T.C.
REPUBLIC OF TURKEY
HACETTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

THE EFFECTS OF ACUTE AND CHRONIC DIPHENHYDRAMINE AND CETIRIZINE USE ON LEARNING AND MEMORY IN RATS

Sadık Taşkın TAŞ, MD

Medical Pharmacology Program

DOCTOR OF PHILOSOPHY THESIS

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Sadık Taşkın Taş, MD

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18/01/2018

Dr. Sadık Taşkın Taş

ETHICAL DECLARATION

I declare that all the information and documents have been obtained within the academic rules and all audio-visual and written information and results in this thesis study have been presented according to the rules of scientific ethics. I did not do any distortions in the data set. In the case of using other works, related studies have been fully cited in accordance with the scientific standards. I also declare that my thesis study is original except the cited references. It was produced by myself in consultation with my supervisor Prof. Mehmet Yıldırım SARA, MD, PhD and written according to the thesis guidelines of Hacettepe University Institute of Health Sciences.

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This work is dedicated to my wife, Güneş.

ÖZET

Taş, S. T., Akut ve Kronik Difenhidramin ve Setirizin İlaç Kullanımının Sıçanlarda Öğrenme ve Bellek Üzerine Etkileri, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Tıbbi Farmakoloji Programı Doktora Tezi, Ankara, 2018. Antihistaminik ilaçlar alerji, nezle ve uykusuzluk gibi birçok endikasyonu olan ve çok yaygın olarak kullanılan bir ilaç grubudur. Bu ilaçların santral sinir sistemi yan etkileri yaptığı bilinmesine rağmen öğrenme ve bellek gibi bilişsel fonksiyonlar üzerine etkileri tam olarak aydınlatılamamıştır. Bu çalışmada *in vivo* hipokampal alan potansiyeli kayıtları kullanılarak akut ve kronik setirizin ve difenhidramin tedavisi ile gelişen nöroplastisite değişikliği etkileri incelendi. Difenhidraminin antimuskarinik etkilerini değerlendirmek için ise skopolamin kullanıldı. Ayrıca, setirizinin kognitif performans üzerinde etkilerini araştırmak için davranış deneyleri yapıldı. Setirizinin patofizyolojik koşullardaki etkilerini araştırmak için de bir REM uyku yoksunluğu modeli kullanıldı. Difenhidramin ve setirizin akut kullanımı kısa dönem plastisitenin potansiyasyon fazlarını engelledi ancak kronik kullanımda bu etkiye tolerans gelişti. Uzun dönem plastisite deneylerinde setirizin tüm gruplarda LTD'yi ve kronik tedavi grubunda LTP'yi inhibe etti. Akut ve kronik setirizin tedavisi sonrası yapılan davranış deneylerinde anlamlı bir sonuç alınamadı. Bu sonuçlar setirizinin uzun dönem plastisite üzerine difenhidraminin oluşturamadığı etkileri olduğunu göstermektedir. Bu bulgulara göre, setirizin hipokampal devreleri H1 reseptör blokajı dışında daha önce bilinmeyen bir mekanizma ile etkiliyor olabilir. Uyku yoksunluğu sırasında setirizin kullanımı kognitif bozukluklara karşı koruyucu olabilir.

Anahtar Kelimeler: Antihistaminik ilaçlar, öğrenme, bellek, setirizin, difenhidramin, REM uyku yoksunluğu

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ABSTRACT

Taş, S. T., The Effects of Acute and Chronic Diphenhydramine and Cetirizine Use on Learning and Memory in Rats, Hacettepe University Institute of Health Sciences Medical Pharmacology Doctor of Philosophy Thesis, Ankara, 2018. Antihistamines are one of the most widely used drug groups in various indications such as allergy, common cold and insomnia. Although these drugs are known to cause central nervous system side effects, their effects on cognitive functions such as learning and memory are not fully elucidated. We used *in vivo* hippocampal field potential recordings to assess the neuroplasticity changes caused by acute and chronic diphenhydramine and cetirizine treatment. Scopolamine is also used to assess the antimuscarinic effects of diphenhydramine. Furthermore, behavioral experiments were conducted to investigate the effect of cetirizine on cognitive performance. An REM sleep deprivation model was used to assess the effects of cetirizine in pathophysiological conditions. Acute use of diphenhydramine and cetirizine affected the potentiation phases of short-term plasticity while chronic treatment caused a tolerance to this effect. In long-term plasticity experiments, cetirizine blocked LTD in all groups and inhibited LTP in chronic treatment. Cetirizine prevented REM sleep deprivation-induced inhibition of LTP. Behavioral experiments with acute and chronic cetirizine treatment yielded no significant results. Our data showed that cetirizine has a significant effect in long-term plasticity that diphenhydramine failed to elicit. Therefore, cetirizine might be affecting the hippocampal circuitry independent of H1 receptor blockage through a previously unknown mechanism. Cetirizine use during sleep deprivation could be protective against cognitive dysfunction.

Key Words: Antihistamines, learning, memory, cetirizine, diphenhydramine, REM sleep deprivation

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Appendix 1: Hayvan Deneyleri Yerel Etik Kurulu Kararı

8. CURRICULUM VITÆ

SYMBOLS AND ABBREVIATIONS

aCTRL	Acute treatment control group
aCTZ	Acute treatment cetirizine group
aDPH	Acute treatment diphenhydramine group
aSCO	Acute treatment scopolamine group
cAMP	3',5'-cyclic adenosine mono phosphate
AKT	Protein kinase B
AMPAR	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
cCTRL	Chronic treatment control group
cCTZ	Chronic treatment cetirizine group
cDPH	Chronic treatment diphenhydramine group
CREB	cAMP responsive element-binding protein
cSCO	Chronic treatment scopolamine group
CTRL	Control
CTZ	Cetirizine
DAG	Diacylglycerol
DPH	Diphenhydramine
EP	Electrophysiology
EPM	Elevated plus maze
FDA	Food and Drug Administration of the United States of America
fEPSP	Field excitatory postsynaptic potential
GABA	γ-Aminobutyric acid
HDC	L-histidine decarboxylase
HFS	High frequency stimulation
I/O	Input/output
ICV	Intracerebroventricular
IP3	Inositol (1,4,5) trisphosphate
IPI	Interpulse interval
LFS	Low frequency stimulation
LTD	Long-term depression

LTP	Long-term potentiation
MWM	Morris water maze
MWMr	Morris water maze reversal learning
OFA	Open field arena
OTC	Over-the-counter
PA	Passive avoidance
PP	Paired-pulse
PS	Population spike
REM	Rapid eye movement
REMD	REM sleep deprived acute treatment control group
REMD+aCTZ	REM sleep deprived acute cetirizine treatment group
SC	Schaffer collaterals
SCN	Suprachiasmatic nucleus
SCO	Scopolamine
SEM	Standard error of mean
TMN	Tuberomamillary nucleus
VHC	Ventral hippocampal commissure

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1. INTRODUCTION

Histamine, originally named beta-iminazolylethylamine, is first synthesized in 1907 by Windaus and Vogt (1). Not soon after, in 1910 and 1911, Dale and Laidlaw published their findings on physiological effects of this new molecule in two articles where they demonstrated its anaphylactic effects (2,3). But it was not clear if this molecule is produced by the body or comes from exogenous sources until 1927, when an article authored by Best, Dale et al. established histamine is, in fact, synthesized by the cells (4).

By 1930s, the interaction between histamine and allergy/anaphylaxis was well-known and first search for agents to block the effects of histamine was initiated in Pasteur Institute in France by Staub and Bovet and was independently followed by researchers in the United States of America during Second World War years (1939 – 1945). The first antihistamine released for therapeutic use was phenbenzamine in 1942, which was subsequently replaced by mepyramine (pyrilamine) in 1945. Diphenhydramine, which is still a widely used agent, was released the same year and many other antihistamines followed them later the same decade (5,6).

Since their first inception, the major side effect of these classical antihistamines has been drowsiness. They interact with the histamine receptors in the central nervous system and cause depression of arousal in the brain (7). When terfenadine and astemizole, two new antihistamines, were being registered in Europe in 1983, they were classified as “second generation” antihistamines; less soluble in lipids therefore penetrating the blood-brain barrier less than preceding “first generation” antihistamines. Even though these two molecules were later withdrawn from the market worldwide because of their cardiovascular side effects, many other safer 2nd generation antihistamines have been released (8). Recently, drugs that reach even lower central nervous system concentrations are named as “third generation” antihistamines (9).

According to the estimates of World Health Organization, there are 400 million allergic rhinitis patients in the world, and these diseases' prevalence is increasing worldwide. Especially juvenile population has a very high risk for allergic rhinitis with a 33.2% worldwide prevalence (10). Second generation oral antihistamines are one of the first line drugs recommended by the guidelines for the treatment of such allergic diseases (11–13). Even though first generation antihistamines are not suggested for the treatment of allergy, urticaria and angioedema in guidelines, they are still commonly used as common cold, insomnia and motion sickness medicines (14). The indications antihistamines are used with or without Food and Drug Administration of the United States of America's (FDA) approval are listed in Table 1.1.

Table 1.1. List of indications for antihistamines and their FDA approval status.

Indication	**Drug**	**FDA Approval**
Allergic rhinitis	Most of the available H1 antihistaminics	+/OTC
Allergy/urticaria	Most of the available H1 antihistaminics	+/OTC
Pruritus	Most of the available H1 antihistaminics	+/OTC
Allergic conjunctivitis	Most of the available H1 antihistaminics	+/OTC
Anaphylaxis (adjunct)	Diphenhydramine, promethazine, cyproheptadine	+
Common cold	Diphenhydramine, chlorphenamine	+/OTC
Hyperemesis gravidarum	Doxylamine	+

Motion sickness	Diphenydramine, promethazine, cyclizine, meclizine	+/OTC
Parkinson's disease	Diphenydramine	+
Preop, postop, obstetric pain	Promethazine, hydroxyzine	+
Nausea, vomiting	Promethazine, cyclizine, meclizine	+/OTC
Anxiety	Hydroxizine	+
Insomnia	Diphenydramine, promethazine	+/OTC
Insomnia	Doxylamine	-/OTC
Eye itching	Ketotifen	-/OTC
Local anesthesia	Diphenhydramine	Counterindicated
Extrapyramidal disease – drug related movement disorders	Diphenhydramine	-
Menstrual pain	Pyrilamine	-/OTC
Constrast matter adverse reaction	Diphenhydramine, promethazine	-/Adjunct

Antihistamines are available as over-the counter drugs for most of their use cases but some of those cases are not approved by FDA or even considered as counterindications (14,15).

As they are indicated for some illnesses with very high prevalence; most notably allergies, common cold and insomnia; antihistamines are among the most used drugs in the world. Because of their relative safety and the commonness of these indications, many of these antihistamine-containing preparations are also available as over-the-counter (OTC) drugs, which further increase their use. Table 1.2 lists the sales figures for highest-selling OTC sleep aids in the USA for the year 2015.

Antihistamines are the active ingredient of the top three and also half of the drugs in the list.

Table 1.2. List of the best-selling OTC sleep aids in the USA for the year 2015.

Drug	Active Agent	Agent Group	Sales
Vicks NyQuil ZzzQuil (syrup)	Diphenhydramine HCl	Antihistaminic	$76.6M
Vicks NyQuil ZzzQuil (tablet)	Diphenhydramine HCl	Antihistaminic	$41.6M
Unisom Sleepgels	Diphenhydramine HCl	Antihistaminic	$30.5M
Alteril	L-Triptophane, melatonine and valerian	Serotonin agonist, sedative/hypnotic, herbal	$15.1M
Unisom Sleeptabs	Doxylamine succinate	Antihistaminic	$13M
Midnite	Melatonin	Sedative/hypnotic	$11.1M
Simply Sleep	Diphenhydramine HCl	Antihistaminic	$10.3M
Neuro Sleep	Melatonin, serotonin and L-teanin	Sedative/hypnotic, serotonin agonist, glutamatergic	$9.9M
Peak Life Somnapure	Melatonin, valerian and L-teanin	Sedative/hypnotic, herbal, glutamatergic	$6.5M
Nature Made Sleep	Melatonin, L-teanin	Sedative/hypnotic, glutamatergic	$5.4M

First generation antihistamine diphenhydramine is the active ingredient of top three ranking best-selling OTC sleep aids in the USA (16). The list also includes doxylamine, which is another first generation antihistamine.

Even though antihistamines are known to affect the central nervous system—as one of their most prevalent side effects is drowsiness—and they are one of the

most used groups of drugs worldwide for a wide variety of indications with or without prescriptions, their effects on cortical functions are not yet fully elucidated.

2. GENERAL INFORMATION

2.1. Histamine

Histamine, also known as 2-(1*H*-Imidazol-4-yl)ethanamine (17), is a molecule involved in a wide variety of physiological and pathophysiological processes. Endogenously synthesized histamine has many roles in mainly immune, gastrointestinal and central nervous systems. Histamine is also found in some foods and high amounts of ingested histamine causes histamine intolerance and food poisoning (18). Histamine does not cross the blood-brain barrier (19).

2.1.1. Chemical Properties of Histamine

Chemically, histamine is a low molecular weight amine. Its chemical formula is $C_5H_9N_3$ and molecular weight is 111.148 g/mol. It is a strong base that exhibits two main structural centers; a primary aliphatic amine (pK_{a1} 9.4) and an imidazole ring (pK_{a2} 5.8) (20). At physiologic pH and temperature, it exists in an equilibrium where tautomers of monocation is the preferred form (96%), with a minor fraction of dicationic form (3%) and a very small amount of neutral tautomers (20,21). The molecular structure of each ionization of histamine is shown in Figure 2.1.

