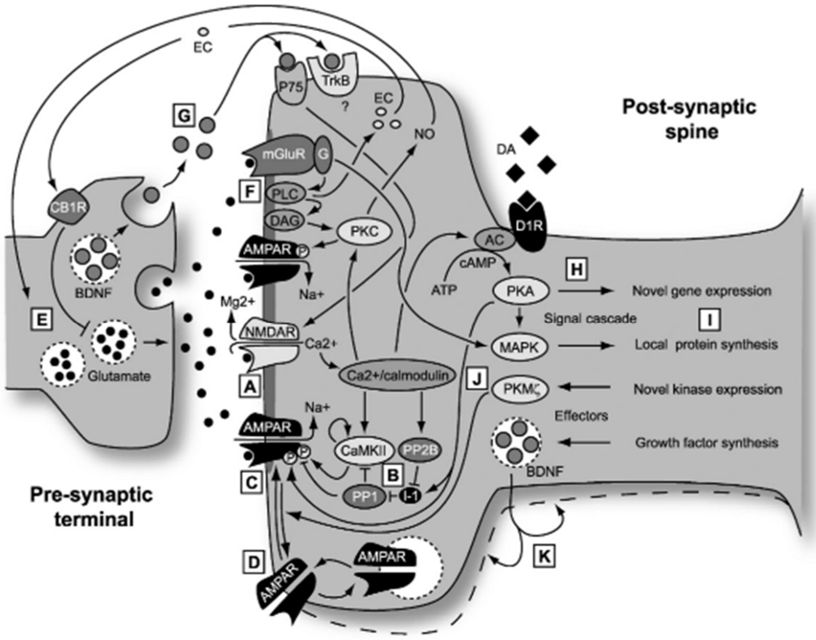

Figure 8. Mechanisms of LTP and LTD at molecular level.

(A) The Stimulation of the NMDAR by binding with glutamate neurotransmitter starts depolarization of postsynaptic membrane (PSM) and dissociates Mg2+ ions that block its ionophore at near-resting membrane potentials. Then the dynamics of Ca2+ through the channel of these receptors initiate the synaptic plasticity.

(B) Abundant phosphatases and kinases such as calmodulin dependent protein kinase II (CaMKII), PKC and Calcineurin (PP2B) (directly) and PKA and PP1 (indirectly) are activated by the binding of Ca2+ with Ca2+/calmodulin as well as the balance between them is dependent on the calcium level. The movement of Ca2+ detects the polarity of the induced plasticity, where a low level of Ca2+ induces LTD and high level of it induces LTP.

(C) Phosphorylation of the AMPA receptor induces LTP expression through NMDAR activations at EPSP. In contrast, the dephosphorylation of the AMPA receptor by phosphatases induces LTD.

(D) Trafficking of AMPA receptors plays an important role in the LTP and LTD inductions by increasing or decreasing the receptors mount in the postsynaptic membrane.

(E) The proportional contributions of pre- and post-synaptic mechanisms differ at various times after induction and different classes of a synapse as well. However, the post-synaptic NMDAR regulates the expression of LTP and LTD, presynaptic component of expression needs a retrograde messenger like nitric oxide (NO) and endocannabinoids (EC) which can signal to the pre-synaptic terminal that coincidence has occurred.

(F) The activation of group 1 metabotropic glutamate receptors (mGluR) induces the expression of LTD and starts signal transduction in post synaptic neuron. Then, the liberation of the lipid PIP2 from the membrane by phospholipase C (PLC) and converted to IP3, which releases Ca2+ and diacylglycerol (DAG). These activations results in the calcium sensitive kinase PKC stimulation which phosphorylates the AMPA receptor.

G and K) Both LTP and LTD which mediates short and long term neuroplasticity is affected by Brain-derived neurotrophic factor (BDNF) as well as alters the structure of the synapse producing long-term synaptic plasticity

(H) Late phase of LTP and LTD require the de novo proteins synthesis, throughout a gene transcription or translating of existing transcripts. The gene transcription process is started in nuclease as consequence to signal transduction which starts as following. The cAMP-dependent signaling cascade initiated by calcium influx and involving adenylyl cyclase (AC) and cAMP-dependent kinase (PKA), which also acts directly on the AMPA receptor in induction of LTP. Catecholaminergic modulatory input plays a major role in determining the longevity of LTP and LTD, through interaction with AC which increases levels of cAMP and thereby activates PKA. Subsequently, PKA determines a body of signals that transcript new genes, and then translated into proteins. Latterly, these proteins are contributing to the long-term expression of synaptic plasticity.

(I) MAPK can also contribute in new protein synthesis. However, in this case existing transcripts are locally translated into proteins, without further requirement for nuclear signaling. mGluR-dependent LTD is robustly depending to the MAPK pathway.

2.4. DEPOTENTIATION (DP)

DP is a one of synaptic plasticity types that can be induced in vitro or vivo by two general patterns of synaptic stimulation. It can be asked that whether DP and LTD are induced by particularly the same phenomena or they are distinguished procedures. The question arises because both forms of plasticity can be induced by similar LFS type protocols and consequence by an identical event (reduction in neuroplasticity) (126). There are many evidences that LTP has been induced previously at synapses before induction of DP via LFS as priming stimulation. It is considered that this is the main difference between DP and LTD. Previous studies are concluded that DP, as different from LTD, allows the elected DP antagonism allowing its role in behavior to be determined. It was possible to distinguish the role in behavior that these processes played, as it was not possible to selectively antagonize one of them either pharmacologically or by genetic manipulation. Also, if DP is deregulated in neurological diseases, understanding its molecular characteristics may provide pharmacological targets by which it may be modulated (126).

2.4.1. The Induction of DP

Applying LFS to the pathway to synapses which received priming stimulus is the most widely studied induction protocol for DP induction (144-147). In some reports 2 Hz LFS is able to induce DP but is unable to induce LTD (145, 148). The second pattern of stimulation that results in DP is theta frequency stimulation, either delivered after the induction of LTP (149-151), or during the LTP induction protocol itself (4). As theta oscillations are a characteristic of hippocampal neuronal activity induction protocols that are based on them may be said to be more physiologically relevant. So, after inducing LTP, synapses enter a different state compared to the basal state and that is making the differences in the induction requirements for DP compared to LTD.

In some experimental conditions this form of plasticity has been observed to occur only within a short time frame after LTP induction (148, 152, 153). However in other conditions it has also been observed to occur outside this time frame (126, 145, 154). One possible explanation for these divergent findings is that the strength of the LTP induction protocol may determine how long after LTP the synapses remain susceptible to DP. For example, repeated trains of high frequency stimulation, typically two 1 second tetani delivered at 100 Hz 20 seconds apart (148, 152, 153). However where DP is observed to occur more than 30 minutes after LTP induction, weaker protocols are used to induce LTP. These may be a single 100 Hz tetanus 2 or theta bursts (126, 154). Consistent with this hypothesis are the findings that additional signaling mechanisms are recruited if strong LTP induction protocols are employed: for example L-type voltage-dependent calcium channels (155, 156). Potentially these additional signaling mechanisms may result in a form of LTP that becomes resistant to DP more quickly.

2.4.2. Molecular mechanism of DP:

To recognizing the differences between DP and LTD, the involved molecular mechanisms have to be studied. In summary, however, some of the mechanisms in DP and LTD are common; many molecular signaling pathways are distinct. The NMDAR (146, 152, 153) and mGluR stimulations (145, 157) are responsible for induction of DP as in LTD (158). Although NMDA receptors are thought to play a role in the induction of both forms of plasticity, there is some evidence that the NMDA receptor subunit NR2B is involved in LTD whereas NR2A is involved in DP (159, 160).

Adenosine signaling is considered one of the first signaling which induces DP. Exogenously applied adenosine is able to decrease the magnitude of LTP (148, 161) and antagonists of adenosine A1 receptors are able to block the induction of DP by synaptic stimulation (146, 148, 162),(163). This mechanism is thought to be triggered by the efflux of cAMP and its extracellular conversion to adenosine, and to be via A1 receptors coupled to Gi/o proteins that reduce intracellular cAMP concentrations, thus abrogating PKA signaling (148).

The signaling induced by A1 receptor activation in induced DP is thought to converge on mechanisms implicated in LTD induction. however, as in LTD induction (164), antagonists of protein phosphatase 1 (PP1) or protein phosphatase 2A (PP2A) block DP, throughout the regulation of PKA signaling (148). In both LTD and DP, protein phosphatases subsequently dephosphorylates Ca^{2+} calmodulin dependent protein kinase II (CaMKII), leading to the failure of LTP induction (136).

As it is mentioned before, the requirements for protein phosphatases in DP are different compared with them in LTD. For example if PP2A is selectively antagonized LTD is blocked but DP is retained (165). In addition a third protein phosphatase, Calcineurin Aα has been found to be specifically involved in DP, as in Calcineurin Aα knockout mice both LTP and LTD can be induced, whereas DP is absent (166). An additional downstream effector of adenosine A1 receptor activation in DP may be G-protein-activated inwardly rectifying K^+ (GIRK) channels. These channels are stimulated by adenosine A1 receptors (167), and the trafficking of GIRK channels is regulated by similar mechanisms to those involved in DP, involving neuronal activity, NMDARs and PP1(168). Consistent with this, DP is deficient in GIRK2 knockout mice (167). As GIRK channels regulate neuronal excitability, this may suggest that the degree of excitability may be important for determining whether DP can occur, for example by regulating the magnitude of LTP that DP is reversing. Consistent with this, in animal models of disease in which excitability is enhanced, DP is also absent (126) (Figure 9).

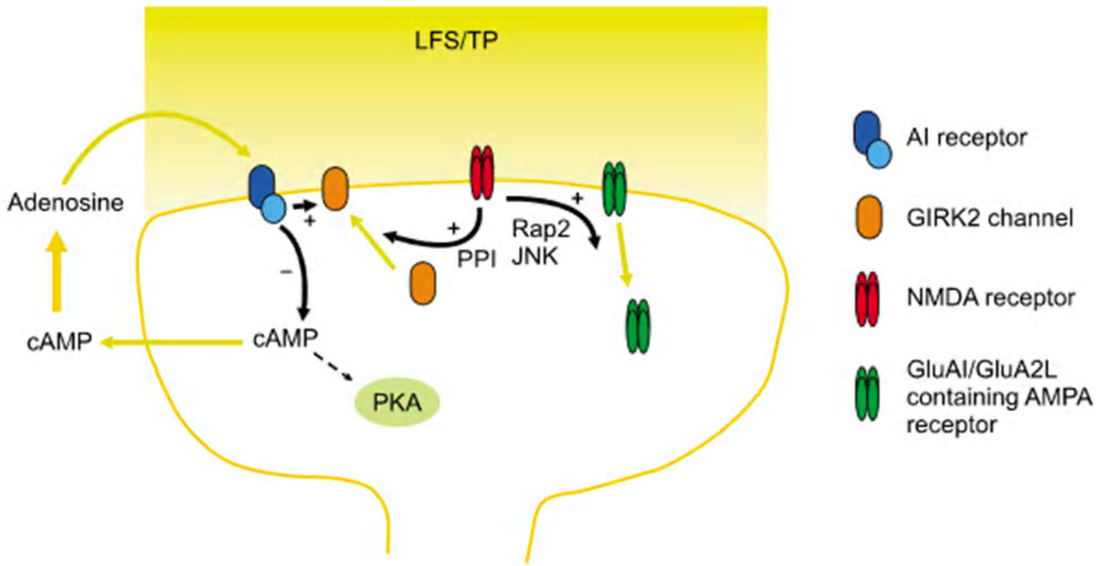

Figure 9. The molecular mechanisms of DP (126), (where movements are indicated by yellow arrows, actions are indicated by black arrows.)

LFS or theta pulse stimulation (TP) is induced DP. This causes outflow of cAMP to the extracellular space, then it transformed to adenosine. Subsequently, Adenosine activates Gi/o linked adenosine A1 receptors that act to decrease cAMP levels, possibly abrogating LTP that is dependent on cAMP dependent mechanisms, like PKA. As well, neuronal activity, NMDA receptors and PP1 cause the trafficking of GIRK channels to the surface, which may then be activated by A1 receptors. NR2A containing NMDA receptors together with Rap2 and JNK also induce AMPA receptor internalization of AMPA receptors containing long C-terminal tails, providing a means by which DP can be expressed.

2.5. MITOGEN-ACTIVATED PROTEIN KINASES (MAPK)

MAPK cascades play a key role in the regulation of gene expression as well as cytoplasmic activities. They typically are organized in a three-kinase architecture consisting of a MAPK, a MAPK activator (MEK, MKK, or MAPK kinase), and a MEK activator (MEK kinase (169) or MAPK kinase kinase). Transmission of signals is achieved by sequential phosphorylation and activation of the components specific to a respective cascade. In mammalian systems five distinguishable MAPK modules have been identified so far. These include the extracellular signal-regulated kinase 1 and 2 (ERK1/2) cascade, which preferentially regulates cell growth and differentiation, as well as the c-Jun N-terminal kinase (JNK) and p38 MAPK cascades, which function mainly in stress responses like inflammation and apoptosis. All MAPKs are serine/threonine protein kinases that phosphorylate the OH group of serine or threonine in proteins.

The Ras family GTPases (Ras, Rap1, and Rap2) and their downstream mitogen-activated protein kinase (extracellular signal-regulated kinase 1/2, Jun NH2-terminal protein kinase, and p38MAPK) signaling cascades control various physiological processes such as hippocampal LTP and LTD. Among of these kinases, ERK1/2 is selectively involved in LTP. Weak HFS protocols induced peak increase in ERK1/2 activation at 3 min - 5 min, reverting to nearly basal levels after 30 min (170) or remaining above the basal level for at least 30 min (171). We therefore investigated whether preventing ERK1/2 activation with a specific inhibitor would modulate priming of LFS-induced plasticity in the dentate gyrus by previous HFS.

2.5.1. Extracellular Signal-Regulated Kinase - ERK

Within the CNS, ERK signaling is essential to brain development and neuritis outgrowth (any projection from cell bodies and neurons) (172), neuronal survival (173, 174), and synaptic plasticity (175, 176). ERK activation is necessary for consolidation and reconsolidation of hippocampal-dependent memory (177). ERK inhibitors impair contextual fear conditioning memory formation (178-180). Furthermore, dysregulation of ERK signaling has been implicated in both neuropsychiatric and neurological disorders including, schizophrenia (181-183), depression (184-190), addiction (191-194), autism (195, 196), mental retardation (197) and AD (198). Although ERK signaling mediates the actions of many CNS drug therapies, more direct pharmacological interventions that target ERK signaling have been limited chiefly to the field of oncology, and may be accompanied by unwanted side effects.