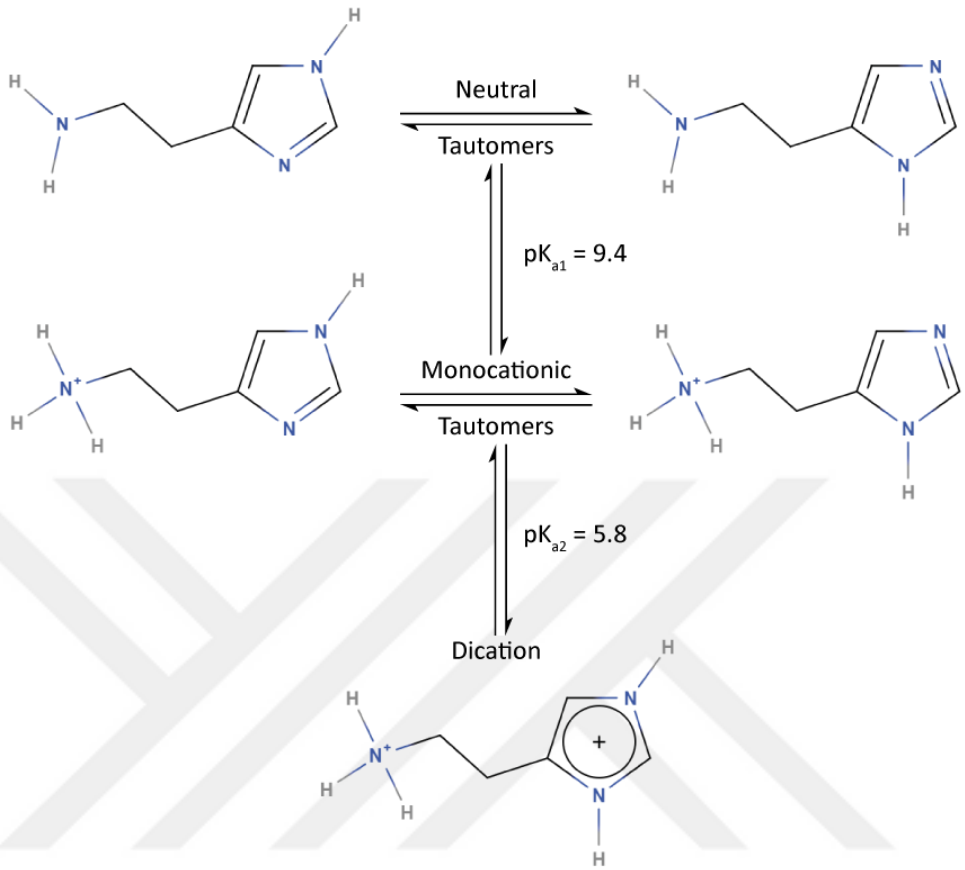

Figure 2.1. Molecular structure of histamine with its different ionizations and tautomers. The aliphatic amine has a pK_a value of 9.4 while the imidazole ring has a pK_a value of 5.8 at 37 °C. Neutral and monocationic forms have two tautomers depending on the location of the proton in the imidazole ring. Majority of histamine is found in its monocationic tautomers at physiologic pH (20,21).

2.1.2. Synthesis of Histamine

Histamine is synthesized from the essential amino acid L-histidine exclusively by the cytosolic enzyme L-histidine decarboxylase (HDC); it cannot be generated by any other enzymatic pathway. After its synthesis, it is stored in special granules in the cells that produce large amounts of histamine, e.g. mast cells, enterochromaffin-like cells; while some cells that produce small amounts of histamine immediately release it after synthesis, e.g. lymphocytes and epithelial cells (18,20). The synthesis pathway of histamine is depicted in Figure 2.2.

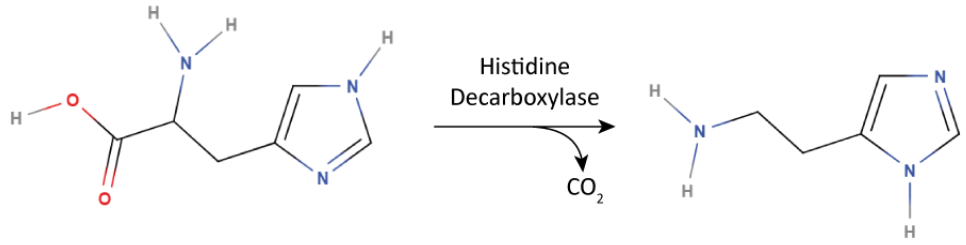

Figure 2.2. Synthesis of histamine from L-histidine. The cytosolic enzyme histidine decarboxylase converts the essential amino acid L-histidine into histamine. During the process, carbon dioxide is yielded as a side product (22).

2.2. Histamine Receptors

Histamine has four membrane-bound receptor subtypes that are all from the G-protein coupled receptor superfamily. Specifically, they belong to the aminergic receptor category of the rhodopsin-like family of G-protein coupled receptors (23). They are named as H1, H2, H3 and H4 receptors and they are numbered in the order of their discovery. Phylogenetically; H1, H3 and H4 receptors belong to subfamily A18 of rhodopsin-like family while H2 receptors belong to subfamily A17 (24). Histamine receptors show high constitutive activity, which means they are partially active even in the absence of the ligand or an agonist (5).

Histamine is also observed to activate chloride conductance in several brain regions and this effect was blocked with picrotoxin and H2 receptor antagonists and was shown to be not G-protein mediated. This ionotropic histamine activity is speculated to be through a receptor similar to γ-Aminobutyric acid A ($GABA_A$) receptor in function (25).

2.2.1. Histamine H1 Receptor

After the first antihistamines were developed to prevent allergy and anaphylaxis, it was observed that these molecules could block the effects of histamine on various smooth muscles, they could not prevent some other effects like increasing heart rate or facilitating gastric acid secretion. Therefore it was deduced

that there are two histamine receptors with different functions (5). The receptor responsible for the allergic and anaphylactic effects, named H1, was later identified pharmacologically as a G-protein coupled receptor in 1966 (26).

H1 receptor is coupled to $G_{\alpha/Q11}$ subtype of G-proteins, which it activates the phospholipase C-β enzyme that catalyzes the hydrolysis of phosphatidylinositol 4,5-phosphate into inositol-(1,4,5)-trisphosphate (IP3) and diacylglycerol (DAG) secondary messengers (27,28). IP3 causes the release of intracellular Ca^{++} deposits through activation of IP3 channels on smooth endoplasmic reticulum that act as Ca^{++} channels. Ca^{++} acts as a secondary messenger and along with DAG they activate protein kinase C enzyme which phosphorylates and regulates downstream proteins (28). βγ subunits of G-protein cause the activation of AMP-activated protein kinase and nuclear factor- κB (26,29).

Histamine H1 receptor is found in most of the smooth muscles, endothelial cells, lymphocytes, adrenal medulla, myocardium, central nervous system and bare endings of unmyelinated type C nerve fibers (26,27). H1 receptor activation in blood vessels result in an increase in vascular permeability largely via nitric oxide-dependent vascular smooth muscle dilation and partly via PKC/rho-associated protein kinase/NO-dependent constriction in endothelial cells (18). Activation of the H1 receptor in bronchial smooth muscles is known to constriction; but *in vitro*, if the H1 receptors are antagonized, histamine causes dose-related bronchial relaxation via H2 receptors. In the adrenal medulla, H1 activation causes stimulation of both adrenaline and noradrenaline and also induces the phosphorylation—therefore activation—of tyrosine hydroxylase enzyme that is part of the catecholamine biosynthesis pathway (20). In the heart, H1 receptor is responsible for a negative dromotropic effect of histamine on the heart and positive inotropic effect of histamine on the atria (30).

In the central nervous system, neocortex layers IV and V, claustrum, hippocampus, thalamus, nucleus accumbens, posterior hypothalamus and globus pallidus have high densities of histamine H1 receptors while cerebellum and basal

ganglia have them in lower densities (27,31). H1 receptors are also found in spinal cord (32). Activation of H1 neurons in many central nervous system neurons causes depolarization and an increase in action potential frequency via the blockade of small leak K^+ channels therefore preventing the polarization of the membrane. In hippocampal pyramidal cells and some neurons of cortex, H1 receptor activation causes hyperpolarization as intracellular Ca^{++} increase opens Ca^{++}-dependent K^+ channels on the membrane of these neurons and intracellular K^+ flows out of the cell through the opened channels. H1 receptors are also shown to enhance the NMDA receptor-mediated currents in the neurons of the cortex (32).

2.2.2. Histamine H2 Receptor

The inability to block cardiovascular, uterine and gastric effects of histamine with the prototypical antihistamines led researchers to conclude that there were two histamine receptors. The second histamine receptor was confirmed to exist when a histamine receptor antagonist that did not block H1 receptors, burimamide, and a selective H2 receptor antagonist, cimetidine, were discovered and shown to block physiological effects of histamine that were not blocked by H1 receptor antagonists (5).

Histamine H2 receptor is coupled to $G_{\alpha s}$ isoform of G-protein which activates adenylyl cyclase that catalyzes the conversion of adenosine triphosphate to 3',5'-cyclic adenosine mono phosphate (cAMP) and pyrophosphate. cAMP in turn activates protein kinase A and transcription factor “cAMP responsive element-binding protein” (CREB) (26,33). H2 receptor is found in brain, gastric cells, cardiac tissue, immune system cells, blood vessels and tracheobronchial, esophageal, uterine and vascular smooth muscles. In the brain it is distributed in high densities in basal ganglia, amygdala, hippocampus, cerebral cortex layers I and II (26,31,32) while it is distributed in low densities in septal areas, hypothalamus, thalamus and cerebellar neurons (27,32). It is also found in spinal cord (32).

H2 receptor activation is a potent stimulator of gastric acid secretion. In the heart, it causes positive inotropic and chronotropic effects (27). H2 receptors are also present on suppressor T cells and neutrophils of the immune system where they stimulate suppressor T-cell activity but inhibit basophil and mast cell histamine release, neutrophil chemotaxis, T-cell proliferation, cell-mediated cytolysis, cytokine production and antibody synthesis (20,26,27). H2 receptor activation causes dilation in vascular, uterine and tracheobronchial smooth muscles while causing contraction in esophageal muscle (18,26). H2 receptors also cause tracheobronchial mucus secretion (26).

Secondary messengers of histamine H2 receptor have important roles for neuronal physiology and synaptic plasticity (29). Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated ion channel 2, also known as HCN2 or I_h, is a type of channel that evoke rhythmic electrical activation in cardiomyocytes and specific neurons. The channel is activated by hyperpolarization but cAMP is a ligand for the channel and facilitates its activation (34). H2 receptor activation, through PKA, blocks high frequency action potential generation through voltage-gated K+ channel Kv3.2 and Ca^{++}-activated K^+ conductance through small K channels. Small K channels are responsible for the post-action potential afterhyperpolarization of neurons (29).

2.2.3. Histamine H3 Receptor

Histamine H3 receptor is first discovered in 1983 as an autoreceptor regulating the histamine release and synthesis (5,29,32). Now it is known that H3 receptor populates both presynaptic and postsynaptic neurons (35). It is an inhibitory receptor that is coupled to $G_{i/o}$ subtype of G-protein. Gi/o activation leads to inhibition of cAMP synthesis (32). Although all histamine receptors show constitutive activity, H3 receptor is known to have a very high constitutive activity that is also demonstrated *in vivo* and this property is important in its physiology (19,35).

H3 receptor is primarily found in the nervous system. It is heterogeneously distributed in all central nervous system but it concentrates primarily in anterior cerebral cortex, hippocampus, amygdala, nucleus accumbens, striatum, olfactory tubercles, cerebellum, substantia nigra and brain stem (29). As it is an inhibitory receptor, its main role in the neurons is to inhibit neurotransmitter release, including glutamate, GABA, dopamine, acetylcholine, serotonin, various peptide neurotransmitters as well as histamine as an automodulator receptor through blockage of high-threshold Ca^{++} channels (19,32).

In the periphery, H3 receptors inhibit gastric mast cells or enterochromaffin-like cells to decrease gastric acid secretion. H3 activation also relaxes cerebral arteries through an endothelium-dependent pathway and stimulates adrenocorticotrophic hormone release from the hypophysis (27).

2.2.4. Histamine H4 Receptor

Histamine H4 receptor is the latest discovered G-protein coupled histamine receptor. It was first reported in late 2000 – early 2001 by several research groups as a receptor that has a high homology with H3 receptor (36–38). Like H3 receptor, H4 receptor is also coupled to the inhibitory $G_{i/o}$ protein (38).

It was first found to be expressed in the peripheral tissues but later it was also reported to be expressed in the central nervous system (19,29). In the periphery, it is found in blood, spleen, lung, liver and gastrointestinal system (29). It is also found in high densities in immune system cells such as eosinophils, mast cells, T cells, dendritic cells and basophils where it plays a role in chemotaxis (5,26). It is observed in thalamus, hippocampus, cerebral cortex layer III and IV, cerebellum, brain stem, amygdala, thalamus, striatum, dorsal root ganglia and spinal cord of various mammalian brains (19,31). Its function in these regions is inducing hyperpolarization and outward rectifying current promotion (19).

2.3. Role of Histamine in Homeostatic Brain Functions

In the brain, all histaminergic neurons originate from tuberomamillary nucleus (TMN), which is located in the posterior hypothalamus and send projections to whole central nervous system to release histamine as a modulator neurotransmitter (Figure 2.3) (19,29,39). In the whole central nervous system, these neurons are the only ones that express histidine decarboxylase, the sole enzyme responsible for the synthesis of histamine (20,29,40). While histamine is exlusively synthesized in TMN, it is not the only neurotransmitter released from tuberomamillary projections. They also release GABA, galanin, enkephalins, thyrotropin releasing hormone and substance P. TMN receives inputs from preoptic area of hypothalamus, septum, prefrontal cortex, subiculum and dorsal tegmentum. (29).

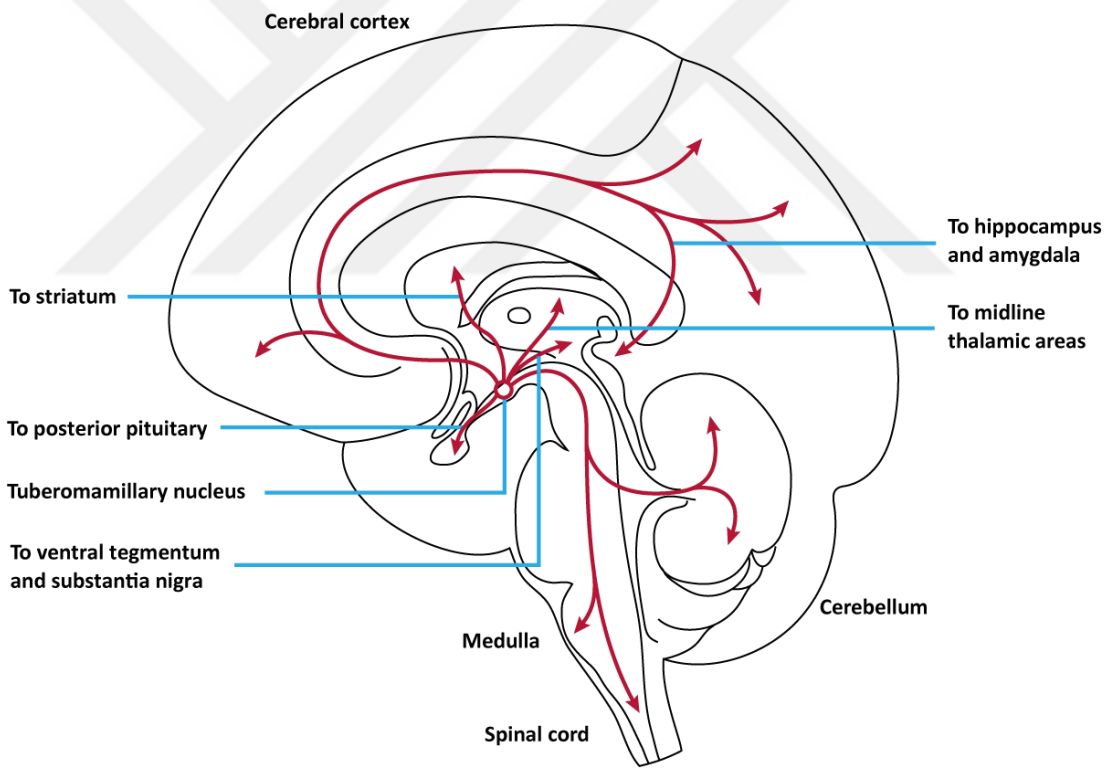

Figure 2.3. The location and projections of tuberomamillary nucleus in the human brain. Tuberomamillary nucleus resides in the posterior hypothalamus. Histaminergic neurons are located only in this region in the brain and sends projections to the whole central nervous system (39).

Histaminergic tonus shows circadian changes with a peak concentration of brain histamine during wakefulness (41). This rhythmicity regulates various

behavioural and physiological patterns such as sleep, feeding, motor activity and hormone release cycles (29,41). Histamine is one of the neurotransmitters regulating the internal clock in the suprachiasmatic nuclei (SCN) of hypothalamus, the central area for circadian rhythm in mammals. Histaminergic projections to hypothalamus (including SCN), thalamus, basal forebrain and mesopontine tegmentum are responsible for control of circadian rhythm via histamine (42). Increased histamine tonus promotes wakefulness via stimulating these regions through H1 receptor (29,42). While seemingly self-regulatory, H3 receptor also plays an important role in the control of circadian rhythm (43). It is observed that after long durations of sleep deprivation, brain histamine levels decrease (29). Histamine also plays a role in central control of biological rhythms that are infradian—longer than a day—such as reproductive and hibernative cycles (29).

Brain histamine also regulates the feeding rhythm. Increased histaminergic tonus in ventromedial nucleus of the hypothalamus suppress food intake and decrease plasma triglycerides through histamine H1 receptor. In addition, histamine regulates body fat percentage by also modulating peripheral energy expenditure; increased H1 activation prevents obesity in diet-indsuced and *db/db* (diabetic dyslipidemia model) obese mice (44). Furthermore, histamine activates supraoptic nucleus neurons through H1 receptors to release vasopressin which causes antidiuresis and water intake (29). Histamine is also involved in body temperature regulation; increase in medial preoptic nucleus causes an increase in the core temperature through H1 receptors while H2 receptor activation in posterior hypothalamus causes heat loss (29,32,45).

Histamine is an important mediator in the stress response of the body. Psychological or metabolic stress activates the neurons in the TMN via neuroendocrine stress signals. In turn, histamine mediates ACTH, β-endorphin and vasopressin release from the hypophysis and controls stress-related activation of aminergic neurons (29,46). Other neuroendocrine pathways interact with histamine. TMN neurons get excited by thyroid releasing hormone and released histamine

shows inhibitory effects on the release of TRH and TSH through H2 receptor in hypothalamus and hypophysis. Similarly, GH release axis is affected by histamine in an inhibitory way; histamine release from the TMN decreases pulsatile GH secretion. Reproduction-related hormones are also affected by the histamine activity. Increased histamine activates gonadotropin releasing hormone release from the supraoptic nucleus. Histaminergic neurons of the TMN seem to be modulated by a feedback mechanism through the α-estrogen receptors they express (29).

Histamine effects cardiovascular functions through central nervous system as well as it does through peripheral systems. Increased central histamine causes hypertension and bradycardia through mechanisms involving both H1 and H2 receptors. Following the histamine release in the brain, plasma catecholamines are observed to increase (29,32).

2.4. Role of Histamine in Higher Brain Functions

Histamine is a very important neurotransmitter for sensory and motor system modulation. It is involved in sensory gating and nociception in the central nervous system. Increase in histamine or H3 receptor inhibition produces an analgesic effect and histaminergic activity blocks nociceptive neuron firing in nucleus lateralis and ventrobasalis of thalamus (32). Increased histamine levels in the brain cause a temporary increase in the locomotion while histamine depletion reduces locomotive behavior (29,47). Knocking out histidine decarboxylase, H1, H2 or H3 all cause in a decrease of locomotion (48).

Histamine is involved in higher cortical functions of mood and cognition. H2 receptor knockout mice show more anxiety while H1 receptor knockout does not produce such a change. H3 deletion causes some anxiety behavior depending on the experimental method used (49). H4 receptor deficiency amplifies anxiety and its activation causes less anxiety (50). Complete inexistence of histamine results in anxiolysis (49). Although dopamine is known to be the main neurotransmitter of the

reward system, histamine plays an important modulatory role on the mesolimbic dopamine transmission.

Furthermore, histamine seems to modify the activation of the reward system by drugs (51). Experiments show that rats that prefer more alcohol have higher histamine concentration in their central nervous systems. H3 receptor expressions in the motor cortex, nucleus accumbens and hippocampus CA1 were lower in high alcohol consumption rats compared to low consumption rats while H3 expressions in lateral septum or TMN were unchanged. Therefore, it can be construed that H3 receptor activity is not responsible for increased histamine concentration but it is responsible for addictive behavior. It was also observed that H1 receptor expression was lower in rats that consume high alcohol, which might be due to downregulation resulting from high histamine concentration. Nevertheless, H1 receptor antagonists did not affect alcohol self-administration whereas H3 inverse agonists significantly reduced it (52).

Histamine has an important role in learning and memory. Brain regions responsible for learning and memory show a high concentration of histaminergic receptors. The central histaminergic system was thought to be interacting with the cognition since the first antihistamines. The first experiment to link histamine to learning and memory was a cocktail of H1 and H2 antagonists blocking the facilitator effect of intracerebroventricular (ICV) histamine in a step-down inhibitory avoidance behavior (53). It is later shown that both H1 or H2 knock-out mice show decreased learning – memory performance and hippocampal long-term potentiation (LTP) (40). Interestingly, studies conducted on histamine signaling find facilitation, inhibition or no effects on learning tasks (32,49,53). Those variances in results can be explained by the species, age and sex variations of animals as well as differences in experimental design such as methods used to alter histamine signaling (receptor or enzyme knock-out, different agonists and antagonists, etc.), dose and route of administration of chemicals, date and time of administrations and experiments and last but not least,

the method used to assess learning and memory. Current knowledge is that all four histamine receptors play significant roles in learning and memory.

H1 receptor increases intracellular Ca^{++} levels and PKC, both of which are required for the induction of early stages of synaptic plasticity. Intracellular Ca^{++} activates membrane Na^{+}-Ca^{++} exchanger and causes depolarization in neurons such as the ones in supraoptic nucleus (53). H1 receptor activation also enhances NMDA receptor currents in the neurons by modulating the Mg^{++} block on the receptor (32). On the other hand, activation of IP3 cascade opens the small conductance K^{+} channels in some neurons, for example the pyramidal cells of hippocampus, causing hyperpolarization and therefore inhibition of excitability. Oral or ICV H1 antagonist administration inhibits active avoidance response (53). Single dose oral H1 antagonist also impairs working memory processing speed without affecting episodic memory (54). Furthermore, increased brain histamine ameliorates stress-induced disruption of LTP in hippocampal slices and this effect is blocked *in vitro* by H1 receptor antagonists (55). Single dose ICV injection of an H1 antihistamine inhibits LTP in awake animals (56). On the other hand, histamine injection immediately after an inhibitory avoidance task training facilitates learning in aged rats (32). So far, there is no *in vivo* electrophysiological data in the scientific literature about the effects of H1 antihistamines on learning and memory when they're administered systemically over short- and long-term periods.

H2 receptor, through increasing cAMP levels, facilitates late phase NMDA receptor-dependent LTP. H2 receptor activation also enhances NMDA receptor currents by inhibiting the calcium-dependent potassium conductivity (32). Both H1 or H2 receptor knock-out animals show impaired performance in non-reinforced exploration tasks (e.g. novel object recognition and Barnes maze) while showing increased acquisition in fear-conditioning tasks. In accordance with this data, LTP in the hippocampal CA1 synapse was impaired in both H1 or H2 receptor deletion (40). Furthermore, H2 receptor blockage also inhibits LTP in the dentate gyrus in vivo (57).

In vitro H3 receptor activation in dentate gyri of hippocampal slices reduced short-term plasticity (58). Some researchers reported that H3 receptor antagonists facilitate learning and memory while the agonists act inhibitory (32). While some others described no change in memory task performance in case of H3 receptor deficiency and impaired performance with H3 receptor antagonists (49). H4 receptor—while also coupled to $G_{i/o}$ like H3—seems to have no effect on learning and memory processes (48,50).

In isolated hippocampal slices, increased histamine concentration facilitates LTP even in the existence of H1 and H2 inhibitors, suggesting a direct effect over NMDA receptors (59). Also histamine caused an LTP of neuronal excitability of CA1 neurons in low Ca^{++} high Mg^{++} medium in isolated slices, mainly through H2 receptors and cAMP/PKA pathway. This effect was modulated by H1 and NMDA receptors (60). Physical or chemical destruction of TMN improves performance in negatively-reinforced learning and memory tasks (e.g. water maze) fear memory and fear conditioning tasks (e.g. passive and active avoidance tests) while disrupting performance in non-reinforced object exploration tasks (32,49). Moreover, HDC knock-out mice show improved LTP and marginally reduced long-term depression (LTD) (49). There is also strong evidence toward histamine's major involvement in learning and memory through mechanisms of forgetting by specific activation of histamine receptors (53).

An alternative mechanism for histaminergic system to affect learning and memory is through modulatory effect of histamine on cholinergic signaling. Histamine release from TMN modulates the activity of many neurons including the cholinergic system and cholinergic activity plays a crucial role in synaptic plasticity (40). Histidine administration ameliorates learning deficits induced by muscarinic blocker scopolamine through H1 receptors (32). Another mechanism for interaction of histamine and learning – memory is directly through altering the glutamate signaling. Presynaptic glutamate release is observed to be higher in HDC knock-out animals after a contextual fear conditioning task compared to wild-type. But as stated

before, histamine facilitates hippocampal synaptic transmission through stimulating NMDA receptors. This paradoxical effect can be explained by HDC knock-out mice losing the histamine blocking the release of dopamine through H3 receptors (49).

2.5. Antihistamines

The word “antihistamine”, without denoting a histamine receptor subtype, is exlusively used for histamine H1 receptor blockers (5). Antihistamines can be classified according to their chemical structure (Table 2.1). A more frequently used classification in clinical practice is separating them into three generations, which were defined according to their side effect profiles and follow a chronological release order. Table 2.2 lists the antihistamines according to their generations.

Table 2.1. Classification of antihistamines according to their chemical structure.

Alkylamines	Etanolamines	Ethylenediamines	Phenothiazines	Piperazines	Piperidines
Brompheniramine Chlorpheniramine Dexchlorpheniramine Dexbrompheniramine Pheniramine Dimetindene Triprolidine Acrivastine	Carbinoxamine Clemastine Dimenhydrinate Diphenhydramine Doxylamine Phenyltoxamine Orphenadrine Bromazine	Antazoline Mepyramine (Pyrilamine) Tripelennamine Chloropyramine	Promethazine Mequitiazine Trimeprazine (Alimemazine)	Buclizine Cyclizne Meclizine Oxatomide Hydroxizine Cetirizine Levocetirizine	Azatadine Cyproheptadine Ketotifen Loratadine Desloratadine Bilastine Ebastine Terfenadine Fexofenadine Levocabastine Mizolastine Rupatadine Norastemizole Descarboethoxyloratadine

Antihistamines are separated into six different groups when classified according to their chemical structure (61).

Table 2.2. Classification of antihistamines according to their generation.

First generation	Second generation	Third generation
Mepyramine (pyrilamine)	Astemizole	Desloratadine
Chloropyramine	Mizolastine	Fexofenadine
Antazoline	Ketotifen	Levocetirizine
Tripelennamine	Acrivastine	
Diphenhydramine	Loratadine	
Carbinoxamine	Terfenadine	
Doxylamine	Quifenadine	
Orphenadrine	Rupatadine	
Bromazine	Olopatadine	
Clemastine	Bepotastine	
Dimenhydrinate	Cetirizine	
Pheniramine	Bilastine	
Chlorpheniramine	Azelastine	
Dexchlorpheniramine	Ebastine	
Dexbrompheniramine	Levocabastine	
Brompheniramine		
Triprolidine		
Dimetindene		
Cyclizine		
Chlorcyclizine		
Hydroxyzine		
Meclizine		
Promethazine		
Trimeprazine		
(Alimemazine)		
Cyproheptadine		

Antihistamines are classified as generations according to their central side effect profiles and selectivities. They also follow a chronological order; first generation antihistamines being the first and third generation antihistamines currently being the last H1 antagonist compounds.

2.5.1. First Generation Antihistamines

Search for antagonists for the histamine H1 receptor was started in 1937, after histamine's anaphylactic and allergic effects had clearly been laid out. The first discovered compounds with potent antihistaminic properties were too toxic for in vivo use. Soon, more viable molecules were developed by modifying those first discovered ones. First of these molecules was phenbenzamine, which was marketed in 1942 as the first antihistamine under the brand name Antergan. Soon after,

mepyramine or also called pyrilamine, a derivative of phenbenzamine with better clinical properties was discovered and released for clinical use as Neoantergan. After the release of these two compounds, of which the latter is still in use today, more antihistamines such as diphenhydramine, chlorpheniramine, brompheniramine and promethazine followed (5,6,62).