Within the brain, ERK signaling may be initiated by the activation of ionotropic glutamate receptors (175, 176), D1 dopamine receptors (191), or L-type Ca^{2+} channels (199). By transmitting the signals received by the receptor, Ras/Raf/MEK/ERK cascade reaction activates transcription factors and regulates gene expression. Briefly, the process involves: (i) Ras recruits and activates the protein kinase Raf; (ii) Raf serine/threonine protein kinase promotes MEK1/2 (MAPK/ERK kinase) dual-specificity protein kinase and the activation of ERK1/2; and (iii) activated ERK1/2 phosphorylated several substrates and regulate different transcription factors, leading to different gene expression (200-203). Consequently, active ERK1/2 phosphorylates a myriad of intracellular substrates that contribute to neuronal function (204). ERKs are activated by dual phosphorylation on Thr and Tyr by MEKs (MEK1/MEK2) and inactivated by dephosphorylating of these sites by MAPK phosphatase (MKP). The inhibitor PD98059 binds inactive MEKs, thereby preventing their activation.

Ca^{2+} influx through activated NMDAR channels activates the Ras–ERK cascade, probably through phosphatidylinositol 3-kinase (PI3K) and Ca2+/calmodulin-dependent protein kinases (CaMKs). Active ERK1/2 proteins translocate into the nucleus to activate their transcription factor targets, cyclic adenosine monophosphate response element–binding protein (CREB) and Elk-1, leading to the facilitation of inducible gene expression implicated in the maintenance of long-lasting LTP (Figure 10).

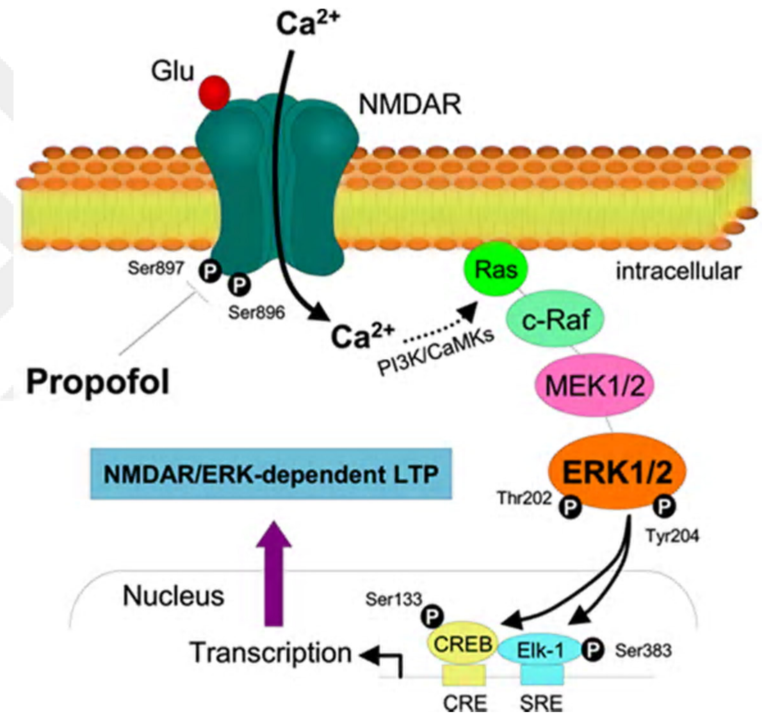

Figure 10. Induction LTP through NMDAR depends on activation of ERK1/2 activation.

2.5.2. P38 MAPK

P38 MAPKs (α, β, γ, and δ) are members of the MAPK family that are activated by a variety of environmental stresses and inflammatory cytokines. Among p38 isoforms, α and β are ubiquitously expressed in most tissues, including the brain (205), whereas γ and δ exhibit tissue-specific variations in expression (206-209). As in other MAPK cascades, the membrane-proximal component is a MAPKKK, typically a MEKK or a mixed lineage kinase (MLK). The MAPKKK phosphorylates and activates MKK3/6, the p38 MAPK kinases. MKK3/6 can also be activated directly by ASK1, which is stimulated by apoptotic stimuli. P38-MAPK is involved in regulation of HSP27, MAPKAPK-2 (MK2), MAPKAPK-3 (MK3), and several transcription factors including ATF-2, Stat1, the Max/Myc complex, MEF-2, Elk-1, and indirectly CREB via activation of MSK1.

p38 MAPK is involved in a wide range of signaling pathways that stimulate different biological functions. In particular, p38 MAPK has been found to play an essential role in the regulation of pro-inflammatory signaling networks and in the biosynthesis of cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in immune cells (210).

In CNS, p38 MAPK is highly expressed in regions that are crucial for learning and memory and is likely a key component in higher brain functions (205). P38 MAPK has been reported to have distinct roles in AD pathologies as activation of the p38 MAPK pathway has been observed in the postmortem brains of AD patients and animal models (211-213), and its specific inhibitors (SB203580) (214) have recently been claimed as novel and potential therapeutics for neurodegenerative diseases, a type of chronic inflammatory disease (205, 215-217). P38 MAPK has also been implicated in inhibition of embryonic stem cell differentiation into neurons, regulation of synaptic plasticity and modulation of neuronal excitability (218-220).

Studies in vertebrates suggest that p38-MAPK inhibits activation of ERK indirectly (Westermarck et al. 2001). Also as mentioned earlier, different models of neuroplasticity require new gene expression and protein synthesis. Guan and colleagues (Guan et al. 2002, 2003) found that blocking CREB2 function using specific antibodies blocked LTD induction by five pulses of FMRFa, and that CREB2 is a substrate of p38-MAPK. Thus, one possibility is that activation of CREB2 via p38-MAPK regulates the expression of genes that are necessary for the induction of LTD (221) figure 11**.**

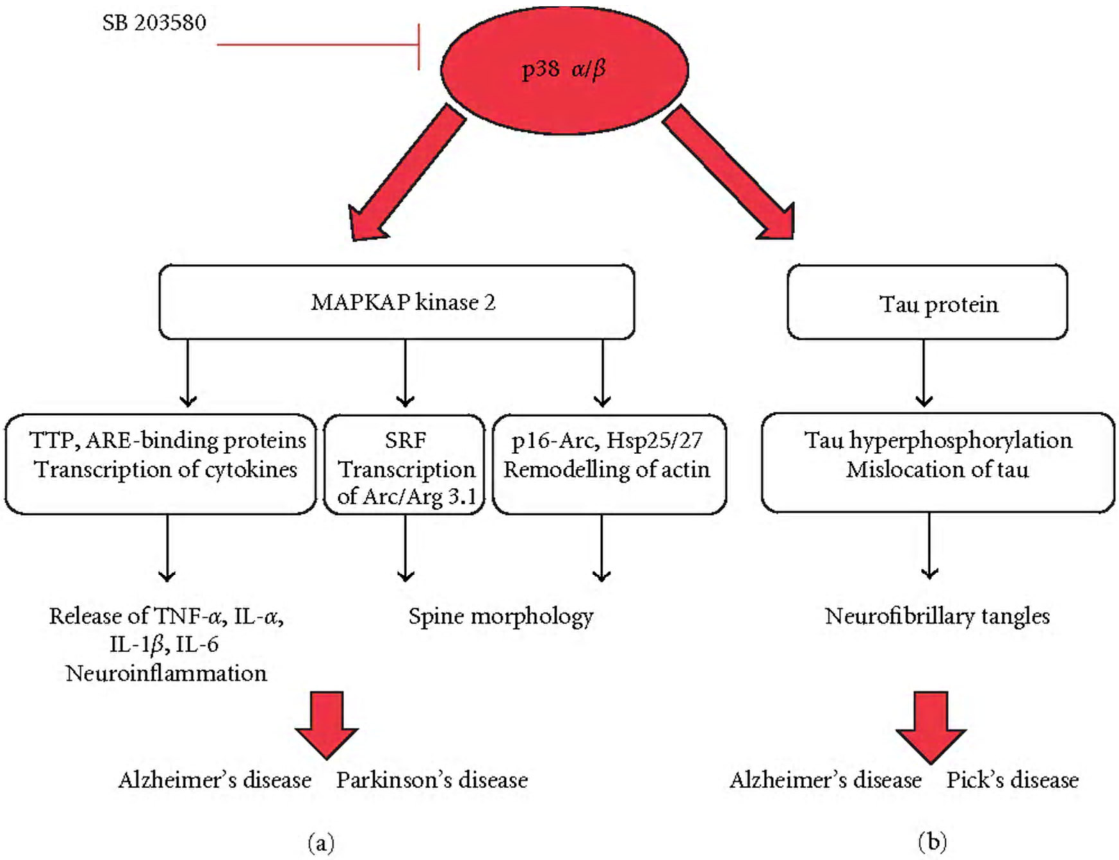

Figure 11. The steps of p38 MAPK substrates which are involved in neurodegenerative disease.

(a) The p38 MAPK-MK2 complex plays a role in neuroinflammation by phosphorylating AU-rich-element- (ARE-) binding proteins, such as tristetraprolin (TTP), which consequently can bind directly or indirectly to ARE sites present in TNF and other cytokine genes leading to transcription, translation, and subsequent release of mediators causing inflammation. The p38 MAPK-MK2 axis potentially plays an important role controlling dendritic spine morphology via direct activation of p16-Arc and Hsp, which are proteins involved in actin remodelling. Activity-dependent induction of p38 MAPK-MK2 axis can play an important role in the expression of the immediate early gene Arc/Arg3.1 which regulates spine morphology in neurons via activation of serum-response-factor- (SRF-) serum response element (160) complex. p38 MAPK-MK2 signalling cascade activation can have an effect on morphological changes observed at dendritic spines, a pattern that is observed during the development of neurodegenerative disease.

(b) p38 MAPK phosphorylates tau protein at several residues. Hyperphosphorylated tau, contributes to the formation of tau oligomers. The aggregation of the tau oligomers forms the paired-helical filaments (PHFs), which then assemble together to form neurofibrillary tangles that are characteristically observed in the brain of patients suffering from Alzheimer's disease.

2.5.3. c-Jun N-terminal kinase - JNK

JNK, a member of the MAPK family, is an important regulatory factor of synaptic plasticity as well as neuronal differentiation and cell death. Recently, JNK has been reported to modulate synaptic plasticity by the direct phosphorylation of synaptic proteins.Three genes, namely jnk1 (MAPK8), jnk2 (MAPK9), and jnk3 (MAPK10), encode for 10 different splice variants with molecular weights of 46 and 55 kDa (222). Whereas, JNK1 and JNK2 have a broad tissue distribution, JNK3 is mainly localized in neurons and to a lesser extent in the heart and the testis.

It has been established that JNK1 and JNK2 have important roles in the modulation of immune cell function and in the development of the embryonic nervous system (221). A study using JNK1 knockout mice demonstrated that JNK1 has a regulatory role and maintains physiological functions in the CNS, while JNK2 knockout established that this isoform may also participate in some physiological functions and, particularly, in the LTP (223). JNK3 is a multifunctional enzyme important in controlling brain functions under both normal and pathological conditions. JNK3 has been implicated in brain development (224), neurite formation and plasticity (225, 226), in addition to memory and learning (227, 228). Under pathological conditions, JNK3 has been considered as a degenerative signal transducer and it seems to be the isoform involved in over-activation of JNK after deleterious stress-stimuli in adult brain (ischemia, hypoxia, epilepsies). This principle is supported by the data on the reduced apoptosis of hippocampal neurons and reduced seizures induced by kainic acid in JNK3 knockout (–/–) mice, and by the notion that JNK3–/– mice are also protected against ischemia (229-231). Therefore, there is now considerable interest in further studying the involvement of this isoform in the development of neurodegenerative disease, such as Alzheimer’s disease (AD). It has been shown an increased expression of phosphorylated JNK (pJNK) in human post-mortem brain samples from AD patients and a positive co-localization with Aβ (232, 233). In particular, JNK3 is highly expressed and activated in brain tissue and cerebrospinal fluid from patients with AD and statistically correlated with the rate of cognitive decline (234).

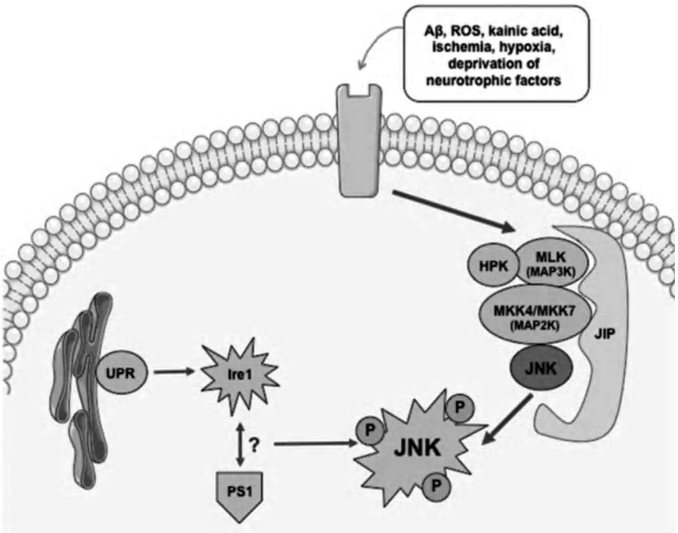

Figure 12. The JNK pathway activation

JNK signaling through scaffold proteins can be activated by different stress cases. Also, the initiation of JNK pathway can be occurred by UPR and an interaction between Ire1 and PS1. ROS, radical oxygen species; Aβ, βamyloid; JIP, JNK interacting protein; UPR, unfolded protein response; Ire, endoplasmic reticulum to nucleus signaling 1 (Figure 12).

2.5.4. Roles of ERK, JNK and p38 MAPK in neuroplasticity

Recent studies have also indicated that MAPKs may play central roles in the development of synaptic plasticity and learning/memory (235-238). First, the ERK signaling pathway has been shown to be required for both NMDA-dependent (239-242) and NMDA-independent (243, 244) LTP in both CA1 and DG regions of the HF. Second, several pieces of evidence have been accumulated suggesting critical roles of ERK-mediated pathway in various forms of memory consolidation (242, 245-248). Third, both p38 MAPK and JNK pathway have also been demonstrated to be tightly associated with DP or LTD of hippocampal synapses (214, 249-252). Albeit with these excitements, so far, little is known about the specialized, particular role of each member of the MAPKs in mediating pain-evoked synaptic plasticity in the hippocampal formation (HF).

Furthermore, AMPA receptor trafficking is included in the DP induction as in LTP and LTD (253, 254). Trafficking of AMPA receptors to and from postsynaptic membrane intercede the majority of neurotransmission stimulations. AMPA receptor subunits have either long cytoplasmic tails (GluR1 and GluR2L) or short cytoplasmic tails (GluR2 and

GluR3) (252). Otherwise, these cytoplasmic tails contain many binding sites which able them to interact with specific binding partners to control their movements. Trafficking of AMPA receptor subunits which have long cytoplasmic tails is especially mediated DP expression. Consequently, Rap2, JNK and the NMDA receptor subunits NR2A mechanism is initiated (252). In fact, the differences of AMPA receptor trafficking mechanisms are different in both DP and LTD. For explaining that, LTD AMPA receptor trafficking is initiated throughout Rap1 and p38 MAPK dependent mechanism not by Rap2 and JNK (252, 255). Along with that there are diverse dephosphorylating patterns on AMPA receptors in LTD and DP. In LTD serine 845 of GluA1 is dephosphorylated, but not serine 831. In contrast, in DP; serine 831 is dephosphorylated and serine 845 is not (154). Thus although signaling mechanism involved in DP and LTD may converge on the same expression mechanism, AMPA receptor trafficking, the regulation is demonstrably different, again pointing to distinct forms of plasticity.