These “first generation” antihistamines have a major drawback; a strong sedation effect that persisted for several days after starting their use as they readily pass the blood brain barrier. Even though that side effect was also beneficial for their use as a medication for acute insomnia; it reduced patient compliance for all other indications.

Furthermore, these drugs were not particularly selective; other than H1 receptors, they interacted and blocked the muscarinic, α-adrenergic and serotoninergic receptors. These interactions added a multitude of other side effects that are listed in Table 2.3 (6,62). For these reasons, first generation antihistamines are no longer suggested as a first choice for urticaria and other allergic diseases (11–13,63).

Table 2.3. Side effects of first generation antihistamines.

Receptor	Side effect
H1 receptor	Inhibition of CNS neurotransmission, sedation, inhibition of cognitive and neuropyschomotor performance, increased appetite
Muscarinic receptor	Dry mouth, urinary retention, sinusoidal tachicardia
α-Adrenergic receptor	Hypotension, dizziness, reflex tachicardia
Serotonergic receptor	Increased appetite
I_{Kr} and other cardiac channels	Prolongation of QT interval, ventricular arrythmia

First generation antihistamines, because of their low selectivity, cause a multitude of side effects through blocking muscarinic, adrenergic, serotonergic receptors and cardiac ion channels (61).

2.5.2. Diphenhydramine

Diphenhydramine is a first generation antihistamine with prominent sedative effects. It is used for a variety of indications (q.v. Table 1.1). Chemically, it contains a diphenylmethane structure like many other antihistamines (64). It is considered to be a part of ethanolamine antihistamines (61). Molecular structure of diphenhydramine can be seen in Figure 3.1. Like most other H1 blockers, it competitively binds to H1 receptors and acts as an inverse agonist (65). Other than H1 receptors, it interacts with and blocks muscarinic, α-adrenergic, serotonergic, IKr and sodium channels (61,66). Through blockade of sodium currents in sensory and dorsal root ganglia neurons, diphenhydramine acts as a very mild anesthetic (66).

Diphenhydramine reaches its peak plasma concentrations 2.5 hours after its oral administration in humans. Its bioavailability is found to be between 42% and 62% in various studies as it is subject to first pass metabolism (59). Diphenhydramine occupancy in the central nervous system H1 receptors was found to be 56.4% after a single 30 mg dose of diphenhydramine (67). The elimination half-life of diphenhydramine varies from 3.4 to 9.3 hours (59). The pA2 value of diphenhydramine is 7.1 – 7.8 on H1 histamine receptors and 6.3 on muscarinic receptors (68,69)

2.5.3. Second Generation Antihistamines

In 1983, when two new antihistamines that were less soluble in lipids and thus had less blood brain barrier penetration were getting registered for the European market, their manufacturers called these new drugs “second generation antihistamines” to differentiate them from former antihistamines (70). These two drugs were terfenadine and astemizole, which were withdrawn from the market lateron because of their cardiotoxicity. These drugs block I_{Kr} rapid delayed potassium rectifier channel. I_{Kr} channel is activated during phase 3 of cardiomyocyte action potential to repolarize the cells. Blockage of these channels prolong the

repolarization, displaying as long QT intervals in electrocardiogram and leads to various arrhythmias including *torsades de pointes* (9,30).

Further research on developing antihistamines with low central nervous system side effects and high H1 selectivity continued and new second generation antihistamines were released; including fexofenadine, ketotifen, cetirizine, loratadine and more. These new molecules—compared to older ones—were safer, needed fewer daily administrations, did not cause serious side effects, suitable for use in children and had higher lethal doses (61).

Even though second generation antihistamines were more selective and less centrally active; they still pass the blood brain barrier and elicit central effects (8). For example, when given orally to people in therapeutic doses, terfenadine was found to occupy about 17% of H1 receptors in the frontal lobe (71). Another experiment in rats found 70% H1 receptor occupancy for terfenadine and 22.5 to 34.2% occupancy for 10 mg/kg and 30 mg/kg doses of cetirizine, respectively (72). Prescription-event monitoring studies for second generation antihistamines show that they differ in their sedative potentials. The odds ratios compared to loratadine were 0.63 for fexofenadine, 2.79 for acrivastine and 3.53 for cetirizine (8). There is virtually zero risk for cardiovascular side effects for second generation antihistamines except toxic doses or continuous use with an inability to metabolize and excrete these drugs (73,74).

2.5.4. Cetirizine

Cetirizine is a second generation antihistamine from the piperazine chemical family. It shares the diphenylmethane structure with diphenhydramine and many other antihistamines (64). The molecular structure of cetirizine can be seen in Figure 3.1. As cetirizine is a second generation antihistamine, it is more selective to H1 and its central nervous system side effects are significantly reduced. Humans treated with a single dose oral 20 mg cetirizine had a relatively higher subjective sleepiness compared to placebo group but this difference was not statistically significant (75).

Cetirizine is one of the suggested drugs by the guidelines for the treatment of allergic diseases in children and adults due to its demonstrated safety (11–13,63). Cetirizine does not cause any cardiovascular side effects unless the patient ingests a very high amount of the drug or there is a severe renal insufficiency (73,74).

Cetirizine is not subject to heavy metabolism. After oral administration, it is rapidly absorbed and its oral bioavailability is at least 70% (76). Its half-life is 24.8±7.7 hours. Cetirizine, especially its dextro stereoisomer, crosses the blood brain barrier; an experiment conducted in the rats found brain H1 occupancy of cetirizine 22.5% and 34.2% after 10 mg/kg and 30 mg/kg doses, respectively (72). In humans, brain H1 receptor occupancy rates of cetirizine were 12.6% and 25.2% after 10 mg and 20 mg single dose oral administrations (75). The elimination half-life of cetirizine is 7 hours (76). The pA_2 of cetirizine on H1 histamine receptors is 9.40 (77). Cetirizine is shown to be inactive on muscarinic receptors for concentrations up to 3×10^4 M (69).

2.6. Third Generation Antihistamines

Third generation antihistamine is a recent nomenclature for some new antihistamines and it is not yet widely accepted (78,79). These molecules are either metabolites or isomers of the second generation antihistamines (9). There are currently three drugs in this category; desloratadine (a metabolite of loratadine), fexofenadine (metabolite of terfenadine) and levocetirizine (L-enantiomer of cetirizine) (9). These drugs are better p-glycoprotein substrates therefore their brain penetration is significantly lower than their antecedents (80).

2.7. Aim and Hypotheses

This thesis aims to improve our understanding on the effects of antihistamines on hippocampal learning and memory. As the central histaminergic system originates from a single nucleus in the brain and has a regulatory role in the whole central nervous system, using an *in vivo* method is preferable to accurately portray real-life conditions. Furthermore, to separate these drugs' effects on learning and memory from their somnolence side effect, an interventional method to directly assess the

relevant neural circuits is a must. Therefore, this thesis investigates the effects of antihistamines on learning and memory using not only behavioral tests but also *in vivo* hippocampal field potential recordings as well.

The first hypothesis for this thesis is defined to be “both first generation (sedating) and second generation (non-sedating) antihistamines affect the learning and memory processes of hippocampus in acute and chronic use”. The other hypothesis that was tested is “cetirizine affects learning and memory impairments caused by REM (rapid eye movement) sleep deprivation”.

3. MATERIALS AND METHODS

3.1. Animals

In this study, 12 weeks-old male Wistar albino rats between 250 and 300 g were used. A total of 98 animals were used for all experiments and groups. The animals were supplied from Kobay D.H.L. A.Ş. (Ankara, Turkey) and were kept in the local shelter of Hacettepe University Department of Medical Pharmacology. After delivery, each animal was hosted for 10 days before experiments for them acclimatize to the new environment and minimize stress. Animals were given *ad libitum* food and water and were subjected to 12-hour dark / 12-hour light cycle. Animals were sacrificed by cervical dislocation after completion of experiments. Number and species of the animals and the research protocols were approved by Hacettepe University Animal Experiments Ethics Committee (q.v. Appendix 1 – Hayvan Deneyleri Yerel Etik Kurulu Kararı, Karar Numarası 2015/99-06).

3.2. Drugs

The aim of this study was to inspect the effects of both first and second generation H1 antihistamines. Therefore, one drug from each generation was chosen. Diphenhydramine and cetirizine were selected from first and second generation antihistamines, respectively. When choosing the drugs, their current frequency of use was considered. Furthermore, scopolamine, a nonspecific muscarinic blocker that readily penetrates the blood brain barrier was used to assess the possible antimuscarinic component of the diphenhydramine's effects. The molecular structure of the drugs used in the experiments can be seen in Figure 3.1.

The dose used for diphenhydramine was 20 mg/kg in a 5 mg/mL solution while scopolamine was administered at 2 mg/kg in a 0.5 mg/mL solution. For cetirizine, two different doses were used; 5 mg/kg in a 1.25 mg/mL solution for REM sleep deprivation and behavioral experiments and 20 mg/kg in a 5mg/mL solution for hippocampal recordings after acute and chronic administrations. All drugs solutions were prepared using saline. Control groups also received saline at 4 mL/kg, which was

equal in volume to treatment groups. All administrations were done through the intraperitoneal route. A duration of 1 hour was given for drug distribution before starting the tests in experiment days.

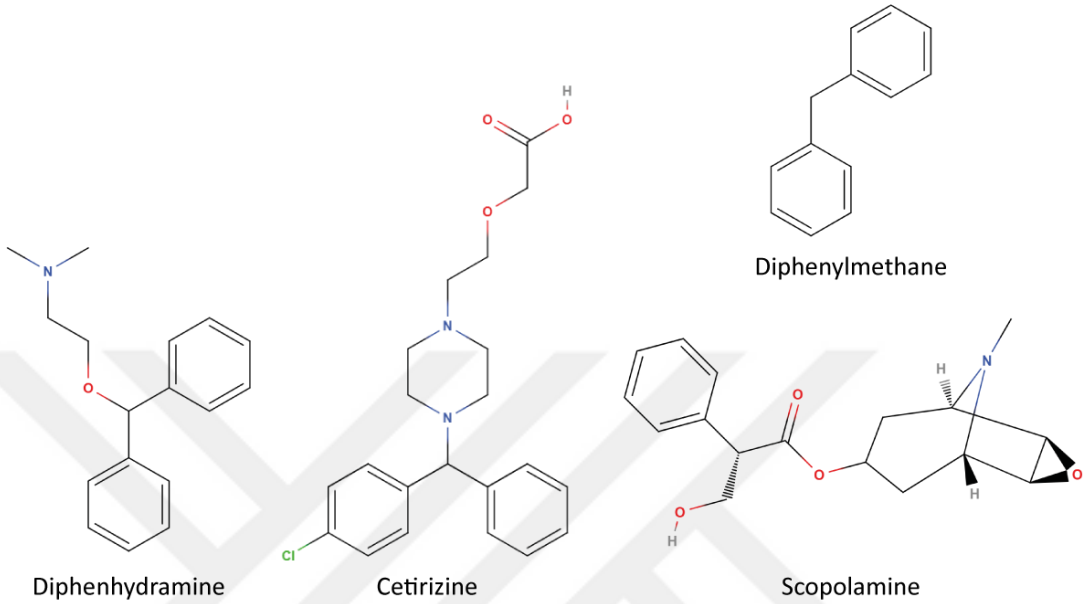

Figure 3.1. The molecular structure of drugs used in experiments. Like many H1 antihistamines, both diphenhydramine and cetirizine share a common group called diphenylmethane in their structure. Scopolamine is used to assess the possible antimuscarinic contribution in diphenhydramine's actions (64).

3.3. Protocols and Groups

The project was conducted as five different protocols. The graphical abstract for timelines of the protocols can be found in Figure 3.2.

3.3.1. Acute Treatment Electrophysiology Groups

It is known that a tolerance develops to sedative effects of antihistamines after 4 days (61,81,82). Depending on this information, the drugs were administered for 4 days to inspect the acute effects of antihistamines to imitate the effects on patients who just started using chronic antihistamines or take these drugs occasionally for indications like common cold or insomnia. For this protocol, diphenhydramine, cetirizine and scopolamine were compared with the control

group. Injections were done every day at the same time at 0, 24, 48 and 72 hours and *in vivo* hippocampal field recordings were started the 4th injection. Required number of animals for each group was determined to be 6 for this protocol via strength analysis using G*Power software.

3.3.2. Chronic Drug Electrophysiology Groups

Most of the time, antihistamines are used for long durations, far exceeding the tolerance development duration of 4 days. For this reason, to evaluate the effects of antihistamines on hippocampal electrophysiological recordings, a chronic drug administration protocol was performed. For this protocol, diphenhydramine, cetirizine and scopolamine were administered for a total of 12 days; and after the last dose, in vivo hippocampal field recording experiments were executed. Required number of animals for each group was determined to be 7 for this protocol via strength analysis using G*Power software.

3.3.3. Acute Cetirizine Behavioral Experiment Groups

After the completion and evaluation of the electrophysiology data for acute and chronic drug groups, behavioral experiments were conducted to compare cetirizine's effects on locomotion, learning – memory, and stress. First, like in the electrophysiology groups, acute effects of cetirizine were examined and were compared to the control group. The behavioral experiments conducted were open field arena (OFA) for locomotive activity (q.v. Section 3.5.1.), elevated plus maze (EPM) for stress behavior (q.v. Section 3.5.2.), passive avoidance (PA) test (q.v. Section 3.5.3.), forced swim test (FST) (q.v. Section 3.5.4.) and Morris water maze (MWM) (q.v. Section 3.5.5.) for evaluation of spatial memory. For this protocol, the animals were given 4 days of cetirizine treatment and at the same time, training phase for MWM was started to last all four days of the protocol. OFA and training phases of PA and FST were conducted at the 3rd day and EPM and the test phases of PA and FST were conducted at the 4th day alongside the last day of MWM. Cetirizine treatment group had 8 animals and the control group had 6 animals for this protocol.

3.3.4. Chronic Cetirizine Behavioral Experiment Groups

Drug administration and behavioral experiments for chronic cetirizine groups took a total of 18 days. Experiments started with PA training phases at the 10th day. 11th day was PA test phase and the beginning of MWM and FST tests. OFA was conducted at the 13th day with the second day of FST. After the last day of regular MWM at 14th day, MWM reversal learning (MWMr) paradigm started at 15th day to last 4 days and end at 18th day with a probe test for reversal MWM paradigm (q.v. Section 3.5.6.). Both cetirizine and control groups had 9 animals for this protocol.

3.3.5. Acute Cetirizine REM Sleep Deprivation Groups

REM sleep deprivation is known to disrupt hippocampal learning and memory and impair electrophysiological recordings (83). After examining the effects of antihistamines on hippocampal learning – memory and behavior of healthy animals, cetirizine's effects on REM sleep deprived rats was examined. The inverted flower pot method was used to deprive rats of REM sleep for 72 hours (q.v. Section 3.6.). Animals received intraperitoneal cetirizine or vehicle injections at hours 0, 24, 48 and 72. At the end of 72 hours, animals were removed from the cage and experiments were started. Required number of animals for each group was determined to be 6 for this protocol via strength analysis using G*Power software.

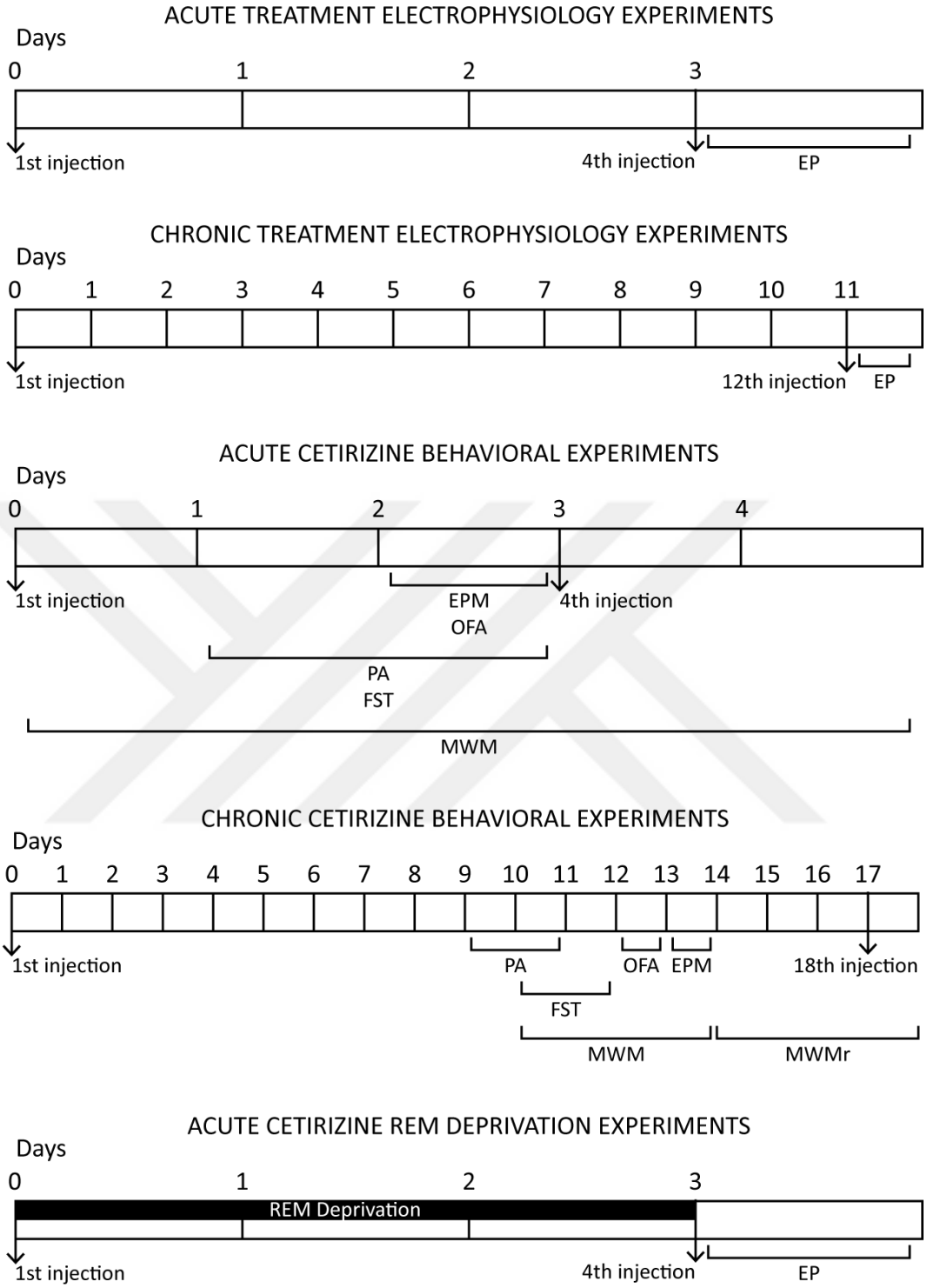

Figure 3.2. Graphical timelines for the protocols. All animals received injections throughout the experiment days except the last day of acute cetirizine treatment behavioral experiments protocol.

3.4. *In Vivo* Hippocampal Electrophysiology

To evaluate the effects of the drugs on plasticity of hippocampal synapses, evoked postsynaptic local field potentials in the CA1 region of the hippocampus in anesthetized rats were recorded through stimulation of Schaffer collaterals (SC) originating from the CA3 region.

One hour after the last drug injection, animals were anesthetized using 25% m/v urethane solution in isotonic saline. Urethane was administered at 1.5 g/kg dose intraperitoneally. After the depth of anesthesia was assessed to be suitable via no response to rear paw pinching, top of the head was shaven and the animals were placed in a stereotaxic frame. Body temperature was maintained at 37 °C with a homoeothermic blanket control unit (Harvard Apparatus, MA, USA). A midline incision coronally through the scalp was made to expose the skull. Burr holes for stimulus and recording electrodes were opened at the predetermined coordinates using bregma as a reference point (84). A borosilicate glass capillary recording microelectrode filled with artificial cerebrospinal fluid solution was aimed at stratum pyramidale of CA1 region of left hippocampus with coordinates 4.6 mm caudal, 3.4 mm lateral and 2.85 mm ventral from bregma. A stimulus electrode made from bipolar insulated stainless steel wires with exposed tips was inserted into two regions. First, for input/output (I/O) and paired-pulse (PP) recordings, the electrode was placed into ventral hippocampal commissure (VHC) fimbria with coordinates 1.3 mm caudal, 0.2 mm lateral and 4.0 mm ventral from bregma. Then, for LTP and LTD recordings, the electrode was placed to the CA3 Schaffer collaterals contralateral to recording electrode with coordinates 4.3 mm caudal, 3.3 mm lateral and 4.1 mm ventral from bregma. A gold-plated ground electrode was placed in the occipital region above cerebellum. The field potentials were evoked with rectangular pulses using stimulators (S44 and S4400, Grass Instruments, RI, USA) with durations of 100 µs at VHC and 200 µs at contralateral SC every 20 seconds. The stimuli were isolated with a passive constant voltage stimulus isolation unit (SIU5, Grass Instruments). The evoked potentials were picked up with a 1× headstage (Batıray, YSED, Turkey),

amplified with a DC amplifier (Kaldıray, YSED), digitized by a data acquisition system (PowerLab/8sp, ADInstruments, Australia) and recorded electronically using a software (LabChart 7, ADInstruments). The recorded field potentials were population spikes (PS) from the stratum pyramidale and field excitatory postsynaptic potentials (fEPSP) from the stratum radiatum of the CA1 (Figure 3.3). PS magnitudes—which denote the cumulative activity of neurons reaching action potential threshold—and fEPSP 10%-90% slopes—which denote the cumulative postsynaptic depolarization—were analyzed using the recording software (LabChart 7, ADInstruments). Three recordings were obtained for each variable point in the experiments and the average for these was accepted as the value for the variable point.

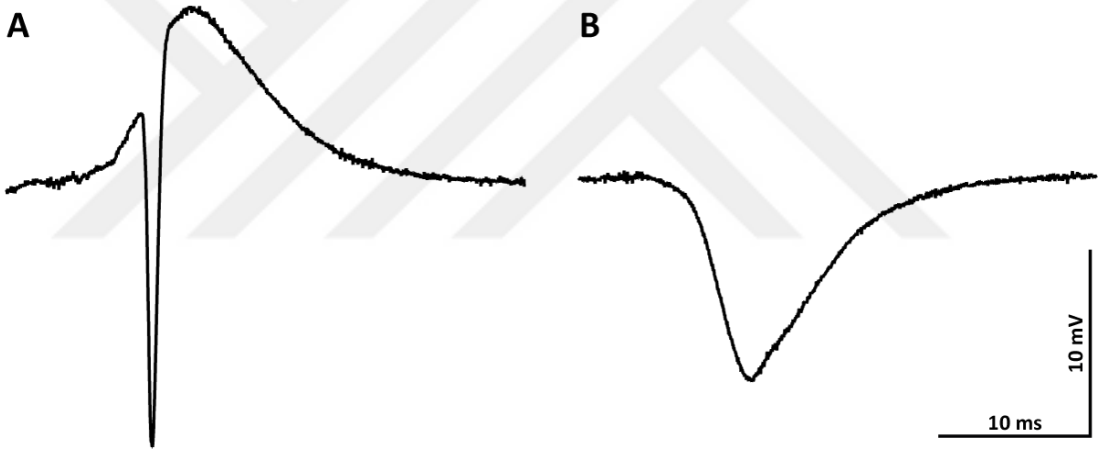

Figure 3.3. Sample field potential recordings from strata pyramidale and radiatum. A) The amplitudes of population spikes were measured for stratum pyramidale recordings. **B)** The slope of the depolarization phase were calculated and used for stratum radiatum recordings.

3.4.1. Stratum Pyramidale Recordings

First, I/O recordings from the stratum pyramidale region were conducted to evaluate the excitability of CA1 neurons. For this, stimuli with increasing intensities from 1 V to 15V with 1 V steps were given from the VHC and PS amplitudes were measured. After the I/O recordings were completed, the stimulus strength evoking

50% of the maximal PS amplitude was determined. PP experiments from the stratum pyramidale were conducted using this input strength. For PP experiments two pulses with increasing interpulse intervals (IPI) were sent and the resulting two PS responses were recorded. IPIs were 20 ms, 40 ms, 60 ms, 80 ms, 100 ms, 150 ms, 200 ms, 300 ms, 400 ms, 500 ms, 600 ms, 700 ms, 800 ms, 900 ms and 1000 ms. To evaluate the potentiation or inhibition of second PS relative to first PS; PS2/PS1 was calculated for each IPI.

3.4.2. Stratum Radiatum Recordings

After the stratum pyramidale recordings, the recording electrode was lowered to stratum radiatum region of CA1 for the remainder of experiments. I/O recordings were conducted to evaluate the synaptic strength. As done in the stratum pyramidale recordings, stimuli from 1 V to 15 V with 1 V steps were given from the VHC and fEPSP slopes were recorded. After the stimulus strength that evoked the 50% of the maximal slope is determined, PP experiments were conducted with the same IPI values as stratum pyramidale PP recordings. To evaluate the synaptic facilitation or inhibition, second fEPSP slope amplitude is divided to the first fEPSP slope.

3.4.3. Long-Term Potentiation Recordings

After the completion of I/O and PP recordings, stimulus electrode was moved to contralateral CA3 Schaffer collaterals and the stimulus duration was increased from 100 µs to 200 µs. Before the LTP recordings, the stimulus strengths that evoked 50% and 90% of the maximal fEPSP slope were determined. A neutral recording for 15 minutes was completed to determine the baseline fEPSP slope. After the baseline, LTP was induced using “high frequency stimulation” (HFS) method; three 1-second 100 Hz stimulus trains with 3-minute intervals at the stimulus strength that evoked 90% of the maximal slope. Post-induction, the recordings continued at 50% stimulus strength for 120 minutes to trace the strength of LTP. The slope amplitudes were binned into 3-minute sets and averages were used for analyses.

3.4.4. Long-Term Depression Recordings

For the baseline of LTD recordings, the last 15 minutes of LTP recordings were used. After the LTP recordings were completed, LTD was induced using the “low frequency stimulation” (LFS) method; 900 PP stimulations at 1 Hz and 40 ms IPI for a total of 15 minutes with the 90% stimulus strength. When the LTD induction was completed, the stimulus strength was taken to 50% and LTD recordings were continued for 60 minutes. The slope amplitudes were binned into 3-minute sets and averages were used for analyses.

3.5. Behavioral Experiments

To inspect implications of electrophysiological changes caused by cetirizine on behavior, various behavioral experiments were conducted; namely, open field arena for locomotion, elevated plus maze for stress, passive avoidance and Morris water maze for spatial learning with Morris water maze reversal paradigm to assess relearning capacity.

3.5.1. Open Field Arena

To assess their locomotive behavior, animals were placed in the center of a 45 × 45 cm arena with 45 cm high glass walls and left to freely explore the arena for 60 minutes. The animals were recorded from above and total traveled distance and distance traveled in the center 50% of the arena were calculated using a tracking software (ANY-maze 5, Stoelting Co., IL, USA).

3.5.2. Elevated Plus Maze

A one-meter-high plus-shaped platform with four perpendicular 40 × 15 cm arms with two opposing ones are sheltered with 45 cm high walls was used for this test. Animals were placed in the center 15 × 15 cm region with their heads looking toward one of the open arms to explore the maze for 5 minutes while they were recorded from above. Times spent in open arms, distance traveled in open arms, time

spent in extreme open arms and distance traveled in extreme open arms were calculated using ANY-maze 5 to assess anxiety behavior.

3.5.3. Passive Avoidance

A shuttlebox with one matte black and one transparent two 26 × 30 × 30 cm chambers with stainless steel grid floors connected with a guillotine door was used for passive avoidance test. The floor of the dark chamber is connected to a shock device that gives a non-harming mild foot shock. The transparent chamber was illuminated with a lamp from above. The experiment was conducted over two days; the first day had habituation and training phases while the second day had the test phase. In habituation phase, the animal was placed in the light chamber and 5 seconds later the guillotine door separating the chambers was raised. When the animal passed completely to the dark chamber, the door was lowered again and the animal was removed from the box 5 seconds later. After a 5-minute waiting period, the animal was placed in the light chamber again for the training phase and the door was raised 5 seconds later. This time, when the animal crossed to the dark chamber, the door was lowered and a foot shock was delivered for 3 seconds. 5 seconds after the foot shock was delivered the animal was removed from the dark chamber until next day. In the third and last test phase, the animal was placed in the light compartment and the door was raised. When the animal passed completely to the dark compartment, the door was lowered again and the animal was removed from the dark compartment 5 seconds later. For each phase the latency to cross to the dark chamber was measured with a chronometer after the door was raised with a cutoff duration of 300 seconds.