Moreover, p38 MAPK signaling showed to be an important mediator of AMPA receptor surface trafficking during synaptic plasticity, a process crucial for rapid altering synaptic strength (255, 256). Given the potential role of LTP in learning and memory formation and the processes disrupting the formation of stable LTP may be the possible cause for the loss of memory (3, 152), the previous study imply a possibility that p38 MAPK is a potential common signaling molecule to exert the mechanism of memory loss.

It would be interesting to explore whether the selective p38 MAPK inhibitor may be implicated in enhancing cognition in the treatment of some dementia disorders.

2.5.5. MAPK and Alzheimer's disease

In AD brain, activation of three MAPK pathways has been demonstrated in neurons and dystrophic neuritis: c-Jun N-terminal kinase (JNK) (257, 258), p38 (Hensley et al., 1999; Zhu et al., 2000), and extracellular signal-regulated protein (259) (260, 261). Furthermore, ERK activation was reported in hippocampal slices after treatment with soluble Aβ1–42 (262), whereas JNK pathway activation occurred in both cortical neurons treated with Aβ25–35 and differentiated PC12 cells after exposure to aggregated Aβ1–42 (263-265).

Inhibition of the JNK pathway significantly reduced the toxicity attributable to Aβ in all previous studies. Increased p38 activity has been reported after a treatment of microglia (266, 267) figure 14.

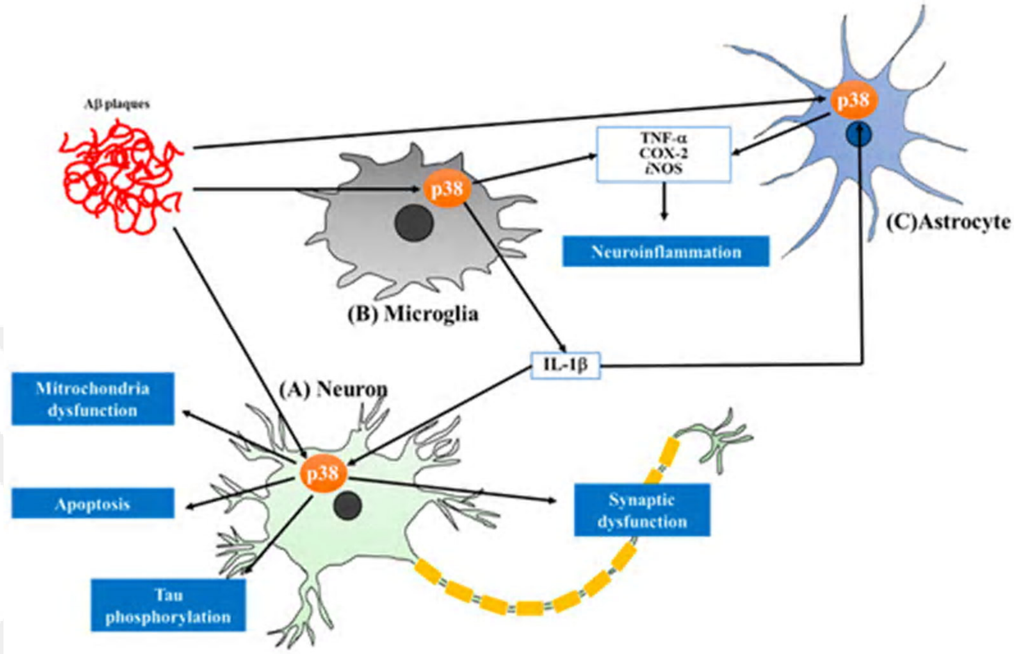

Figure 13. Diverse roles of p38 MAPK in AD

(A) Amyloid-β (Aβ) plaques evoke neuronal damages including mitochondria dysfunction, apoptosis, tau phosphorylation and synaptic dysfunction via p38 MAPK activation; (B) Increased microglial p38 MAPK signaling induced by Aβ is a main driver of neuroinflammation in AD, leading to production of pro-inflammatory mediators, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). Especially, IL-1β released from microglia stimulates p38 MAPK signaling of neuron and astrocyte in AD and exacerbates the AD brain pathology; (C) P38 MAPK activation in astrocyte is enhanced by Aβ plaques and IL-1β produced by microglia. This activation accelerates the neuroinflammation by releasing iNOS, COX-2 and TNF-α.

MAPK pathways have also been implicated in tau hyperphosphorylation. Cell-free assays have demonstrated that JNK, p38, and ERK phosphorylate tau at sites found in AD (268, 269), yet cell-associated phosphorylation of tau by these kinases has not been reported.

2.6. HYPOTHESIS

After listing the importance and role of thyroid hormone and MAPKs proteins on brain and especially for neuroplasticity, the effects of the thyroxin (T4) on MAPKs as well, it was interesting to investigate the effect of both of them on decaying the LTP which induced in hippocampal dentate gyrus.

This study is planned to show the effect of different doses of T4 which infused during high frequency stimulation for inducing depotentiation of long term potentiation on its magnitude.

To investigate the signaling pathways underlying the effect of T4 infusing on DP, we did the following next protein analysis after electrophysiological recording:

Total levels MAPK (ERK, JNK and P38).

Phosphorylated levels MAPK (ERK, JNK and P38).

Total and phosphorylated tau protein.

We wondered that these effects of T4 would be in parallel with those of MAPK inhibitors.

For that we infused specific MAPKs inhibitors (SB203580, SP600125 and PD98059 that target MAP kinase signaling pathways of ERK1/2, JNK, and P-38 respectively) during high frequency stimulation and evaluated its effects on DP magnitude

3. MATERIALS AND METHODS

3.1. Animals

The experiments were carried out on 2-3 months old adult male Wistar rats in accordance with the European Communities Council Directive of 24 November, 1986 (86/609/EEC). The protection of animals which used for experiments is occurred according to the guiding principles for the care and use of laboratory animals approved by Erciyes University. All rats were obtained from the Experimental Research and Application Center of Erciyes University (ERAC, Kayseri, Turkey) and were housed in plastic cages (4 per cage, cage size 42 x 24 x 19 cm) under constant environmental conditions of temperature (25±3◦C), relative humidity (50-60%) and light: dark cycle of 12:12. Food and drinking water were available with ad libitum. On the day that the experiment was performed, they were anesthetized and transported from ERAC to the experimental room for LTP/LTD/DP recording.

3.2. Drugs

All drugs were purchased from Sigma–Aldrich Company, and 1 M stock solutions of these drugs were prepared by dissolving them in a minimal volume of DMSO and diluting them with distilled water.

- L-Thyroxin (T4, Na salt pentahydrate, Catalogue No.: T2501-5G) was purchased from Sigma– Aldrich Company, and 1 M stock solutions of this drug was prepared by dissolving it in a minimal volume of 0.1 N NaOH and diluting with saline. T4 was diluted to 100 pM or 100 nM (final concentration) when used.
- SB203580 (a specific inhibitor of the p38 MAP kinase) (4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole, catalogue No.: 104M4605V) was purchased from Sigma– Aldrich Company, and 10 µM stock solution of this drug was prepared by dissolving 0.4 mg in a minimal volume of

DMSO and diluting by distilled water. 10 ml of stock solution diluted by distilled water to 1µM (final concentration) when used.

- SP600125 (a specific inhibitor of the JNK) (1,9-Pyrazoloanthrone, Anthrapyrazolone, catalogue No.: SLBQ9854V) was purchased from Sigma–Aldrich Company, and 20µM solution of this drug was prepared by dissolving 1.1 mg of this drug in a minimal volume of DMSO and diluting by distilled water till 250 and used as final concentration.

- PD98059 (a specific inhibitor of p42/44 MAP kinase) kinase)2-(2-Amino-3-methoxyphenyl)-4H-1-benzopyran-4-one, PD98059, catalogue No.: MKBX1068V) was purchased from Sigma– Aldrich Company, and 50µM solution of this drug was prepared by dissolving 1.336 mg of this drug in a minimal volume of DMSO and diluting by distilled water till 100 ml and used as final concentration.

3.3. Electrophysiology

For recording the field potential from right hippocampal dentate gyrus, rats were anesthetized with intraperitoneally urethane injection (1.2 g/kg). Subsequently, a double-barrel glass micropipette (Borosilicate, outer diameter 1.5 mm, length 10 cm length; World Precision Instruments) was integrated into the DG (in 3 mm, from bregma: anteroposterior: – 2.13; mediolateral: 2.50; dorsoventral: 2.5–3 mm below the dura) (as shows in figure 14). Stimulation of the medial perforant path (PP) of the right hemisphere is occurred through the insertion of bipolar tungsten electrode (stainless steel, Teflon-coated, 127 µm in diameter, insulated except at its tips) (from bregma, in mm: anteroposterior: −8.0; mediolateral: 4.2; dorsoventral: 2–2.5 below the dura) (as shows in figure 14). Previously, the both electrodes positions were verified to be in the dentate gyrus granule cell layer and in the PP (270, 271). The recognition of the correct depth (dorsoventral coordinate) of recording and stimulating electrodes was confirmed with achieving a large positive EPSP followed by a negative-going PS in response to perforant path stimulation. The recording barrel was filled with 3 M NaCl (tip resistance: 2–10 MΩ), and the other was filled with the infused material whatever it were (T4 (100 pM or 100 nM), PD98059, SP600125 and SB203580). After a stable EPSP was obtained, the PP was stimulated by pulses at an intensity that ranged from 0.1

to 1.5 mA at 0.05Hz three times and by increasing the intensities from a 0.1 mA to a 1.5 mA step by 0.2 mA per step to create an input-output curve (figure 15), which was stored for off-line analysis. The stimulus intensity produced by half of the maximum PS amplitude was determined (test stimulus) and then used throughout the experiment. After 10-min baseline recording followed by 5-min intrahippocampal infusions, LTP was induced by delivering a high frequency stimulus (100Hz, 1 sec, four times, HFS) to the perforant pathway. For depotentiation, a LFS that consisted of 900 stimuli at 1 Hz was delivered 5 min after completing tetanization. Following the delivery of LFS, he test stimulus was repeated every 30 s for up to 60 min.

Figure 14. R: reference electrode. **S:** Stimulating electrode. **C:** Recording electrode with infusing canal.

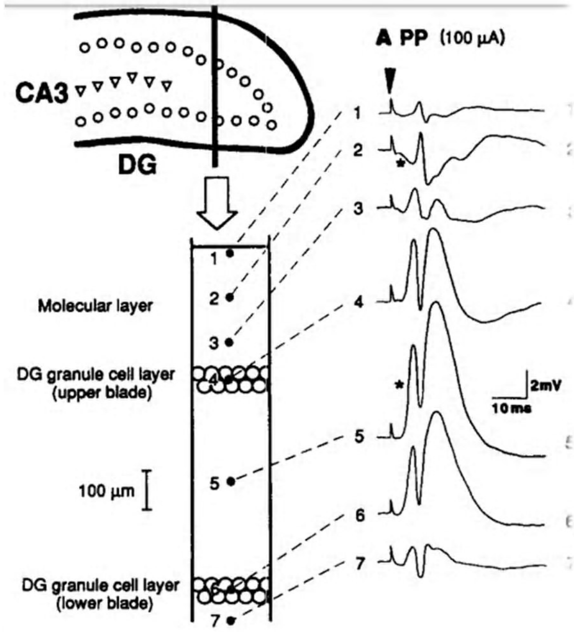

Figure 15. The Excitatory Post-synaptic Potential or "EPSP", which represents the number of granule cells synchronously depolarizing. The EPSP is measured as rise/run or slope, such that the steeper the slope, the greater the number of granule cells depolarizing

3.4. Western blotting analysis

Whole hippocampus was removed 60 min after LFS, and lysed in RIPA buffer (sc-24948; Santa Cruz Biotechnology, Santa Cruz, California, USA) with thorough homogenization. This buffer was supplemented with a protease inhibitor cocktail, PMSF, and sodium orthovanadate (sc-24948; Santa Cruz Biotechnology) by adding 10 μl of each to 1 ml of RIPA on ice immediately before use. The homogenates were centrifuged at 15 000 × g for 20 min at 4°C. Forty micrograms of total protein from each sample were separated on a 10% SDS-polyacrylamide gel and transblotted onto a nitrocellulose membrane (Millipore, Billerica, Massachusetts, USA), which was blocked with 5% non-fat dry milk or bovine serum albumin in TBST (10 mM TRIS-HCl pH 7.4, 150 mM NaCl, 0.1% Tween20) for 1 h. After overnight incubation at 4°C with primary antibodies (Table 1), the membranes were washed briefly with TBST and incubated for 1 h at room temperature in a 1 : 4000 dilution of goat anti-rabbit IgG (sc2004; Santa Cruz Biotechnology) or goat anti-mouse IgG (sc-2005; Santa Cruz Biotechnology) antibodies conjugated to horseradish peroxidase label. The bound antibodies were visualized using an enhanced chemiluminescence substrate (Cat: 170–5061; Biorad, Hercules, California, USA) according to the manufacturer’s instructions.

The blots were subsequently stripped and reprobed with a β-actin mouse monoclonal antibody to confirm equal loading of protein samples in the gel. Optical density of the blots was measured with Image J software (National Institutes of Health, USA), was normalized to the expression levels of β-actin and was expressed as an arbitrary unit relative to a sample of control group in each membrane. Kruskal-Wallis test followed by Mann- Whitney U tests was used to compare differences between groups.

Table 1 Primary antibodies used for Western blot (WB) analysis

Antibody	Host	Immunogen	Supplier/Catalog number	Dilution	Reactivity
ERK	Rabbit	A synthetic peptide corresponding to residues near the C-terminus of rat p44 MAP kinase.	Cell Signaling/#4695	1:1000	human, mouse, rat, hamster, monkey, mink, D. melanogaster, zebrafish, bovine, dog, pig, C. elegans
pERK1/2	Mouse	A synthetic phosphopeptide corresponding to residues surrounding Thr202/Tyr204 of human p44 MAP kinase.	Cell Signaling/#9106	1:1000	human, mouse, rat, hamster, monkey, mink, D. melanogaster, zebrafish, bovine, pig
JNK	Rabbit	A synthetic phosphopeptide corresponding to residues surrounding Thr183/Tyr185 of human SAPK/JNK or with a recombinant human JNK2 fusion protein.	Cell Signaling/#9250	1:1000	human, mouse, rat, monkey, S. cerevisiae, bovin, D. melanogaster
P-JNK	Rabbit	A synthetic phosphopeptide corresponding to residues surrounding Thr183/Tyr185 of human SAPK/JNK.	Cell Signaling/#9251	1:1000	human, mouse, rat, hamster, monkey, D. melanogaster, bovine, S. cerevisiae
p38-MAPK	Rabbit	a synthetic peptide corresponding to the sequence of human p38 MAPK	cell signaling #9212	1:1000	Human, Mouse, Rabbit, Monkey, GP
p-p38-MAPK	Rabbit	a synthetic phosphopeptide corresponding to residues around Thr180/Tyr182 of human p38 MAPK	cell signaling #9211	1:1000	Human, Mouse, Rabbit, Monkey
Tau	Mouse	Monoclonal antibody is produced by immunizing animals with native bovine tau and the epitope maps to the carboxy-terminus of the protein.	Cell Signaling/#4019	1:1000	human, mouse, rat
p-Tau (Ser416)	Rabbit	A synthetic phosphopeptide corresponding to residues surrounding Ser416 of human Tau protein.	Cell Signaling/#15013	1:1000	human, mouse, rat
p-Tau (Thr231)	Mouse	Raised against highly purified PHF-Tau preparation of human origin	Santa Cruz Biotechnology Inc./sc-32276	1:1000	Human, rat, mouse
β-Actin (AC-15) monoclonal	Mouse	A synthetic peptide corresponding to β-cytoplasmic Actin	Santa Cruz Biotechnology Inc./sc-69879	1:500	broad species

3.6. Data analysis and statistics

The EPSP slope is calculated as the change capacity at 20-80% of the voltage difference between the beginning and the peak of the waveform. The calculation of PS amplitude was done by calculating the average of the two potential differences of the negative spike peak to the preceding and following positive peaks. The EPSP slope and PS amplitude in I / O curves were analyzed for significance by using repeated-measures ANOVA with stimulus intensity (8 levels of intensity) as a within-subjects factor.