3.5.4. Forced Swim Test

Forced swim test is a behavioral despair test utilized to assess antidepressant effects of interventions in animals. A 45 cm high 25 cm wide cylindrical glass tank that is filled with 30 cm high is used for this test. The experiment continues for two days. On the first day, the animals were gently placed inside the tank to stay in water for

15 minutes. At this first phase, animals discover the tank and see that no escape routes are found. The behavior observed in the test phase next day is animal actively trying to stay on top of the water or passively floating on the water. This 5-minute phase is recorded with a video camera then manually timed for the total immobility duration; shorter immobility denoting antidepressant activity.

3.5.5. Morris water maze

The water maze used for this paradigm was a black cylindrical water tank with 180 cm diameter and 75 cm height. The tank was divided into quadrants named N, S, W and E and a sign with a different shape and color was placed in each quadrant. A 30 cm high circular platform with 10 cm diameter was placed in the center one of the quadrants. Each animal was tested in the water maze for four days and each day had 4 trials in the acquisition phase. For these trials, the animals were placed in the water from a random point at least half a quadrant away from the quadrant target platform is in. On the first day, to train the animals, the tank was filled below the platform level with water and the animals were given 120 seconds to find the platform themselves. If the animals would not find the platform, they were gently directed towards it after the cutoff. For following days, the tank was filled until the platform submerged 2 cm below the water surface and the experiments continued without animals seeing the platform. The latency to reach the platform was recorded for each trial with a cutoff duration of 120 seconds. For the fifth day, the platform was removed for a probe test. The animals were placed in the water for 60 seconds and the time spent in the target quadrant where the probe previously placed was measured for each trial. Water temperature was kept constant at around 20 to 22 °C for the duration of the experiments.

3.5.6. Morris water maze reversal learning

Morris water maze tests were continued with reversal learning after the end of the acquisition phase for the chronic cetirizine behavioral experiments instead of the probe test. This paradigm is used as the behavioral equivalent of LTD as reversal

learning is mediated by hippocampal LTD (85). For this test, the platform is moved to another quadrant after the fourth day of MWM test. Then, testing was continued as regular MWM experiments with the platform's new place never being shown to the animals for three more days. Latency to find the platform was measured for each trial. For the fourth day, the platform was removed for the probe test. The animals were placed in the water for 60 seconds and the time spent in the target was measured for each trial.

3.6. REM Sleep Deprivation

Inverted flower pot technique, which is also called columns-in-water, is used to induce REM sleep deprivation in the animals (86). A 25 × 25 × 45 cm glass tank with one 8 cm diameter 30 cm high platform in its center was filled to 29 cm with water and the animal was placed in the platform. The water temperature was kept constant at 35 °C with an aquarium heater and the top of the tank was closed by a metal grid. Animals were kept in this cage for 72 hours each with ad libitum water and food pellets. With this method, when the animal goes into flaccid paralysis due to REM sleep, it drops into water to wake up and climb back to the platform again.

3.7. Analysis

The data from the electrophysiological experiments were first analyzed using Excel 2016 (Microsoft, WA, USA). All statistical analyses were done with Prism 6 (GraphPad Software, CA, USA). Student's t test was used to analyze experiment results with only two groups. For experiments with multiple groups, ANOVA was used; one-way ANOVA for experiments with one independent variable and two-way ANOVA for experiments with two independent variables. Dunnett Tukey or Sidak's methods were used for ANOVA post-hoc analyses. $P \leq 0.05$ is considered statistically significant. Error bars and plus–minus signs denote standard error of mean (SEM).

4. RESULTS

4.1. Electrophysiology Experiments of Acute Treatment Groups

4.1.1. Input/Output Results from Strata Radiatum and Pyramidale of Acute Treatment Groups

In order to evaluate acute drug effects on CA3-CA1 transmission efficacy input/output characteristics of this circuitry was studied (Fig 4.1A). Acute cetirizine and diphenhydramine treatment for 4 days did not alter the I/O curves and the fEPSP amplitudes in the stratum radiatum of the rat hippocampus. However, acute scopolamine treatment significantly shifted the curve to the left compared to that of the control group and increased the maximal fEPSP slopes. Maximum EPSP slope for the control group was 3.7±0.4 mV/ms. Maximum fEPSP slope for scopolamine was 4.8±0.1 mV/ms (two-way ANOVA treatment effect $F(3,300)=56.3$, $p\leq0.001$). To further assess drug induced shifts of I/O curves normalized data were also analyzed (Figure 4.1 B). The stimulation strength that produced the EPSP amplitudes closest to half-maximal were 6 V for cetirizine and diphenhydramine, 5 V for scopolamine and 7 V for the control group. Scopolamine-induced synaptic facilitation was significant between 5 to 15 V (at 7 V DPH vs CTRL $p\leq0.001$ and at 15 V $p\leq0.01$; two-way ANOVA treatment effect $F(3,300)=23.6$, $p\leq0.001$; $n=6$).

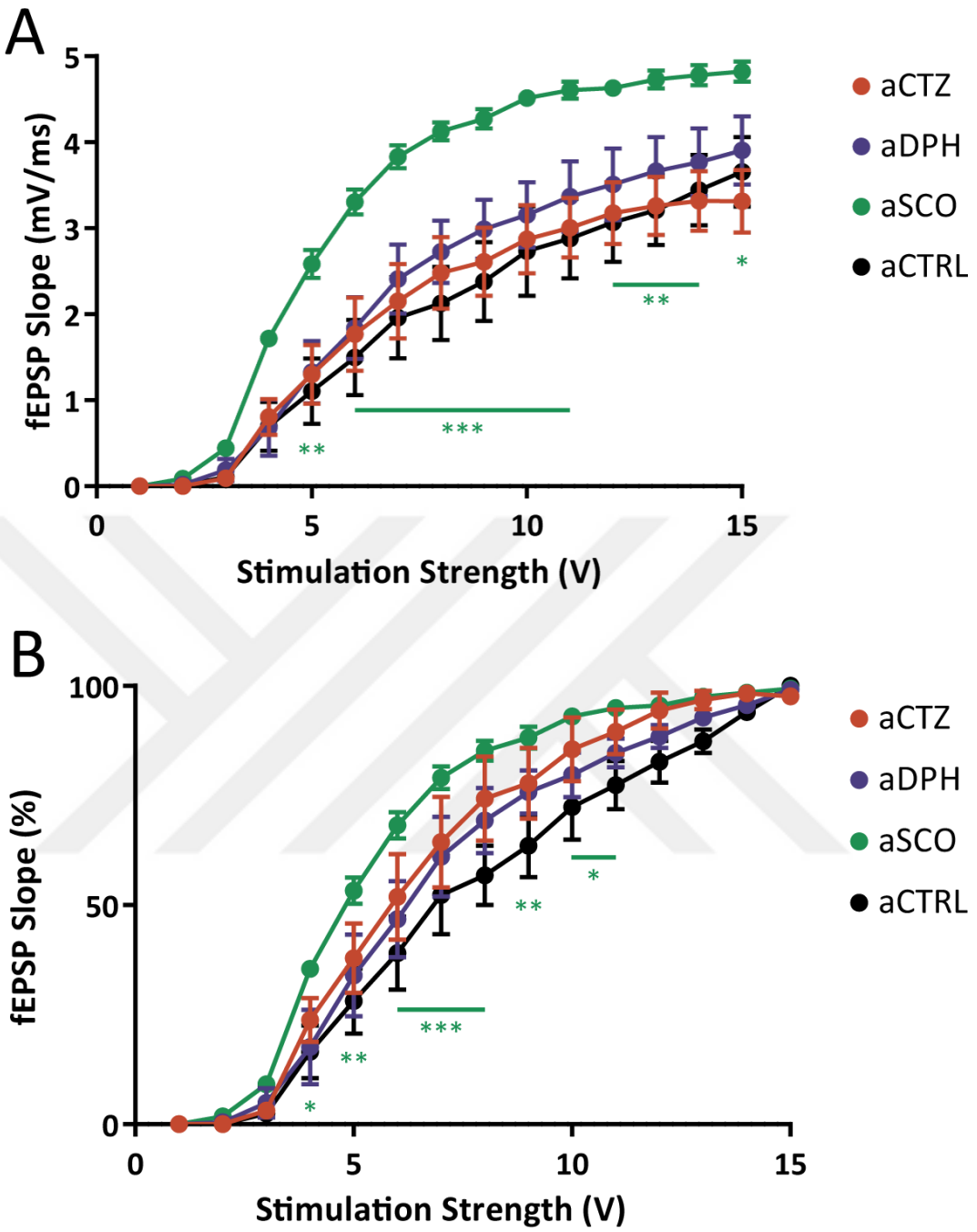

Figure 4.1. The effects of acute drug treatment on the I/O curves from CA1 stratum radiatum. A) Cetirizine and diphenhydramine did not affect I/O characteristics while scopolamine significantly shifted the curve to the left. Furthermore, scopolamine increased the maximum fEPSP slope. (Two-way ANOVA treatment effect $F(3,300)=56.3$, $p \leq 0.001$; post-hoc Dunnett's multiple comparisons test) **B)** Normalized I/O curves displayed no significant shifts by cetirizine and diphenhydramine treatments but acute scopolamine treatment shifted the curve to the left. (Two-way ANOVA treatment effect $F(3,300)=23.6$, $p \leq 0.001$; post-hoc Dunnett's multiple comparisons test) (4-day acute treatment; aCTZ: acute cetirizine,

aDPH: acute diphenhydramine, aSCO: acute scopolamine, aCTRL: acute control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$; green * CTRL vs SCO; n=6)

In stratum pyramidale of rats, acute cetirizine caused a minor shift in PS amplitude curves to the left, most notably at stimulation strengths of 8 to 10V. (two-way ANOVA treatment effect $F(3,300)=18.7$, $p \leq 0.001$; n=6). In control group animals, maximum stimulation yielded in average 14.1±0.6 mV PS amplitudes at stratum pyramidale (Figure 4.2 A). Cetirizine-treated animals displayed insignificantly higher mean PS amplitudes at maximal stimulation (18±0.9 mV) while PS amplitudes for diphenhydramine (14.7±1 mV) and scopolamine (15.8±0.7 mV) at that level were closer to that of the controls. Normalized PS amplitude graph also demonstrated that cetirizine and scopolamine shifted the I/O curve to the left but the shift induced by the cetirizine was not as drastic as the scopolamine-induced I/O shift (Figure 4.2 B; two-way ANOVA treatment effect $F(3,300)=13.5$, $p \leq 0.001$; n=6). The right shift caused by cetirizine treatment was significant between 8 to 10 V ($p \leq 0.05$). On the other hand, scopolamine-induced I/O shift was more pronounced and statistically significant between 5 to 10 V ($p \leq 0.05$ at 5, 9 and 10V; $p \leq 0.01$ at 6 to 8 V; n=6). Acute diphenhydramine treatment did not change the I/O curves.

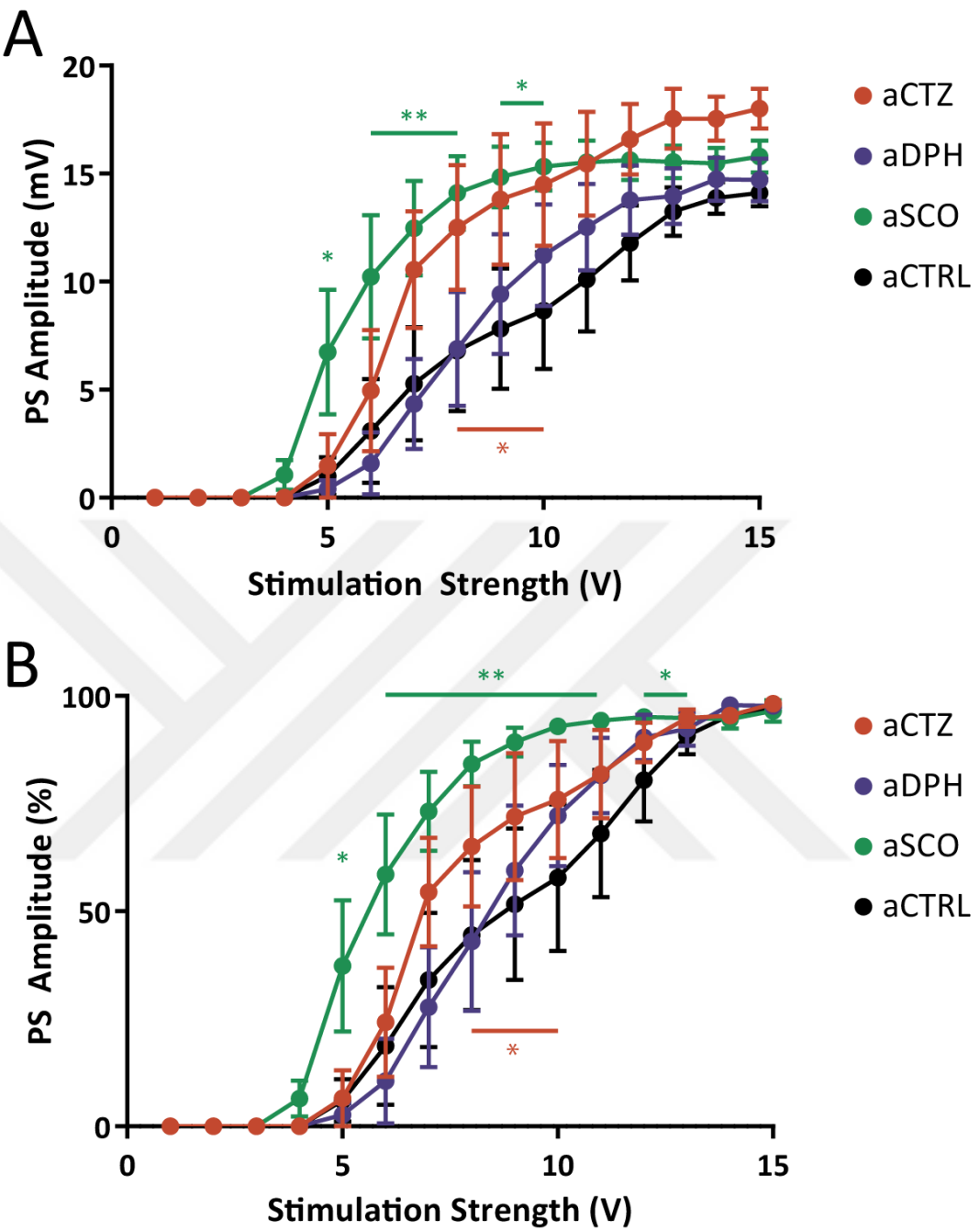

Figure 4.2. The effects of acute drug treatment on the I/O curves from CA1 stratum pyramidale. A) Both scopolamine and to some extent cetirizine caused stratum pyramidale I/O curve to shift right. Diphenhydramine did not cause any alteration from the control group. (Two-way ANOVA treatment effect $F(3,300)=18.7$, $p \leq 0.001$; post-hoc Dunnett's multiple comparisons test) **B)** These changes were also visible in the normalized I/O graph. (two-way ANOVA treatment effect $F(3,300)=13.5$, $p \leq 0.001$; post-hoc Dunnett's multiple comparisons test) (4-day acute treatment; aCTZ: acute cetirizine, aDPH: acute diphenhydramine, aSCO: acute scopolamine, aCTRL: acute

control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; * $p \leq 0.05$, ** $p \leq 0.01$; red * CTRL vs CTZ, green * CTRL vs SCO; n=6)