The mean value of the EPSP slope and the PS amplitude during baseline recording was chosen to represent 100 percent, and each EPSP and PS was expressed as a percentage of this value. For the analysis, the EPSP slope and PS amplitude values were averaged over 5-min data bins at two time windows, T1=15–20 min, and T2=85–90 min. LTP decay and DP magnitude were calculated by the formula of (T1−T2)/T1 × 100 (Kang-Park et al., 2003). These data were analyzed for significance by using Student's t test. Significance was set at p < 0.05 (two-tailed).

All statistical analyses were performed using SPSS software (SPSS, Chicago, IL). Synaptic PTP and LTP were quantified as the average of the fEPSP slopes in the first 5 minutes and in the last 5 minutes of the 60 minutes post-induction recording period. Non-synaptic PTP and LTP were quantified as the average of the PS amplitude / fEPSP slope ratio in the first 5 minutes and in the last 5 minutes of the 60 minutes post-induction recording period.

For the analysis, The EPSP slope and PS amplitude values were averaged over 5-min data bins at two time windows, T1=15–20 min, and T2=85–90 min. LTP decay and DP magnitude were calculated by the formula of (T1−T2)/T1 × 100 (272).

4. RESULTS

4.1. I/O curves

Each experiment started with an input-output (I/O) curve (maximal stimulation 1500 μA) to determine the stimulus intensity required to elicit a PS that was of 50% of the maximum obtained in the I/O curve (Fig. 16). These I/O curves were analyzed to verify whether the overall synaptic strength in the beginning of experiments is comparable across stimulation intensities. Repeated measures of ANOVA revealed that fEPSP slope ($P < 0.001$) and the PS amplitude ($P < 0.001$) increased as a function of stimulus intensity and that there was no significant difference at any stimulus intensity (group effect: $Ps > 0.05$; interaction effect: $Ps > 0.05$). Therefore, possible differences between groups might not be the resulting from baseline synaptic strength.

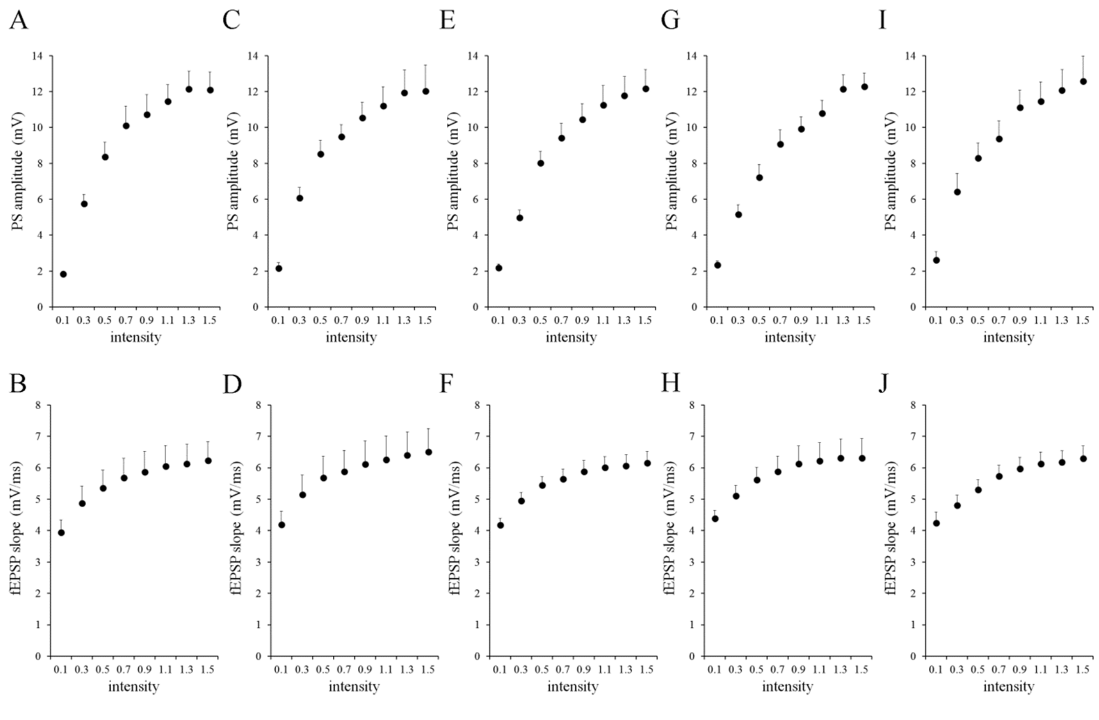

Figure 16. Absolute input–output (I/O) curves of the population spike (PS) amplitude (top) and field excitatory postsynaptic potential (fEPSP) slope (bottom) in the dentate gyrus (DG) area as a function of stimulus intensity.

I/O curves were taken before baseline recording. A and B: Saline infusion group C and D: T4 (100 nM) infusion group. E and F: DP98059 infusion group. G and H: SB203580 infusion group. I and J: SP600125 infusion group. n = 10/ group. Bars are standard errors of the means. I/O curves indicate no significant difference in synaptic strength across stimulation intensities.

4.2. Effects of T4 on reversal of LTP (DP)

The synaptic component of plasticity, measured by the slope of the EPSP, was increased by 131.4 ± 8.6% of baseline within 5-min, and down to a value of 105.5 ± 10.8% of baseline within 75-min after the last HFS in the saline group (DP: 20.3 ± 5.3%). When T4 were infused during HFS application the EPSP slope increased to somewhat lower value (100 nM T4: 115.2 ± 3.2%; 100 pM T4: 118.2 ± 11.4% of baseline), but completely returned to baseline values within 75-min after the last HFS in 100 pM T4 infusion group (105.2 ± 5.2%; DP magnitude: 8.7 ± 3.8%), whereas more depressed fEPSP slope below baseline was observed in the 100 nM T4 infusion group (62.1 ± 13.9%; DP magnitude: 49.8 ± 6.9%) (Figure 17: A).

One-way ANOVA revealed that the groups differed significantly in their ability to reverse LTP ($F_{2,18} = 15.08$; $P < 0.001$). Notably, DP magnitude of EPSP slope was significantly increased in 100 nM T4 infusion group compared with saline infusion group and 100 pM T4 infusion group (Ps < 0.001) (Figure 17: B). No significant difference between saline and 100 pM T4 infusion groups. These data show that thyroxin increase the weakening of amplified synaptic strength or efficacy higher doses, but not lower doses.

The somatic component of plasticity, measured by the amplitude of the PS, was increased by 168.2 ± 8.0% of baseline and 168.3 ± 13.7% of baseline within 5-min, and down to, but remained potentiated within 75-min after the last HFS in the saline group (141.8 ± 14.8% of baseline; DP: 16.4 ± 6.8%) and 100 nM T4 infusion group (154.3 ± 17.7%; DP magnitude: 7.1 ± 9.7%) (Figure 17: C).

When T4 were infused at the dose of 100 nM during HFS application, the PS amplitude increased to a similar extent within 5 min (177.5 ± 14.6% of baseline), but completely returned to baseline values within 75-min after the last HFS (102.5 ± 18.4%; DP magnitude: 41.8 ± 8.4%) (Figure 17: C).

One-way ANOVA revealed that the groups differed significantly in their ability to reverse LTP ($F_{2,18} = 4.62$; $P = 0.024$). Notably, DP magnitude of PS amplitude was significantly increased in 100 nM T4 infusion group compared with saline infusion group ($P = 0.045$) and 100 pM T4 infusion group ($P = 0.009$) (Figure 17: D). These data show that thyroxin increase the weakening of amplified neuronal output at higher doses, but not lower doses.

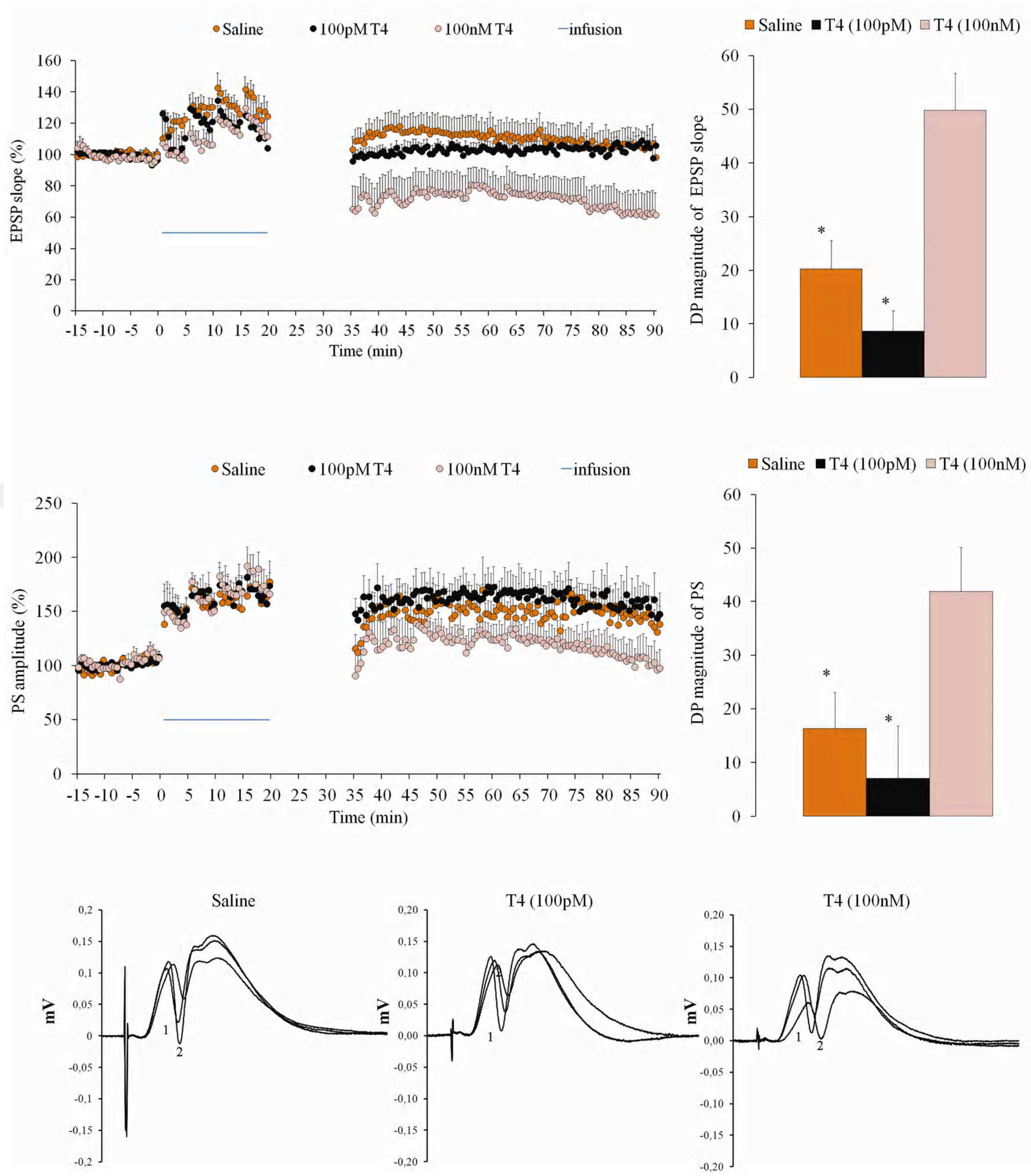

Figure 17. The effect of T4 on depotentiation magnitude

Magnitude of both dendritic EPSP (A and B) and population spike (PS) (C and D) in hippocampal dentate gyrus are increased with higher dose of T4 (100nM), but not lower dose (100 pM). Infusions of saline or T4 (100 nM and 100 pM), were made during the high frequency stimulation protocols (horizontal bar). Error bars denote the standard errors of the means n=10 for each group. (E) Representative traces of field potential recordings made immediately before tetanization (no-depicted), and 5-minutes (depicted with “1”) and 65 min after tetanization (depicted with “2”) in saline, 100 pM T4 and 100 nM T4 infusions, respectively.

4.3. Effects of T4 on total levels of MAPKs

To investigate the signaling pathways underlying the difference in reversal of LTP between the saline infused and T4 infused rats, we next examined the activity of several proteins known to be involved in plasticity: ERK1/2, p38 MAPK, and JNK.

The results of the Kruskal-Wallis test revealed that total levels of ERK protein were similar between groups ($\chi^2 = 3.74$; df = 3; $P > 0.05$) (Figure 18: A and D), but total levels of P38 MAPK and JNK proteins differ among group ($\chi^2 = 11.18$ and 13.69; $P < 0.011$) (Figures 18: B, C and D).

Independent samples Mann–Whitney U tests (Bonferroni-corrected for three comparisons) revealed a reduced P38 MAPK levels in the 100 pM T4 infusion group compared to the non-stimulated group or neutral group ($Z = 2.74$, $p = 0.006$), and saline infusion group ($Z = 2.40$, $p = 0.016$) (Figure 18: B and D).

A reduced JNK level compared to the neutral group ($Z = 2.90$, $p = 0.004$), saline infusion group ($Z = 2.56$, $p = 0.010$), and 100 nM T4 infusion group ($Z = 2.72$, $p = 0.006$) were also observed in the 100 pM T4 group (Figure 18: C and D). There was no difference between other group pairs (Figure 18: C; $P > 0.05$).

These observations may suggest that weakening of potentiated synapses is not associated with significant changes in total levels of MAPKs, but L-thyroxine at lower doses induces a reduction of JNK and P38 MAPK proteins.

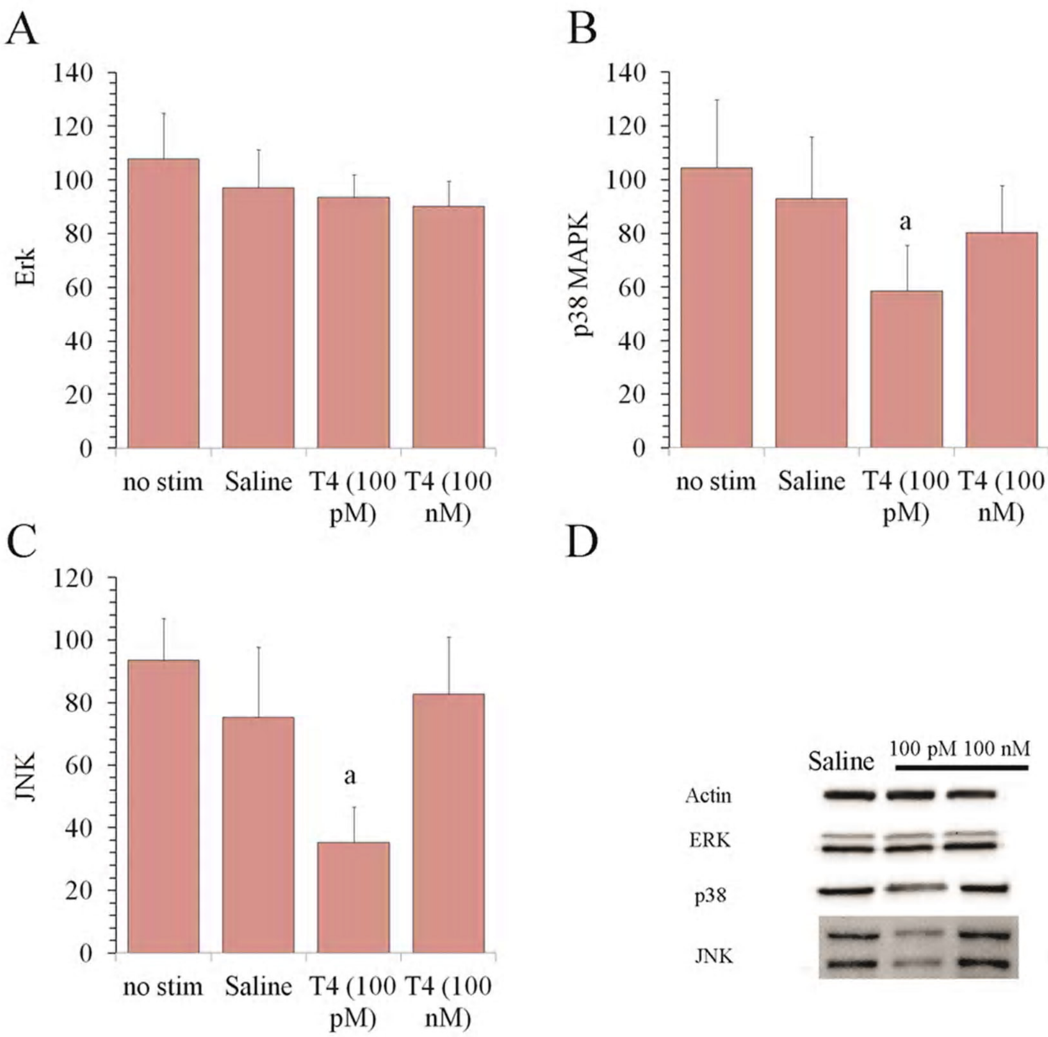

Figure 18. The effect of T4 infusion on Total MAPKs

L-thyroxine at 100 pM induces a reduction of total level JNK and P38 MAPK proteins. According to Kruskal-Wallis test, total levels of Erk protein were similar between groups (A; $P > 0.05$) but total levels of P38 MAPK (B) and JNK (C) proteins differ among group ($Ps < 0.011$). D shows western blot bands of total ERK, p38 and JNK which were normalized to β-actin.

4.4. Effects of T4 on phosphorylated levels of MAPKs

Analysis of the ratio of phosphorylated-to-total protein showed that the fraction of phosphorylated P38 MAPK was constitutively similar in all rats irrespective of their group (χ^2= 4.89; df=3; P > 0.05) (Figure 19: B and D), while there are significant differences between groups at ERK1/2 and JNK proteins (χ^2= 15.22 and 8.31; df=3; P = 0.002 and 0.040) (Figure 19: A, C and D).

Mann–Whitney U tests (Bonferroni-corrected for P < 0.05/4) confirmed that increased pErk-to-Erk ratios in saline infusion group compared to the non-stimulated group (neutral group) (Z = 2.76, p = 0.006) was partially inhibited by 100 nM T4 infusion, but not significantly after Bonferroni correction (Z = 2.21, p = 0.027) (Figure 19: A and D). Increased pErk-to-Erk ratios were observed in both T4 infusion groups compared to the non-stimulated group (neutral group) (Z = 2.76 and 2.59, p = 0.010 and p = 0.006) (Figure 19: A and D). In addition, a trend of increase in saline infusion group compared to the non-stimulated group (neutral group) (Z = 1.69; P = 0.097) was reached to statistically significant levels by low-dose T4 infusion (Z = 2.74; P = 0.006), but not by high-dose T4 infusion (Z = 0.55; P > 0.05). There was no difference between other group pairs (Figure 19: A; P > 0.05). These observations may suggest that weakening of potentiated synapses is associated with an obvious Erk1/2 phosphorylation, which is decreased by T4 infusion, especially at higher doses, during LTP induction.

In addition, weak phosphorylation of JNK could be significantly increased by T4 infusion, especially at lower doses, in response to depotentiation stimulus protocol (Figure 19: C, P > 0.05).

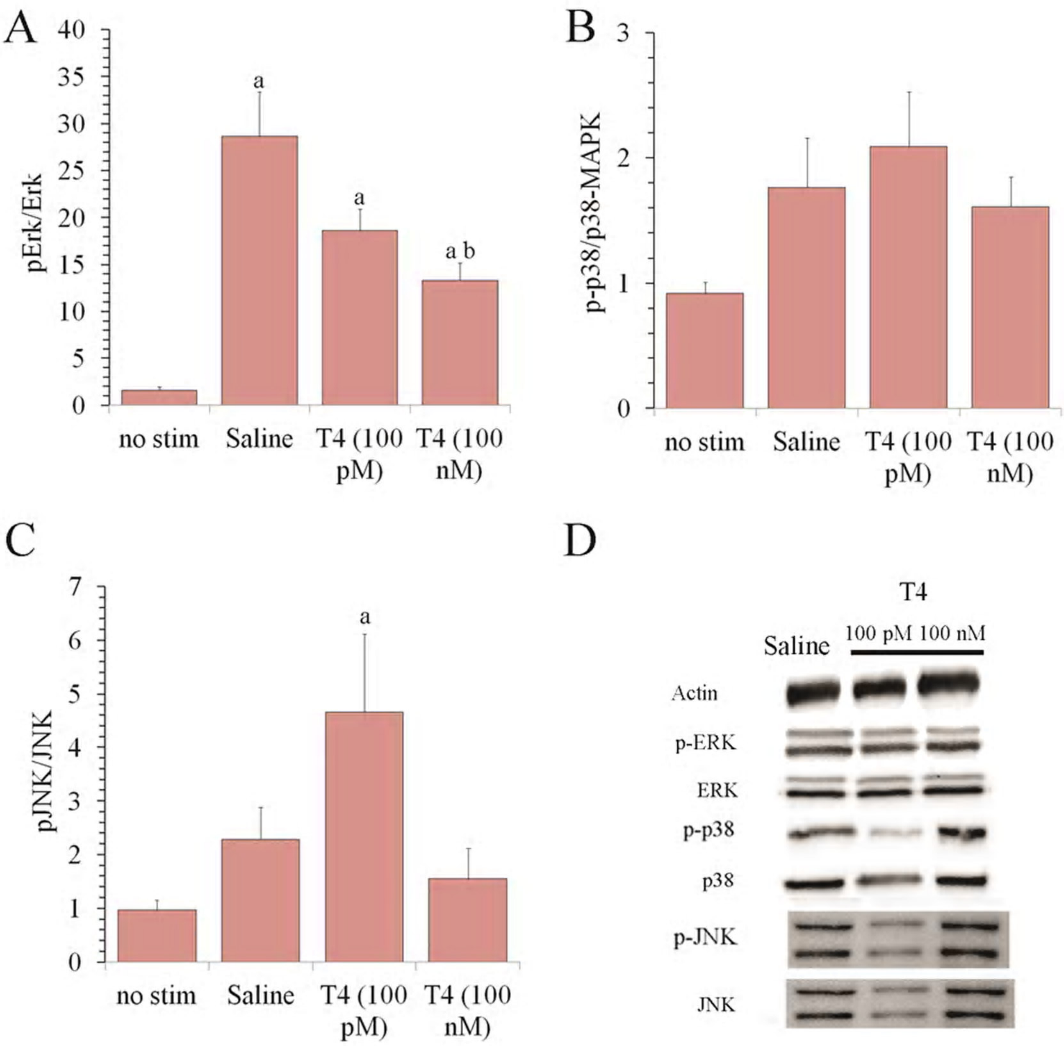

Figure 19. The effect of T4 infusion on phosphorylated MAPKs level

(A) shows a decrease in p-Erk1/2 to t-ERK1/2 protein level through T4 (100 nM) infusion. For p-P38 to t-P38 protein level, there is no any significant difference between different groups (B). p-JNK to t-JNK protein level is significantly increased by T4 (100pM) infusion in response to depotentiation stimulus protocol (C). D shows western blot bands of phosphorylated and total ERK, p38 and JNK proteins which were normalized to β-actin.

4.5. Effects of T4 on Protein Tau

The results of the Kruskal-Wallis test revealed that total levels of Tau protein differ among group ($\chi^2 = 10.23$; P = 0.017) (Figure 20: A and D). Independent samples Mann–Whitney U tests (Bonferroni-corrected for four comparisons) revealed an increased Tau levels in the saline group (Z = 2.64, p = 0.008), 100 pM T4 and100 nM T4 infusion group (Z = 2.76, p = 0.006) compared to the non-stimulated (neutral) group. The difference between saline group and T4 infusion groups did not reach to significance level after Bonferroni correction (Z = 2.39, p = 0.017). There was no difference between other group pairs (Figure 20: A; P > 0.05).

Analysis of the ratio of phosphorylated-to-total Tau protein showed that the fraction of phosphorylated tau at Thr231 was constitutively similar in all rats irrespective of their group (χ^2= 4.55; df=3; P > 0.05) (Figure20: C), while there are significant differences at Ser416 (χ^2= 12.86; df=3; P = 0.005) (Figure 20: B).

Mann–Whitney U tests (Bonferroni-corrected for tour comparisons; P < 0.054) confirmed that increased p^{Ser416}Tau-to-t-Tau ratio in the saline infusion group compared to the non-stimulated (neutral) group (Z = 2.76, p = 0.006) was inhibited by T4 at the dose of 100 pM (Z = 2.42, p = 0.016, ns after Bonferroni correction) but not at the dose of 100 nM (Z = 2.90, p = 0.004) (Figure 20: B). There was no difference between other group pairs (Figure 20: B; P > 0.05).

These observations may suggest that weakening of potentiated synapses is associated with an obvious Tau phosphorylation at Ser416 epitope, but not Thr231 epitope, which is significantly decreased by T4, especially at lower doses, infused during DP induction.

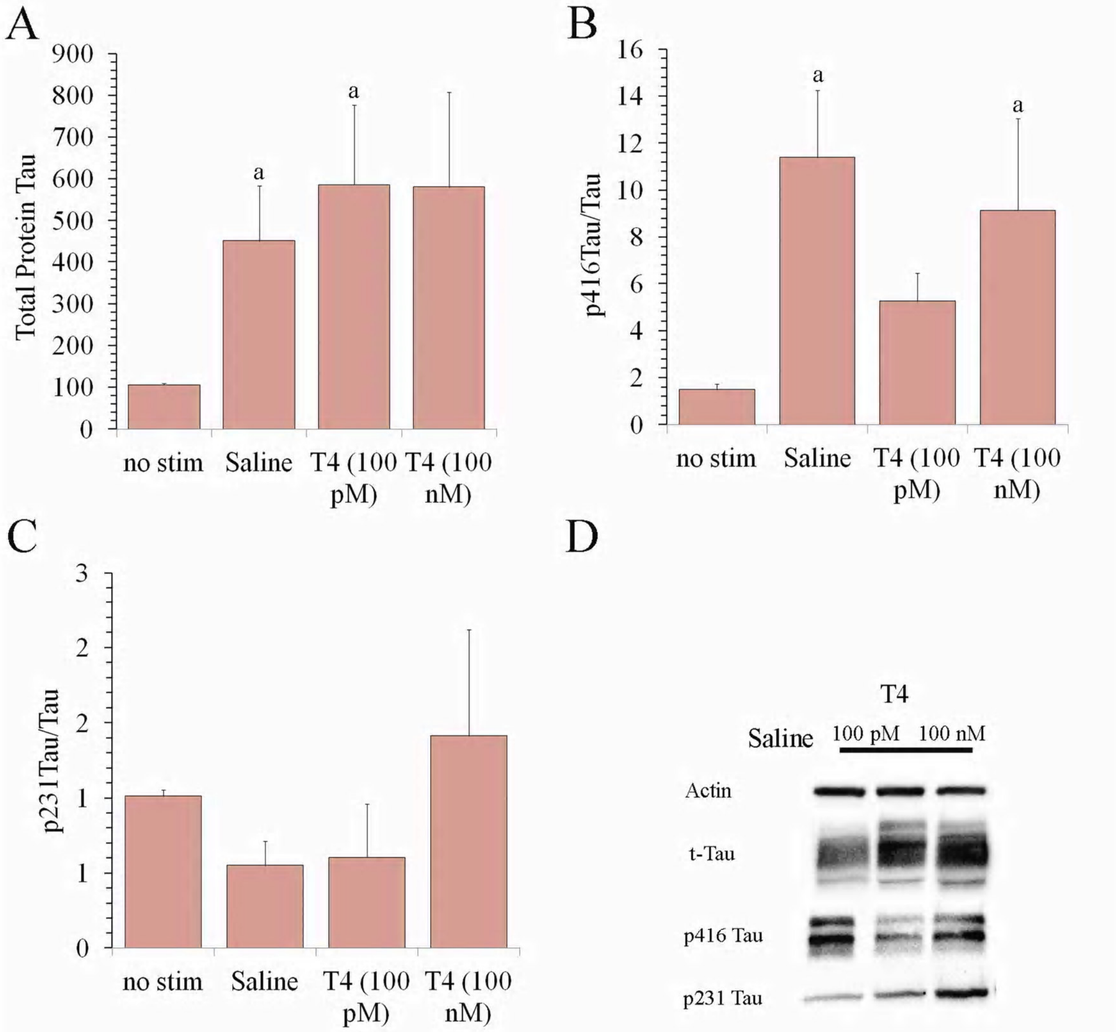

Figure 20. The effect of T4 on the level of total and phosphorylated Tau protein

Total levels of Tau protein are significantly higher in all infused groups compared with non-stimulated group, but there is no significant difference between infused groups (A). p^{Ser416} Tau is significantly decreased by T4 (100 pM) infused during HFS by applaying depotentiation of LTP (B). p^{Thr231} Tau is similar in all groups ($p > 0.05$) (C). D shows western blot bands of total and phosphorylated Tau protein which were normalized to β- actin.