4.1.2. Paired-Pulse Stimulation Results from Strata Radiatum and Pyramidale of Acute Treatment Groups

Paired-pulse recordings from stratum radiatum with increasing IPIs showed a significant decrease in facilitation from 20 ms to 150 ms (Figure 4.3 A; two-way ANOVA treatment effect $F(3,300)=46.7$, $p \leq 0.001$; n=6). At 20 ms, the average ratios of second fEPSP slopes to first fEPSP slopes were 1.5±0.1 for cetirizine, 1.7±0.1 for diphenhydramine, 1.5±0.08 for scopolamine and 2±0.04 for the control group. At 150 ms, the ratios were 1.1±0.08 for cetirizine, 1.2±0.08 for diphenhydramine, 1.2±0.03 for scopolamine and 1.4±0.05 for the control group ($p \leq 0.001$ for 20 to 60 ms IPI, $p \leq 0.01$ for 80 to 150 ms IPI for all the drugs tested with respect to control; n=6).

In stratum pyramidale, paired-pulse stimulation elicited inhibitory response in shorter intervals (20 to 60 ms) then followed by a facilitation at longer intervals (80 to 1000 ms), in the control groups of rats (Figure 4.3 B; two-way ANOVA treatment effect $F(3,300)=81.6$, $p \leq 0.001$; n=6). All the tested drugs did not alter paired-pulse inhibition but significantly blocked paired-pulse facilitation occurring at longer interpulse intervals ($p \leq 0.001$ for scopolamine, cetirizine and diphenhydramine at 150 ms IPI; n=6). At 80 ms, the ratios of the 2nd PS amplitudes to the 1st PS amplitudes were 0.72±0.037 for cetirizine, 1±0.2 for diphenhydramine, 1.2±0.4 for scopolamine and 2±0.2 for the control group. At 1000 ms, the ratios were 1.114±0.042 for cetirizine, 1.4±0.1 for diphenhydramine, 1.4±0.06 for scopolamine and 2.1±0.2 for the control group.

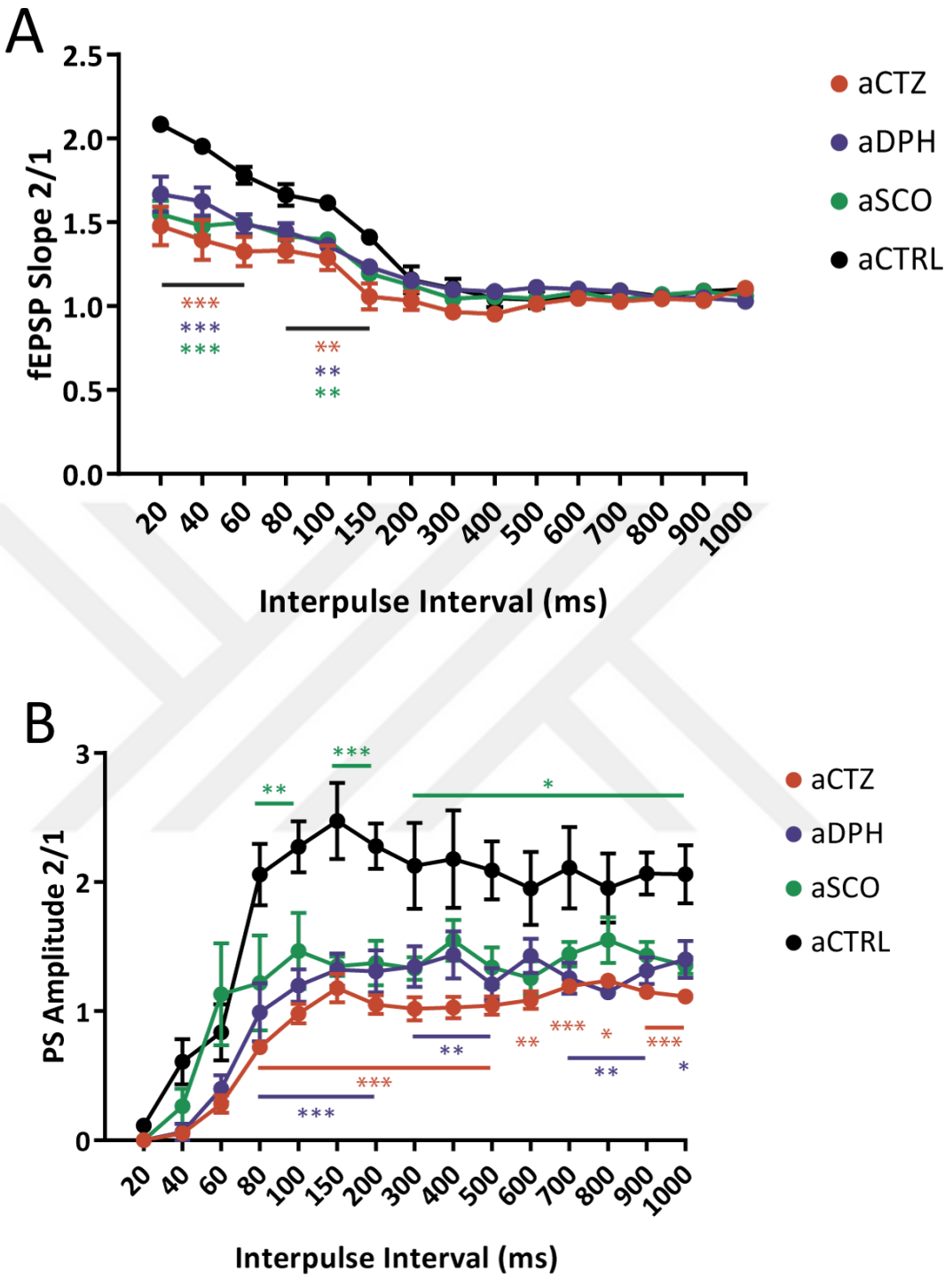

Figure 4.3. Paired-pulse recordings from CA1 strata radiatum and pyramidale of acute treatment groups. A) Cetirizine, diphenhydramine and scopolamine considerably reduced 20 to 150 ms paired-pulse facilitation in stratum radiatum. (Two-way ANOVA treatment effect $F(3,300)=46.7$, $p \leq 0.001$; post-hoc Dunnett's multiple comparisons test) **B)** Acute treatment of either cetirizine, diphenhydramine or scopolamine blocked paired-pulse facilitation observed between 80 to 1000 ms

IPIs in stratum pyramidale. (Two-way ANOVA treatment effect $F(3,300)=81.6$, $p \leq 0.001$; post-hoc Dunnett's multiple comparisons test) (4-day acute treatment; aCTZ: acute cetirizine, aDPH: acute diphenhydramine, aSCO: acute scopolamine, aCTRL: acute control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$; red * CTRL vs CTZ, purple * CTRL vs DPH, green * CTRL vs SCO; n=6)

4.1.3. Long-Term Potentiation and Long-Term Depression Results of Acute Treatment Groups

The effects of the drugs on the long-term plasticity in rat hippocampal CA1 region were evaluated by LTP and LTD paradigms (Figure 4.4). In control rats 3 consequent 100Hz stimulations induced %215 increase in EPSP slopes and this increase was almost stabilized around 160% at the end of 2nd hour. Recordings from cetirizine-treated animals exhibited a lower initial potentiation immediately after the LTP induction when compared to that of the controls ($p \leq 0.05$ from 18 to 33 minutes; n=6). However, this reduction did not prevent establishment of LTP, and cetirizine group of rats displayed similar levels of LTP to the controls. Cetirizine and diphenhydramine-treated animals exhibited more stable LTP which became evident from their highly stable baselines throughout 2 hours of recording. As it was expected from a centrally active antimuscarinic agent, scopolamine significantly occluded LTP from just after its induction up to 120 minutes ($p \leq 0.01$ at all data points; n=6). At the end of 2nd hour, the mean fEPSP slopes were 147.8±11.3% for cetirizine, 163.3±21.7% for diphenhydramine, 84.5 ± 9.7% for scopolamine and 163.6±15.8% for the control group (two-way ANOVA treatment effect $F(3,300)=191.2$, $p \leq 0.001$; n=6).

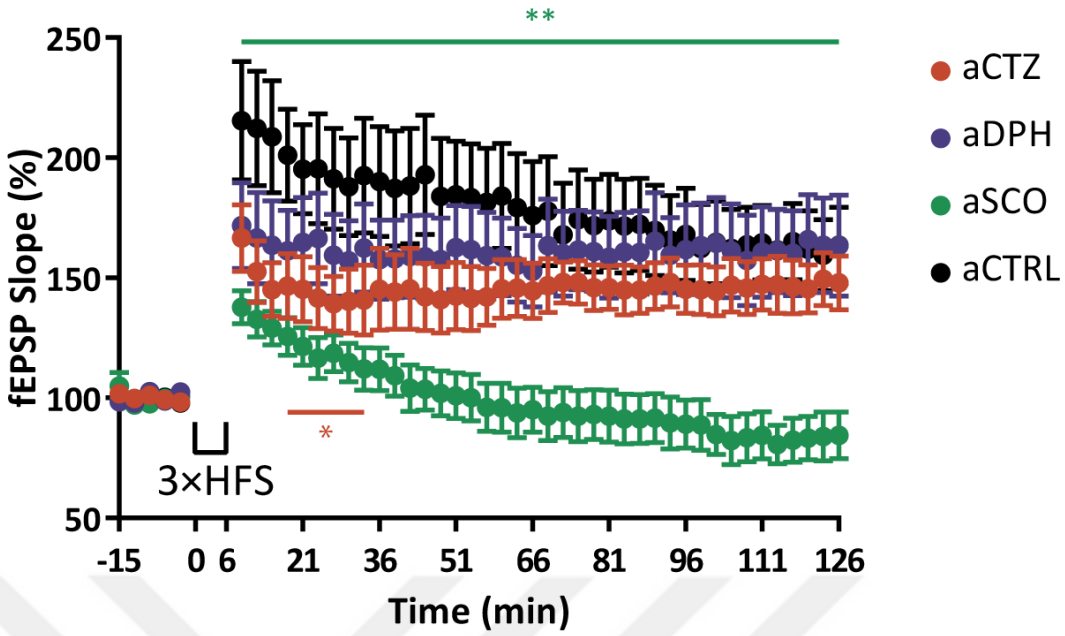

Figure 4.4. Long-term potentiation recordings from CA1 synapses for acute treatment groups. Scopolamine blocked LTP at all data points of post-induction period. While cetirizine and diphenhydramine reduced the early phases of LTP, they did not block the potentiation in the long run. (Two-way ANOVA treatment effect $F(3,900)=191.2$, $p\leq0.001$; post-hoc Dunnett's multiple comparisons test) (4-day acute treatment; aCTZ: acute cetirizine, aDPH: acute diphenhydramine, aSCO: acute scopolamine, aCTRL: acute control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; * $p\leq0.05$, ** $p\leq0.01$; red * CTRL vs CTZ, green * CTRL vs SCO; HFS: 1 s 100 Hz high frequency stimulus trains with 3 minutes between each train; n=6)

Long-term depression studies of acute treatment groups had a rather unexpected result where cetirizine prevented the induction of LTD at all data points (Figure 4.5; two-way ANOVA treatment effect $F(3,300)=104.9$, $p\leq0.001$; CTZ vs CTRL $p\leq0.05$ at 18 min, $p\leq0.01$ at 21 to 30 min, $p\leq0.001$ at 33 to 75 min; n=6). Scopolamine slightly inhibited the LTD at early phases but this inhibition was not statistically significant 57 minutes after the beginning of LTP induction. Diphenhydramine did not produce any significant results. At 75 minutes, normalized fEPSP slope amplitudes were

101.8±4.2% for cetirizine, 76.3±7.4% for diphenhydramine, 83.6±5.9% for scopolamine and 65.001 ± 6.381% for the control group.