4.6. Effects of MAPK inhibitors on DP

As shown above, weakening of potentiated synapses was found to be associated with an obvious ERK1/2 phosphorylation and 100 nM T4 infusions resulted in a marked weakness and inhibition of Erk1/2 phosphorylation. Therefore we wondered that these effects of T4 would be in parallel with those of MAPK inhibitors.

The synaptic component of plasticity, measured by the slope of the EPSP, was increased by 131.4 ± 8.6% of baseline within 5-min, and down to a value of 105.5 ± 10.8% of baseline within 75-min after the last HFS in the saline group (DP: 20.3 ± 5.3%) (Figure 21: A). The EPSP slope increased to somewhat lower value when Erk1/2 inhibitor (122.7 ± 13.1%) and JNK inhibitor (130.7 ± 5.6%) were infused, but to somewhat higher values when p38 inhibitor was infused (144.3 ± 8.6%) (Figure 21: A). However it completely returned to baseline values within 75-min after the last HFS in p38 inhibitor infusion group (109.9 ± 5.9%; DP magnitude: 24.1 ± 1.6%) (Figure 21: A), whereas more depressed fEPSP slope below baseline was observed in the Erk1/2 inhibitor infusion group (64.3 ± 12.3%; DP magnitude: 49.9 ± 4.4%) and potentiated fEPSP slope above baseline was observed in the JNK inhibitor infusion group (124.0 ± 8.2%; DP magnitude: 4.6 ± 6.6%) (Figure 21: A).

One-way ANOVA revealed that the groups differed significantly in their ability to reverse LTP ($F_{2,18} = 15.12$; $P < 0.001$). Notably, DP magnitude of EPSP slope was significantly increased in Erk1/2 inhibitor infusion group compared with saline infusion group ($P = 0.001$), JNK inhibitor group ($P = 0.001$) (Figure 21: B) and P38 MAPK inhibitor infusion group ($P = 0.005$) (Figure 21: B), and decreased in JNK inhibitor infusion group compared with P38 MAPK inhibitor group ($P = 0.040$) (Figure 21: B). No significant difference between other group pairs.

These data show that ERK1/2 inhibition, as similar to the effect of high dose T4, increases the weakening of amplified synaptic strength.

The somatic component of plasticity, measured by the amplitude of the PS, was increased by 168.2 ± 8.0% of baseline within 5-min, and down to, but remained potentiated within 75-min after the last HFS in the saline group (141.8 ± 14.8% of baseline; DP: 16.4 ± 6.8%) (Figure 21: C). The PS amplitude increased to somewhat higher values when Erk1/2 inhibitor (195.1 ± 30.1%), p38 inhibitor was infused (210.4

± 21.3%) and JNK inhibitor (186.5 ± 16.8%) (Figure 21: C). However it completely returned to baseline values within 75-min after the last HFS in Erk1/2 inhibitor infusion group (98.2 ± 31.2%; DP magnitude: 54.9 ± 7.0%), whereas PS-LTP slope was observed in the P38 inhibitor infusion group (163.6 ± 14.6%; DP magnitude: 21.3 ± 2.9%) and the JNK inhibitor infusion group (221.0 ± 17.8%; DP magnitude: -19.6 ± 6.1%) (Figure 21: C).

One-way ANOVA revealed that the groups differed significantly in their ability to reverse LTP ($F_{2,18} = 24.93$; $P < 0.001$). Notably, DP magnitude of PS amplitude was significantly increased in Erk1/2 inhibitor infusion group compared with saline infusion group (P = 0.001) (Figure 21: D), JNK inhibitor group (P = 0.001) and P38 MAPK inhibitor infusion group (P = 0.004) (Figure 21: D), and decreased in JNK inhibitor infusion group compared with P38 MAPK inhibitor group (P = 0.001) (Figure 21: D). No significant difference between other group pairs.

These data shows that ERK1/2 inhibition, as similar to the effect of high dose T4, increases the weakening of amplified synaptic strength.

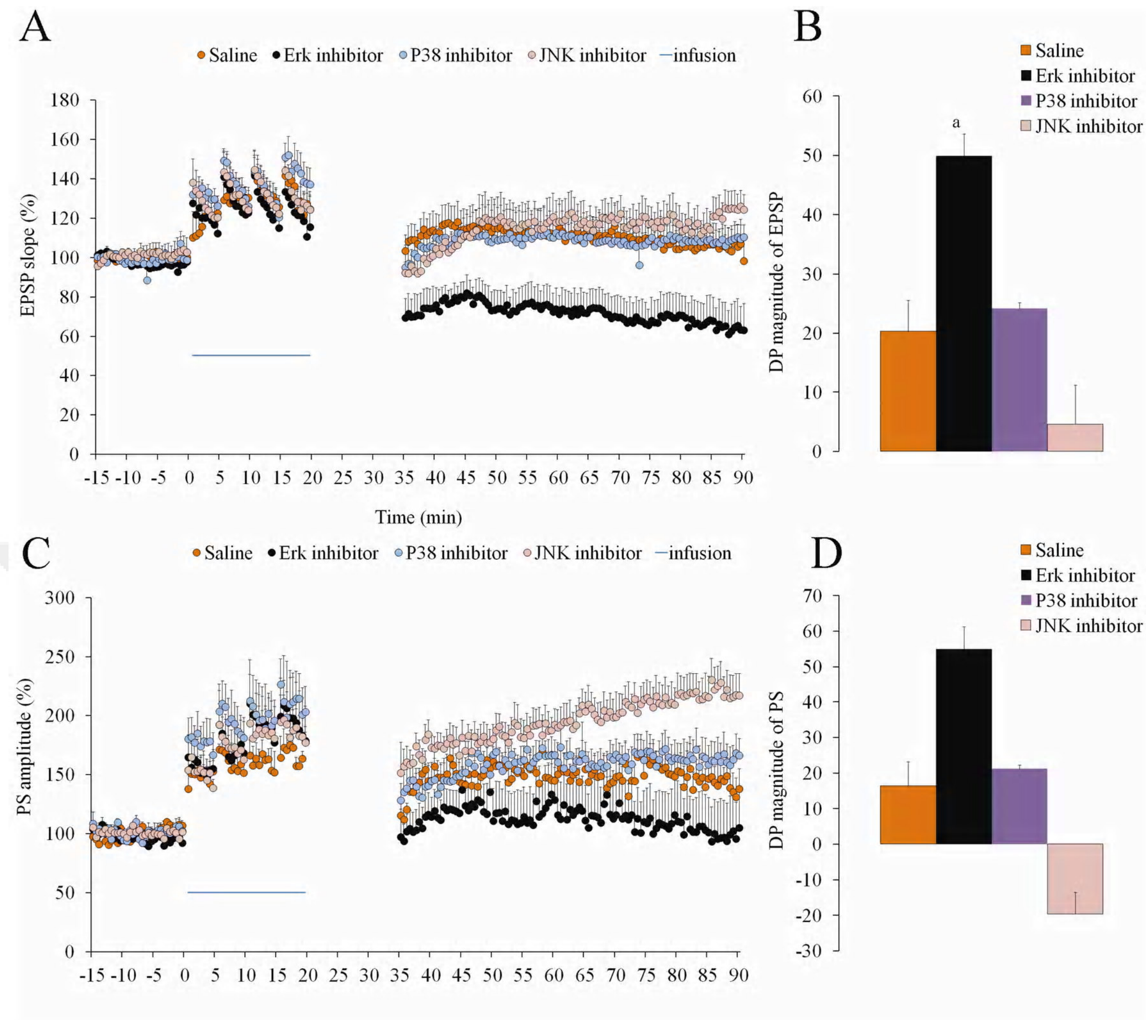

Figure 21. The effect of different MAPKs inhibitors on depotentiation magnitude

As similar to the effect of high dose T4 (100 nM), ERK1/2 inhibition increases the weakening of amplified synaptic strength. Infusions of saline ■ , Erk inhibitor (D980059) ■ , P38 inhibitor (SB203580) ■ and JNK inhibitor (SP600125) ■ were made during the high frequency stimulation protocols (horizontal bar) (A and C). Error bars denote the standard errors of the means n=10 for each group. Depotentiation of LTP magnitude of both dendritic EPSP (A,B,) and the population spike (PS) (C,D) was significantly increased in Erk inhibitor (PD98059) infusion group compared with other infused groups, decreased in JNK inhibitor (SP600125) infusion group compared with other infused groups as well.

5. DISCUSSION

5.1. Effects of T4 on reversal of LTP (DP)

It is well known that triiodothyronine (T3), the active form of thyroid hormone is produced predominantly outside the thyroid parenchyma and is converted in peripheral tissue to thyroxine (T4) by deiodination process, with <20% being secreted directly from the thyroid. In healthy individuals, plasma T3 is regulated by the negative feedback loop of the hypothalamus–pituitary–thyroid axis and by homoeostatic changes in deiodinase expression. T3 regulate genes expression by binding to specific nuclear thyroid receptors but several rapid effects of T4 are not mediated by this mechanism and involve other than nuclear TRs (273, 274). The major result of this study is that rats display impaired depotentiation in the perforant pathway–dentate gyrus synapses when induction protocols were applied simultaneously with 100 nM T4 infusion, although the EPSP slop and PS magnitude of all infused groups (saline, 100 pM T4 and 100 nM T4) were increased nearly similar during HFS applying. As shown in figure 17:A and C, the EPSP slope and PS magnitude were significantly decreased in 100 nM T4 infusion group compared with saline infusion group and 100 pM T4 infusion group. By calculating the magnitude of DP at the end of recording ratio to the magnitude of potentiation during HFS applying in EPSP as well as Ps, we found that this ratio were significantly increased in 100 nM T4 infusion group compared with saline infusion group and 100 pM T4 infusion group (Figure 17: D). These findings suggest that T4 increased the weakening of amplified synaptic strength or efficacy and amplified neuronal output at higher doses, but not lower doses as well. The effect of T4 may be resulted by increasing the AMPA receptor removal (desensitization of AMPA receptor) from post synaptic membrane.

Previous studies in vitro (on hippocampal cell cultures) adduced that the activation of hippocampal NMDAR is reduced by administrating T4 and T3 according to the strength of their concentrations (275) by affecting the inhibitory post-synaptic potential (IPSP)

(276) which is used as a signal that presynaptic mechanisms play an critical role in the modulation of synaptic transmission. Otherwise, T4 infusion in vivo caused a reduction in EPSP. This confirmed that T4 has influences on a presynaptic mechanism because EPSP mirrored an increase in transmitter release from activated synapses.

In fact, the modification threshold (ɵm) is mirrored the critical level for postsynaptic responses which is modulated by the balance between protein kinases/phosphatases. At ɵm, the indicated synaptic modification inverts from negative LTD to positive LTP. Suer et al. 2016 (277) reported that an alternation of ɵm by T4 infusion could be a conceivable construction for reduced LTP and boosted LTD (Figure 22). The binding of T4 to integrin αVβ3-R reduces the increase in [Ca2+]$_i$ throughout the induction of LTP and LTD. This situation results in a raise in phosphatases activities and a reduction in kinases activities. Finally, the ɵm is shifted to the right. Regardless of how, the molecular mechanisms which regulated these effects is required further studying.

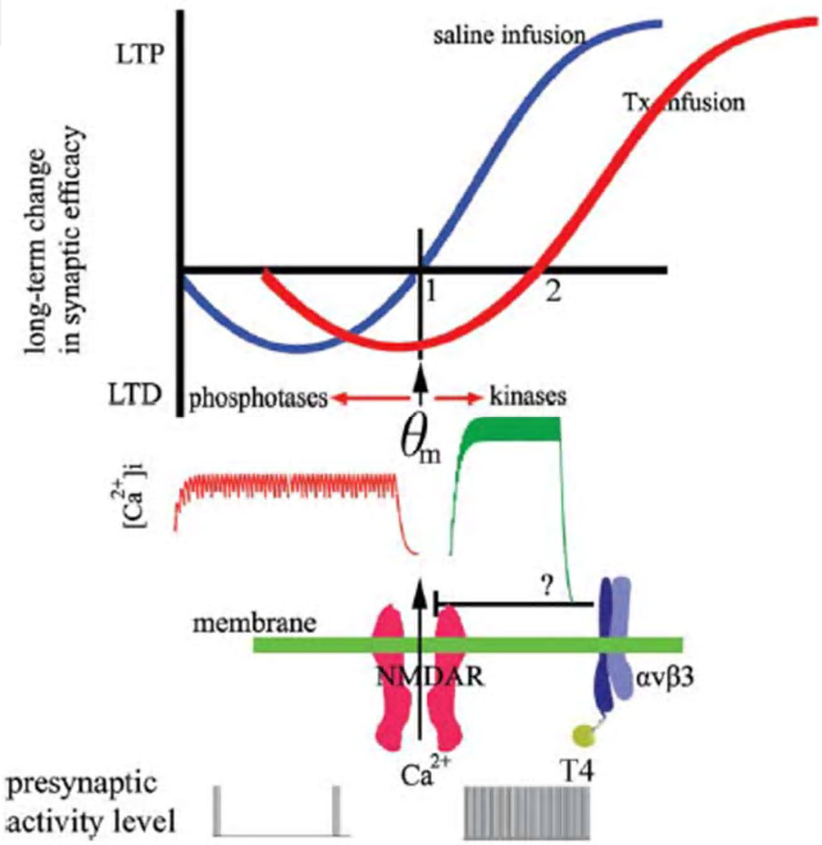

Figure 22. The binding of T4 with integrin αVβ3 receptors regulate the threshold (ɵm) value.

The kinases and phosphatases balances determine a point (ɵm, showed as “1”) in where the sign of the synaptic modification reverses from negative (LTD) to positive (LTP). Binding of T4 to its receptor (**αVβ3**) reduces the calcium influx through NMDAR (depicted as “?”). This leads to an increase in the activity of phosphatases and a decrease in the activity of kinases and ultimately shifts the modification threshold to the right (the point depicted as “2”).

5.2. Effects of T4 on total and phosphorylated levels of MAPKs

There is a body of evidence that hippocampal CA1 and CA3 have strongly involved in the learning and memory generation. Whereas the granule cells in DG are more resistant to conditioned stimulus than the pyramidal cells. Latterly, it has been proven that MAPK genes are the T4 direct targets (278, 279), because the stimulation of MAPK is part of the non-genomic action of thyroid hormone pathways. These pathways typically are activated by physical and chemical signals at the cell surface. However, our findings showed that weakening of potentiated synapses is not associated with significant changes in total levels of MAPKs, but T4 at lower doses induces a reduction of JNK and P38 MAPK proteins. This result revealed that total levels of ERK protein were similar between groups, but total levels of P38 MAPK and JNK proteins differ among group. A reduced P38 MAPK levels in the 100 pM T4 infusion group compared to the neutral group and saline infusion group, a reduced JNK level compared to the neutral group, saline infusion group, and 100 nM T4 infusion group were also observed in the 100 pM saline group. There was no difference between other group pairs. Here these results come opposite to our expectation where low concentration of T4 decreased the t-JNK and t-p38. These decreasing do not have relation to the impairment of DP magnitude which was caused by 100 nM.

Whereas the results also showed that increased pERK-to-t-ERK ratios were observed in all infused groups compared to the non-stimulated (neutral) group, probably because of synaptic potentiation through applies HFS, which was partially inhibited by 100 nM T4 infusion, but not significantly (Figure 19: A and D). In addition, a trend of increase in saline infusion group compared to the non-stimulated (neutral) group ($P = 0.097$) was reached to statistically significant levels by low-dose T4 infusion ($P = 0.006$), but not by high-dose T4 infusion ($P > 0.05$). There was no difference between other group pairs ($P > 0.05$). The data of this study showed that at doses that affect reversal of LTP (DP), T4 reduced the increase in ERK phosphorylation that was observed associated with weakening of potentiated synapses. At doses that not affect reversal of LTP (DP), weakening of potentiated synapses resulted in weak phosphorylation of JNK, which was markedly increased in the presence of T4 as well. The increasing of p-JNK/JNK level in 100 pM T4 group comes opposite to our electrophysiological results.

Several previous studies described the relation between some MAPKs proteins and thyroid hormones. It was reported that the type 2 iodothyronine deiodinase (*Dio2*) gene as a target of JNK signaling that negatively regulates the hypothalamic–pituitary–thyroid (HPT) axis (280). Also, Hara et al. (281) demonstrated a stimulatory effect of low and physiological TSH concentrations on JNK activity imply a significant involvement of the JNK pathway. Bi-directional regulation of JNK activity by TSH, low TSH concentration enhanced but higher dose of TSH suppressed JNK activity, was observed. It has been recently showed JNK mediates AMPA receptor endocytosis.

ERK1/2 is an important protein involved in memory generation and learning. When we look at the previous studies on the relation of ERK to thyroid hormones, we found the following. The binding of T4 to its receptor activates G-protein-coupled protein which consequently phosphorylates ERK1/2. These signals transductions promote the transcription of some proteins that involved in the formation of memory (282). In the case of hypothyroidism, the basal p-ERK1/2 in hippocampal CA1 region is reduced without affecting t-ERK1/2 (282, 283). These results are matched with Davis and colleagues (15, 284) studies, where they reported that the stimulation of ERK1/2 pathway is mediated by the binding of T4 with integrin αVβ3. Furthermore, the basal p-ERK1/2 in hippocampal CA1 region is decreased by hypothyroidism without affecting t-ERK1/2 (282). Gerges, Alkadhi and colleagues as well as previous studies provided molecular evidence that a reduction in thyroid hormone leads to a reduction in phosphorylated levels of hippocampal ERK1/2, which is critical for hippocampus-dependent learning and memory (285-287).

Further, there is also a relation between thyroid hormones and p38-MAPK where THs is able to regulate it (288) and protein kinase C as well (289, 290). These messengers' activations mediate the phosphorylation of a sodium channel. One potential hypothesis that the implicated intracellular kinases and phosphatases directly modulate the sodium channel phosphorylation. Likewise, phosphorylation of voltage-gated sodium channels by MAPK (p38) reduces $(Na)_i$ amplitude by 50% (291). Another set of studies revealed a significant disruption of LTP in hippocampal tissue from hypothyroid rat pups (292). Overall, deficient thyroid hormone signaling leads to disrupted synaptic plasticity.

5.3. Effects of MAPK inhibitors on DP

As shown above, weakening of potentiated synapses was found to be associated with an obvious decrease in ERK1/2 phosphorylation level with 100 nM T4 infusions. Therefore, the hypothesis was these effects of T4 would be in parallel with those of MAPK inhibitors as (PD98059, SP600125, and SB203580) that target MAPK signaling pathways (ERK1/2, JNK, P-38 respectively). Many of the MEK1/2, JNK and p38 MAP kinase pharmacological inhibitors have been shown to be quite specific in their kinase inhibition profiles (293). SP600125 have been shown to inhibit JNK isoforms by competing with the ATP-binding site (294). However, SP600125 has also been shown to inhibit a number of other kinases (295), which must be considered when using this compound for evaluating JNK pathway regulation. Also, for PD98059 which is considered a p42/44 MAP kinase specific inhibitor (296). PD98059 is considered a highly selective inhibitor against MEK where inhibits either basal MEK1 or a partially activated MEK without inhibiting JNK and P38. PD98059 potently prevents the activation of MEK1 by Raf or MEK kinase with IC50 of 4 μM, and weakly inhibits the activation of MEK2 by Raf with IC50 of 50 μM. PD98059 does not inhibit the activation of MEK homologues MKK4 and RK kinase that participate in stress and interleukin-1-stimulated kinase cascades in KB and PC12 cells, and the activation of p70 S6 kinase by insulin or epidermal growth factor in Swiss 3T3 cells (297). SB203580 (P38-MAPK specific inhibitor) (214) has recently been claimed as novel and potential therapeutics for neurodegenerative diseases, a type of chronic inflammatory disease (205, 215-217).

This hypothesis are approved by our findings which showed that depotentiation of LTP (DP) magnitude (at the end of the recording) of both PS amplitude and dendritic excitatory post synaptic potential (EPSP) was significantly increased in ERK1/2 inhibitor infusion group compared with saline infusion group ($P = 0.001$), JNK inhibitor group ($P = 0.001$) and P38 MAPK inhibitor infusion group ($P = 0.004$), and decreased in JNK inhibitor infusion group compared with P38 MAPK inhibitor group ($P = 0.001$) as well. At doses that not affect reversal of LTP, weakening of potentiated synapses resulted in weak phosphorylation of JNK, which was markedly increased in the presence of T4 as well. No significant difference between other group pairs. On the other hand, EPSP slop and PS magnitude of all infused groups (PD98059, SP600125, and SB203580 which target MAPK signaling pathways ERK1/2, JNK, P-38 respectively) increased nearly similar during HFS applying.

These data shows that ERK1/2 inhibition, as similar to the effect of high dose T4, increases the weakening of amplified synaptic strength. So it can be suggested that ERK1/2 inhibition, as similar to the effect of high dose T4, increases the weakening of amplified synaptic strength.

Researches in the last ten years showed that few small molecule inhibitors of JNKs have entered clinical trials for different indications, but none for the treatment of AD. In this topic some studies reported that intracerebroventricular infusion of SP600125 improved escape latency in the Morris Water Maze (298). In AD transgenic mouse model, administration of SP600125 improved spatial learning impairment in the Morris Water Maze, and reduced pTau and Aβ oligomeric burden (299).

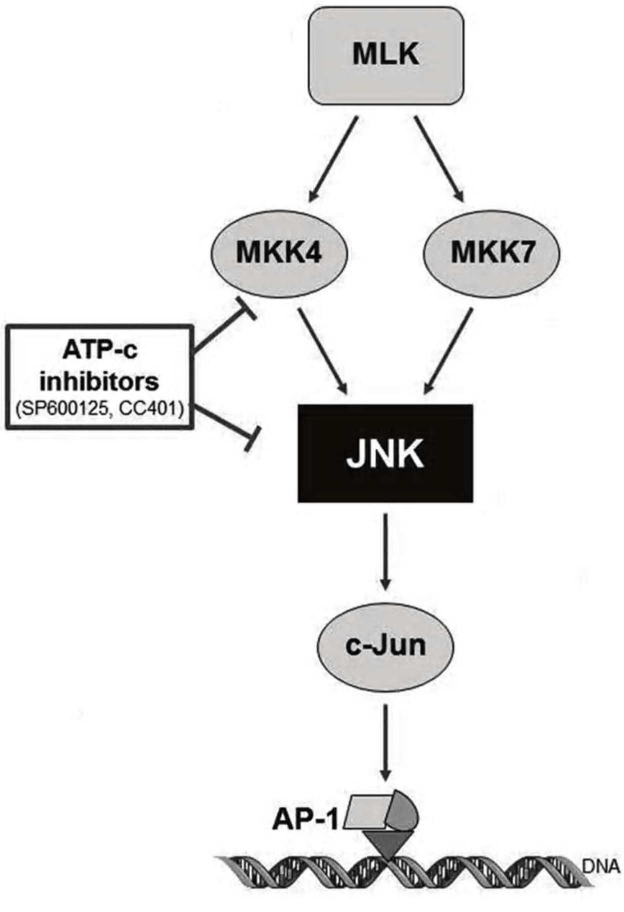

Figure 23. Pharmacology of JNK inhibitors, targets and mechanism of action.

However, it is to note that SP600125 has shown a limited specificity toward JNK, as also inhibits not only MKK4 and MKK7 (Figure 23), but also other protein kinases unrelated to JNK, such as SGK, p70 ribosomal protein S6 kinase (S6K1), AMPK, CDK2, CK1d, and DYRK1A (295).

In both in vitro and in vivo models of AD, SP600125 has demonstrated to prevent the pathological mechanisms triggered by the up-regulation of pJNK. In vitro, SP600125 has demonstrated that this inhibitor prevents βAPP induced neuronal cell death as well as down-regulation of ASK1 in F11 cell-lines (300). Interestingly, the study did not find neuroprotection against βAPP induced neuroapoptosis when exposing cultures to a p38 inhibitor, which highlights the importance of JNK within this process. Other in vitro experiments showed decrease of Aβ-induced cytokine expression (IL6, IL8, MIP1β, TNFα, Groα, GM-CSF;(301)).

Our results that JNK inhibition significantly impaired depotentiation are consistent with the study by H.Yang et al., 2011 (302), where their data suggested that suppression of JNK-dependent signalling may serve to enhance synaptic depression, and indirectly promote LTP through impairment of depotentiation. It is also showed that JNK is involved in STD, LTD and depotentiation in the dentate gyrus of freely behaving rats and also with the study by Zhu et al. 2005 (252), which showed that Rap2- dependent activation of the JNK pathway leads to depotentiation of hippocampal synapses in CA1.

It should be kept in mind that PSD-95 is a member of the membrane-associated guanylate kinase (MAGUK) family. With PSD-93 it is recruited into the same NMDA receptor and potassium channel clusters. These two MAGUK proteins may interact at postsynaptic sites to form a multimeric scaffold for the clustering of receptors, ion channels, and associated signaling proteins. It is almost exclusively located in the post synaptic density of neurons, and is involved in anchoring synaptic proteins. Its direct and indirect binding partners include neuroligin, NMDA receptors, AMPA receptors, and potassium channels. The regulation of the number or density of postsynaptic AMPA receptors is essential for hippocampal synaptic plasticity. Postsynaptic density 95kD protein (PSD 95) is required for targeting AMPA receptors to approximately 50% of excitatory synapses (303). As PSD95–ser295, a site of phosphorylation by JNK, regulates the localization of PSD95 and functional AMPA receptors to synapses (304), JNK activity may influence synaptic plasticity. Furthermore, JNK activity may contribute to the reinsertion of GluR 2L⁄4 AMPA receptors into the plasma membrane after neuronal stimulation by direct phosphorylation of the receptors (305). It has been proposed that the different MAPK subgroups engage in different roles. Induction of LTP is associated with activation of the NR2A subunit of N-methyl-D-aspartate receptors, a downstream activation of Ras, extracellular-regulated kinase 1⁄2 and subsequent synaptic delivery of the GluR1⁄2L subunit of the AMPA receptor. Induction of LTD is associated with activation of the NR2B subunit of the N-methyl D- aspartate receptor, downstream activation of Rap1 and p38, leading to stimulation of Rab5-dependent endocytosis and removal of GluR2 ⁄3 (22, 251).In spite of the reports mentioned above that JNK contributes to LTP and LTD, the detailed studies of Zhu et al. (2005) implicated involvement of JNK, not in LTP or LTD, but rather in depotentiation, where it mediates internalization of GluR1 ⁄ 2L downstream of NR2A and Rap2. So PSD95 is important for synaptic AMPA receptor currents (303), PSD95 ser295 regulates the synaptic localization of this protein, and JNK-dependent phosphorylation of PSD95 ser295 is proposed to promote retention of PSD95 at synapses and synaptic localization of functional AMPA receptors (304). Our results which showed that JNK inhibition converted decaying LTP to nondecaying LTP (weakening of potentiated synapses) may be explained by dephosphorylating of PSD95 on ser295 and subsequent loss of synaptic PSD95 and functional AMPA receptors. Therefore, the inhibitor will increase the phosphatase: kinase activity ratio leading to rapid dephosphorylation of JNK phosphorylation sites on substrates; and we indeed observed reduction in the specific phosphorylation of two known JNK substrates. JNK has also been reported to directly phosphorylate GluR2L⁄4 (305). However, a possible contribution of this to the regulation of LTD by JNK inhibition appears less likely, as JNK regulation of GluR2L ⁄ 4 may only promote recycling at extrasynaptic sites rather than functional synaptic receptors, and mutation of the JNK phosphorylation site in GluR2 does not lead to slowed recycling(305).

5.4. Effects of T4 on Protein Tau

Previous studies reported that reduced thyroid hormone levels in brain may result in the AD progression directly by raise expression of AβPP followed by amyloid- β peptide and amyloid- β levels. On a parallel note, TRH depletion has been associated with enhanced phosphorylation of tau proteins (306). In this study, total and phosphorylated Tau were measured to explain the relation between impairment of T4 to depotentiation magnitude and Tau protein. Our study showed that the level of total Tau protein in all infused groups was significantly higher compared to the non-stimulated (neutral) group, probably because of potentiation of synapses by HFS applying. This result was built on the analysis of the ratio of phosphorylated-to-total Tau protein showed that the fraction of phosphorylated tau at Thr231 was constitutively similar in all rats irrespective of their group ($P > 0.05$), while there are significant differences at Ser416 ($P = 0.005$). Also, increased p^{Ser416}Tau-to-t-Tau protein level ratio in the saline infusion group compared to the non-stimulated (neutral) group ($p = 0.006$) was inhibited by T4 at the dose of 100 pM ($p = 0.016$, but not at the dose of 100 nM ($p = 0.004$). There was no difference between other group pairs ($P > 0.05$). It is thought that increased p^{Ser416}Tau-to-t-Tau protein level ratio in infused groups (saline and 100 nM T4) also because of potentiation of synapses by HFS applying. Weakening of potentiated synapses resulted in obvious phosphorylation of Tau at ser^{416} epitope, which was markedly decreased in the presence of low concentration (100 nM) T4. So we can thought that if artificial potentiation (tetanic stimulation =100HZ) causes pathological increasing of p^{Ser416}Tau, it can be decreased by administrating low dose of T4.

The present study showed that at doses that not affect reversal of LTP, weakening of potentiated synapses resulted in obvious phosphorylation of Tau at ser^{416} epitope, which was markedly decreased in the presence of T4. Erk1/2 phosphorylation, but increased tau phosphorylation at Ser416 epitope that are was observed associated with weakening of potentiated synapses. This result was built on the analysis of the ratio of phosphorylated-to-total Tau protein showed that the fraction of phosphorylated tau at Thr231 was constitutively similar in all rats irrespective of their group, while there are significant differences at Ser416. Also, increased p^{Ser416}Tau-to-JNK ratio in the saline infusion group compared to the neutral group was inhibited by T4 at the dose of 100 pM.

Previous studies confirmed that phosphorylation of serine 416 could be used as an indicator for phosphorylation of tau by CaM kinase II in vivo (307). Also it was found that serine 416 was phosphorylated in rat brain extract. As addition of protein phosphatase inhibitors was necessary to detect the phosphorylation, this site appeared to be dephosphorylated rapidly during the preparation of brain extract. It was interesting that fetal-tau in the early post-natal period was much more strongly phosphorylated at serine 416 than higher molecular weight forms of tau in adult brain. Immunostaining of primary cultured hippocampal neurons indicated that both PS416-tau and CaM kinase IIα were localized mainly in neuronal soma. These results suggested that fetal-tau is phosphorylated by CaM kinase IIα in neuronal soma (307). Furthermore, when CaM kinase IIα was overexpressed in GT1-7 cells, CaM kinase IIα was localized mainly in neuronal soma and phosphorylation at serine 416 was clearly detected in neuronal soma. Phosphorylation of tau at serine 416 in wild-type cells was weaker than that in the stable clones (Y. Hiragami and H. Yamamoto, unpublished data). These results indicate that tau in neuronal soma is strongly phosphorylated by CaM kinase II, when CaM kinase II is abundant in neuronal soma. In this context, it is of interest that the accumulation of CaM kinase IIα has been noted in neuronal soma of CA1 and subiculum in AD brain (308).

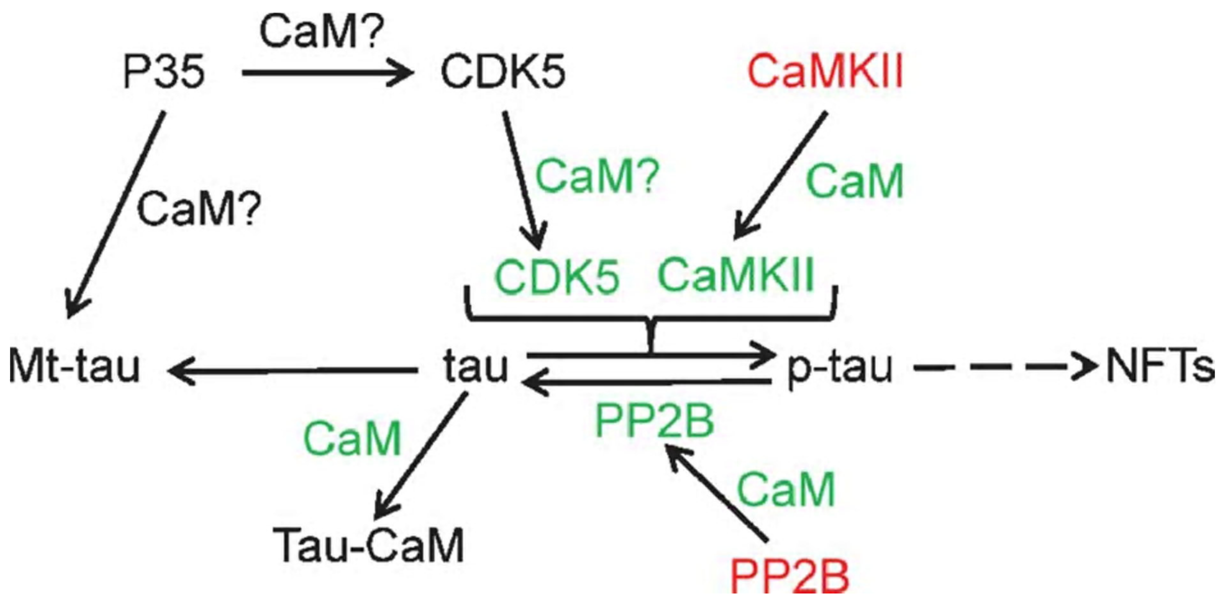

Figure 24. Calmodulin binding proteins linked to tau phosphorylation (309).

It is known that Calcium/Calmodulin-Dependent Protein Kinase IV (CaMKIV) belongs to the family of multifunctional calmodulin-dependent protein Ser/Thr kinases including also CaMKI, CaMKII and CaMKK (310). The enzyme is highly expressed in specific tissues, especially in brain (here next to the granular cells of the cerebellum, also in other neuronal subpopulations, especially in neuron nuclei of the hippocampus (311). Regulatory roles of CaMKIV are probably dependent on the induction by the thyroid hormone T_3 (312) (Figure 24) since maternal hypothyroidism results in a significant reduction of CaMKIV in the fetal brain (313) leading to the impairment of its neurodevelopment. Therefore it would be important to analyze in more detail the role of CaMKIV, whether its expression is influenced by hypothyroidism or by T4. But the role of this enzyme in depotentiation remained unstudied.

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T.C.
ERCİYES ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
(EÜHADYEK)

Tarih: 12.08.2015 **Toplantı Sayısı: 07** **Karar No:15/114**

Erciyes Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu 12.08.2015 tarihinde Prof. Dr. Fahri OĞUZKAYA'nın başkanlığında toplanmıştır.

Üye Adı/Soyadı	Ünvanı	Bölümü	İmza
Fahri OĞUZKAYA	Prof. Dr.	Tıp Fakültesi	
Coşkun TEZ	Prof. Dr.	Fen Fakültesi	
Gültekin ATALAN	Prof. Dr.	Veteriner Fakültesi	KATILMADI
Füsun Ferda ERDOĞAN	Prof. Dr.	Tıp Fakültesi	
Ahmet ÖZTÜRK	Doç. Dr.	Tıp Fakültesi	
Çağrı ŞAKALAR	Doç. Dr.	Tıp Fakültesi	KATILMADI
M. Betül AYCAN	Doç. Dr.	Eczacılık Fakültesi	KATILMADI
Nükhet KÜTÜK	Doç. Dr.	Diş Hekimliği Fakültesi	KATILMADI
Serpil SARIÖZKAN	Doç. Dr.	Veteriner Fakültesi	KATILMADI
Çağrı Çağlar SİNMEZ	Yard.Doç. Dr.	Veteriner Fakültesi	
Hamiyet ÜNAL	Yard.Doç. Dr.	Eczacılık Fakültesi	
Osman İBİŞ	Yard.Doç. Dr.	Ziraat Fakültesi	
Serap ALTUNTAŞ EROĞLU	Avukat	Kurumla İlişkisi Olmayan Üye	
Asiye GÖKBELEN	Yardım Sevenler Derneği Başkanı	Sivil Toplum Kuruluşu Temsilcisi	KATILMADI

Erciyes Üniversitesi Tıp Fakültesi Fizyoloji A.D.'dan **Prof. Dr. Cem SÜER** tarafından sunulan **"NMDA Reseptor Aracılıklı Depotansiyasyon Yanıtlarına Tiroid Hormonlarının MAPK Aracılıklı Etkisi: Tiroid Hormonu ve Alzheimer Hastalığı İlişkisi."** başlıklı proje incelenerek çalışmanın yapılmasının uygun olacağına ve Rektörlük makamına sunulmasına oybirliğiyle karar verildi.

Tarih : **12.08.2015**
Etik kurul Başkan Vekili : **Prof. Dr. Fahri OĞUZKAYA**
İmza :

Investigation of NMDA receptor-mediated depotentiation responses in rats with intra-hippocampal infusion of thyroid hormone: The role for MAPK signaling pathway

ORIJINALLIK RAPORU

%28	%9	%30	%
BENZERLIK ENDEKSI	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCI ÖDEVLERI

BIRINCIL KAYNAKLAR

1	Thomas M Sanderson. "Molecular Mechanisms Involved in Depotentiation and Their Relevance to Schizophrenia", Chonnam Medical Journal, 2012 Yayın	%8
2	Soner Bitiktaş, Burak Tan, Şehrazat Kavraal, Marwa Yousef, Yeliz Bayar, Nurcan Dursun, Cem Süer. "The effects of intra-hippocampal L-thyroxine infusion on long-term potentiation and long-term depression: A possible role for the αvβ3 integrin receptor", Journal of Neuroscience Research, 2017 Yayın	%7
3	www.frontiersin.org İnternet Kaynağı	%4
4	www.ncbi.nlm.nih.gov İnternet Kaynağı	%4

CURRICULUM VITAE

PERSONAL DATA

Surname:	Yousef
Name:	Marwa
Adress:	Bahcelievler Mah., Kanuni Cad.7/10, B lock Talas, Kayseri
Date of birth:	17. August 1985
Place of birth:	Alexandria, Egypt
Nathionality:	Egyptian
Phone:	+90 546 603 38 65
E-Mail:	marwa.w.sadek@gmail.com
Social Status:	Married

PROFESSIONAL EXPERIENCE

04/2016 – 10/2018

-Assistant and researcher on scientific and technological research projects (TUBITAK) entitled with "Investigation of NMDA receptor-mediated depotentiation responses in rats with intra-hippocampal infusion of thyroid hormone: The role for MAPK signaling pathway." with Prof. Dr. Cem Suer, Physiology Department, Medicine Faculty, Erciyes University, Turkey

- Teaching physiology student laboratory for 2nd year medical student at Erciyes University, Turkey

10/2013 – 04/2016

Assistant and researcher on scientific and technological research projects (TUBITAK) entitled with " Investigation The Genomic and Non-Genomic Effects of Thyroid Hormone On Behavioral, Molecular and Electrophysiological Indicators on Sinaptic Plasticity in Implanted Cannula Adult Rats" with Prof.Dr. Cem Suer, Physiology Department, Medicine Faculty, Erciyes University, Turkey

09/2007 – 09/2011

Assistant at Dentistry Faculty, Pharous University, Alexandria, Egypt

09/2007 – 09/2011

Assistant at Dentistry Faculty (Malysian Student), Alexandria University, Alexandria, Egypt

Assistant at Science Faculty, Alexandria University, Alexandria, Egypt

09/2006 – 06/2010	<u>Tesching practical courses</u> • Anatomy (vertebrate and invertebrate) • Taxonomy (vertebrate and invertebrate) • Physiology • Neurophysilogy • Histology • Parsitology

EDUCATION

10/2018	Preparation for Telc German A1 exam in 15/11/2018
Since 02/2016	Plan, start and write PhD thesis under supervising of Prof. Dr. Cem Suer, Physiology Department, Medicine Faculty, Erciyes University, Turkey
2017	Experimental animal certificate from Erciyes University, Turkey
12/2015	Qualification Exam for PhD
02/2014 – 06/2015	Pre-doctoral courses
09/2013 – 06/2014	Turkish language (C1 certificate) from ERSEM, Erciyes University, Kayseri, Turkey
09/2008- 07/2013	Master degree from Science Faculty, Alexanderia University, Egypt Entitled with "Evaluation The Effect of Lithium Treatment alone and along with Vitamin C on Rat Brain and Associated Glands"
11/2011 – 11/2012	With Neuromed Exchange Programme, I did two projects in Prof. Suer's Physiology Laboratory, Physiology Department, Medicine Faculty, Erciyes University, Turkey • Lithium Treatment Disrupts Behavioral and Electrophysiological Indicators Of Hippocampal Function in Rats • The effect of sleep deprivation on Behavioral and Electrophysiological Indicators Of Hippocampal Function in Rats
09/2007-06/2008	Premaster courses included German basic language
09/2002 –	Science Faculty, Alexandria University, Egypt

06/2006	Graduated with excellent grade from Special Zoology Department (Biology)
2003-2004	Computer course (Word, Excel, Photoshop and Power Point) at the Northern Military Command in Alexandria, Egypt

LANGUAGES

Arabic: Native tongue
English: Very good (1st foreign language in Egypt)
Turkish: Very good
German: Good
Frensh: Basic knowledge

MEMBERSHIPS AND INTERESTS

2009 – 2013	Member and researcher at Mediterranean Neuroscience Society (M.N.S.)
2010-2013	Researcher at FP7 Capacity Building Project
2010	Researcher South Mediterranean Group.

SKILLS AND COMPETENCES

Technical skills acquired through my master:

ELISA to measure several hormones levels in plasma

Immunohistochemistry technique on brain frozen section to detect nitric oxide producing cells on cortex and hippocampus.

Histological techniqu

oTechnical skills acquired through my Ph.D.:

Stereotactic surgery in rats and applying the following protocols:

 Long-Term Potentiation (LTP), Long-Term Depression (LTD)

, Metaplasticity and Depotentiation

Different protein level measurements via gel electrophoresis- western blot and ELISA

Other skills

Target-oriented

Analytical and conceptual thinking

Strong perception of details with stron intercultural competences

Teamwork and empathy

Enjoy to learn new protocols and methods for new researches

Ability to work under pressure with accuracy and commitment

LIST OF PUPLICATIONS

A paper "Depotentiation of long-term potentiation is associated with epitope specific tau hyper/hypo phosphorylation in the hippocampus of adult rats" on Journal of Molecular and Cellular Neuroscience

A paper entitled "The Effects of Intra-hippocampal L-Thyroxine Infusion on Long-term Potentiation and Long-term Depression: a Possible Role for the $\alpha v\beta 3$ Integrin Receptor" on Journal of Neuroscience Research.

A paper entitled " Chronic Lithium Treatment Impairs the Long term Potentiation and Spatial Memory Function in Adult Rats" on Journal of Clinical Psychopharmacology and Neuroscience.

Through confrences

Published a poster entitled (Inhibition of c-Jun N-terminal Kinase Decelerates the Reversal of Induced Long-Term Potentiation: Implication on Treatment of Memory Loss.) 31st ECNP Congress in Barcelona, Spain, on 6-9 October 2018

Published a poster entitled (Inhibition of Extracellular Signal–Regulated Kinases 1/2 Rescues the Decay of Long-term Potentiation in the Dentate Gyrus in vivo: Implication on Alzheimer's disease.) 31st ECNP Congress in Barcelona, Spain, on 6-9 October 2018

Published a poster entitled (The effects of P38-MAPK inhibitor on long-term potentiation, long-term depression and depotentiation in the perforant pathway – dentate gyrus synapses), 30th ECNP Congress, 2-5 September 2017. Paris, France

Published a research by symposium entitled (The Effectiveness of Spreading Depression Wave On Long Term Potentiation Magnitude On Dentate Gyrus) 15th National Neuroscience Congress, Sakarya University, Sakarya, 7 - 10 May 2017

Published a poster entitled (Adult Onset Hyperthyroidism Enhances LTD and Up-regulates P38-MAPK and PP1 Expression in Rat Hippocampus) on 28th ECNP CONGRESS in Amsterdam, The Netherlands 29 August-1 September 2015.(win a CDE grant)

Participate by poster (Lithium Treatment Disrupts Behavioral and Electrophysiological Indicators Of Hippocampal Function in Rats.) 13th National Neuroscience Congress, Selçuk University Neuroscience Research Center, Konya, 30 April 2015 - 3 May 2015

Symposium entitled (Evaluation of lithium chloride treatment on brain and synaptic plasticity) in 4th conference of the Mediterranean Neuroscience society. (September 30- October 3, 2012, Istanbul, TURKEY)

Published via symposium a research entitled (Evaluation of Lithium treatment alone and along with Vitamin C on rat brain and associated glands) the Second IBRO-Kemali Mediterranean School of Neuroscience, which will be held at the Naples/ Italy international laboratory "Stazione Zoologica Anton Dohrn" July 6-13, 2011

Published a poster entitled (Evaluation of Lithium treatment alone and along with Vitamin C on rat brain and associated glands) 7th International Congress of Neuroendocrinology in Rouen/ France July, 2010

Marwa Yousef