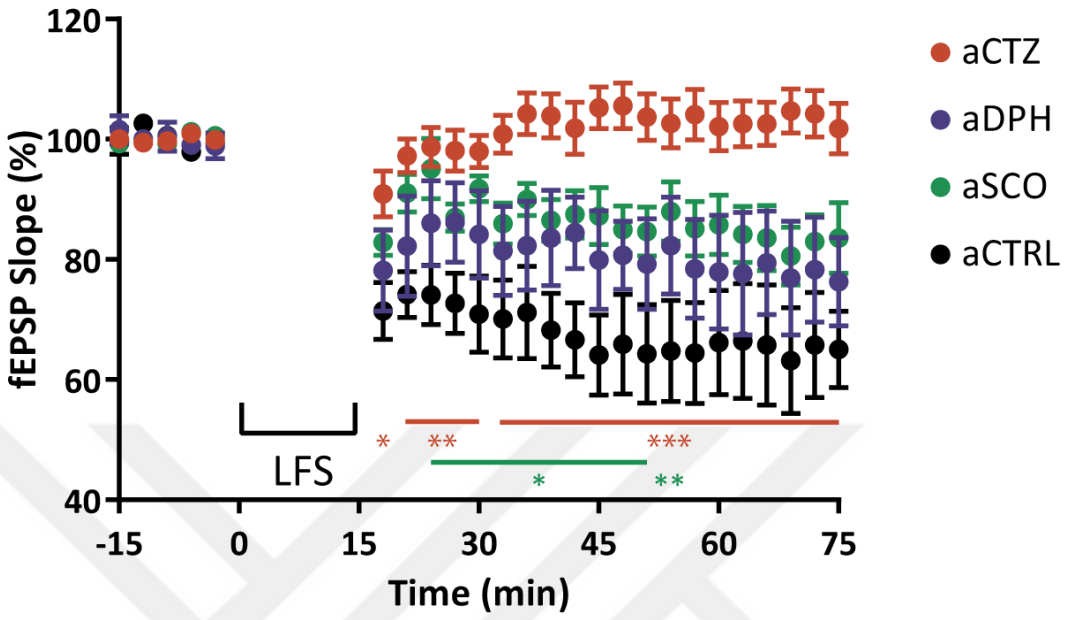

Figure 4.5. Long-term depression recordings from CA1 synapses of acute treatment groups. LTD was entirely blocked by cetirizine throughout the recordings. Diphenhydramine did not alter the LTD. Scopolamine caused some disruption in depression in its early phases but its effects were not long-standing. (Two-way ANOVA treatment effect $F(3,500)=104.9$, $p \leq 0.001$; post-hoc Dunnett's multiple comparisons test) (4-day acute treatment; aCTZ: acute cetirizine, aDPH: acute diphenhydramine, aSCO: acute scopolamine, aCTRL: acute control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$; red * CTRL vs CTZ, green * CTRL vs SCO; LFS: 15 minute 1 Hz low frequency paired-pulse stimulation with IPI of 40 ms at a total of 900 paired-pulses; n=6)

4.2. Electrophysiology Experiments of Chronic Treatment Groups

4.2.1. Input/Output Results from Strata Radiatum and Pyramidale of Chronic Treatment Groups

Chronic cetirizine, diphenhydramine and scopolamine treatment for 12 days showed no shifts of I/O curves in either direction; each treatment yielded a similar curve to the control group's curve (Figure 4.6 A and B). At the 7 V stimulation level that almost yielded half maximal response for the groups, average fEPSP slopes were 2.1±0.3 mV/ms for cetirizine, 2.5±0.3 mV/ms for diphenhydramine, 2.6±0.2 mV/ms

for scopolamine and 2±0.2 mV/ms for the control group. The effects of scopolamine on input-output characteristics observed in the acute treatment group were completely abolished in chronically scopolamine-treated animals.

The averages of maximal fEPSP slopes measured at maximal stimulation of 15 V were 3.9±0.3 mV/ms for cetirizine, 4.4±0.3 mV/ms for diphenhydramine, 4.446 mV/ms for scopolamine and 4±0.4 mV/ms for the control group. The stimulation voltages that evoked almost 50% of maximal fEPSP slope were 6V for scopolamine and 7 V for cetirizine, diphenhydramine and control groups.

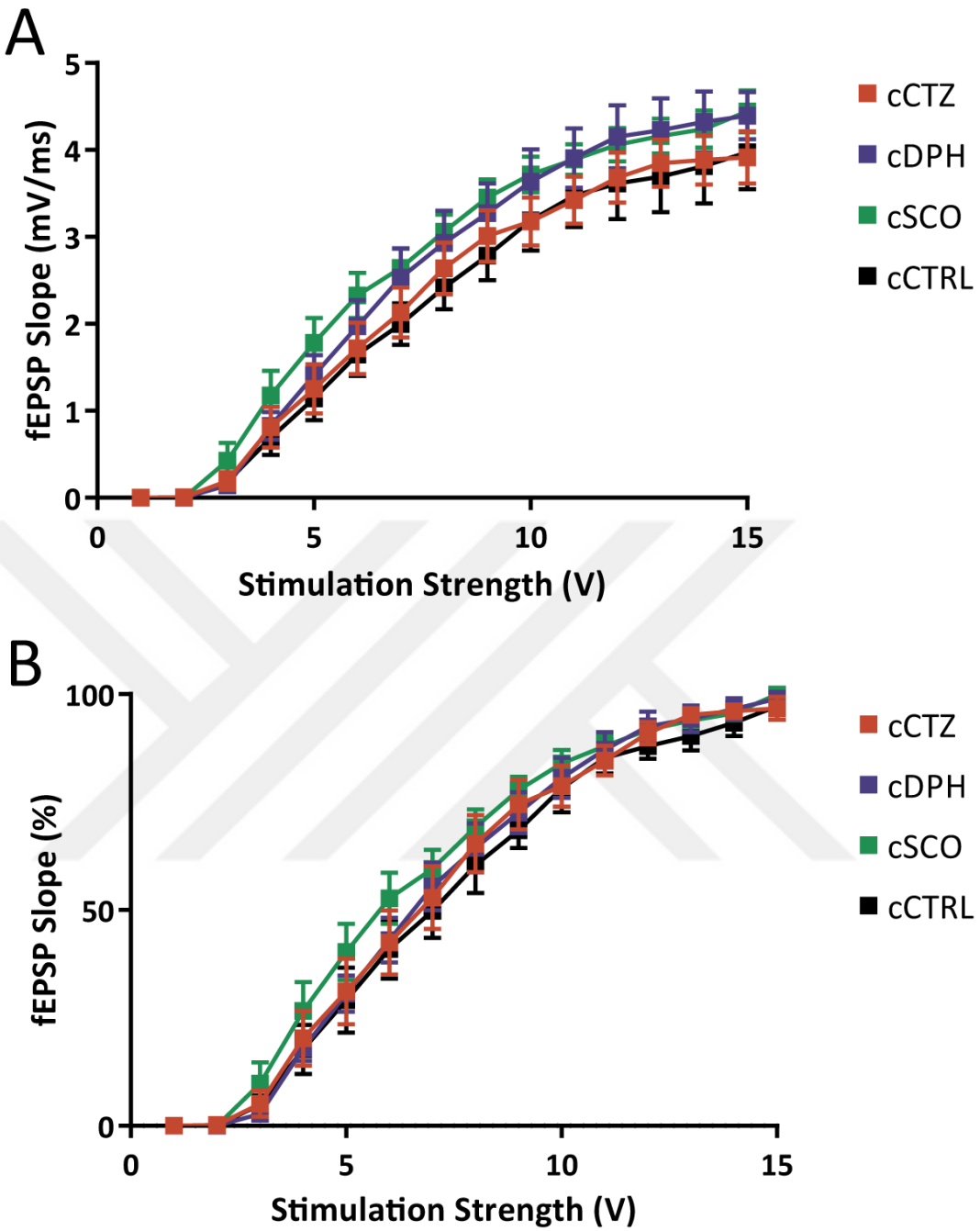

Figure 4.6. The effects of chronic drug treatment on the I/O curves from CA1 stratum radiatum. A) None of the drugs elicited a significant shift in I/O curves and alteration of maximal amplitudes from the stratum radiatum suggesting no change in the strength of the synapses. (Post-hoc Dunnett's multiple comparisons test) **B)** Normalized fEPSP slopes also showed no significant shifts in I/O curves. (Post-hoc Dunnett's multiple comparisons test) (12-day chronic treatment; cCTZ: chronic cetirizine, cDPH: chronic diphenhydramine, cSCO: chronic scopolamine, cCTRL: chronic control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; n=7)

In the stratum pyramidale, the results did not demonstrate a significant curve shift for any group (Figure 4.7 A). The rightward shift of the curve observed in acute scopolamine treatment group was lost in chronic treatment. At maximal stimulation of 15 V, measured PS amplitudes were 15.9±0.6 mV for cetirizine, 16±1.4 mV for diphenhydramine, 13.8±0.9 mV for scopolamine and 17.1±2.1 mV for the control group. Scopolamine decreased the maximal PS amplitude but the result was not found to be statistically significant. When the obtained PS amplitudes were normalized to the maximal value, only cetirizine displayed a slight shift to the left at 6-7 V stimulation points (Figure 4.7 B; $p \leq 0.05$; two-way ANOVA treatment effect $F(3,360)=3.5$, $p \leq 0.05$; $n=7$). The stimulation strength in order to obtain half maximal PS amplitudes were about 6V for cetirizine, 7 V for diphenhydramine and 8 V for both scopolamine and the control group.

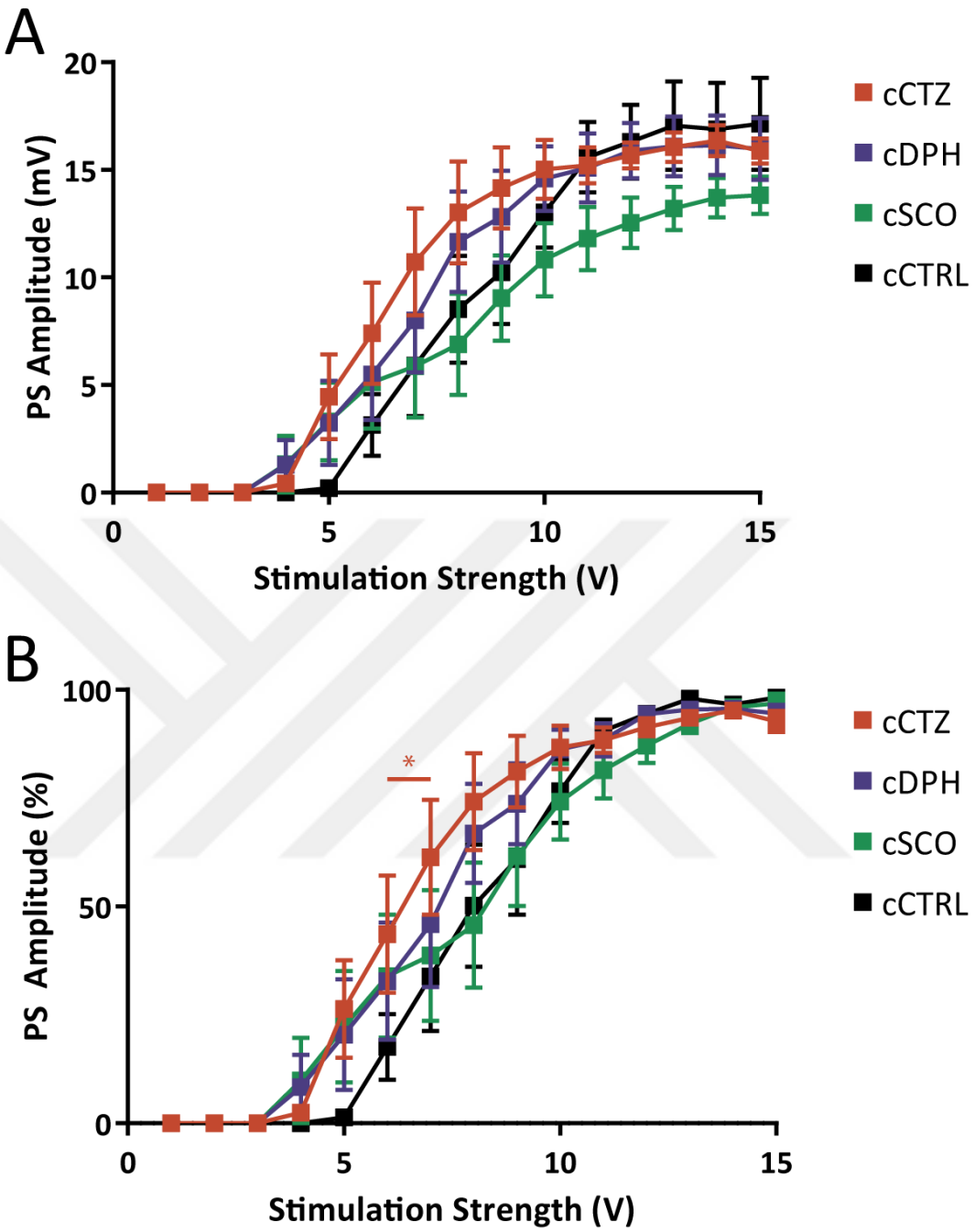

Figure 4.7. The effects of chronic drug treatment on the I/O curves from CA1 stratum pyramidale. A) Mean PS amplitudes displayed no shift for any of the drugs. While maximal PS amplitude for scopolamine was lower than the control group, this result was not statistically significant. (Post-hoc Dunnett's multiple comparisons test) **B)** Normalized PS amplitudes revealed a slight shift of I/O curve to the left for cetirizine treatment at 6 to 7 V. (Two-way ANOVA treatment effect $F(3,360)=3.5$, $p \leq 0.05$; post-hoc Dunnett's multiple comparisons test) (12-day chronic treatment; cCTZ: chronic cetirizine, cDPH: chronic diphenhydramine, cSCO: chronic scopolamine,

cCTRL: chronic control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; * p≤0.05; red * CTRL vs CTZ; n=7)

4.2.2. Paired-Pulse Stimulation Results from Strata Radiatum and Pyramidale of Acute Treatment Groups

In stratum radiatum, chronic treatment with cetirizine did not alter the early facilitation observed in paired-pulse paradigm that was significantly reduced in acute treatment (Figure 4.8 A). Similar to that of the acute treatment, diphenhydramine and scopolamine decreased in early phase facilitation of synaptic transmission. The differences were statistically significant from 20 ms to 60 ms for diphenhydramine ($p \leq 0.001$ for 20-40 ms and $p \leq 0.01$ for 60 ms) and between 20 to 80 ms for scopolamine ($p \leq 0.001$ for 20 ms and 60 ms; $p \leq 0.05$ for 40 ms and 80 ms). After 80 ms, the curves for all groups were similar (Two-way ANOVA treatment effect $F(3,360)=31.9$, $p \leq 0.001$; n=7).

The loss of inhibition of paired-pulse facilitation was also observable in stratum pyramidale (Figure 4.8 B). Chronic treatment with all three drugs did not cause a significant change in differences compared to that of controls in stratum pyramidale, unlike the acute treatment.

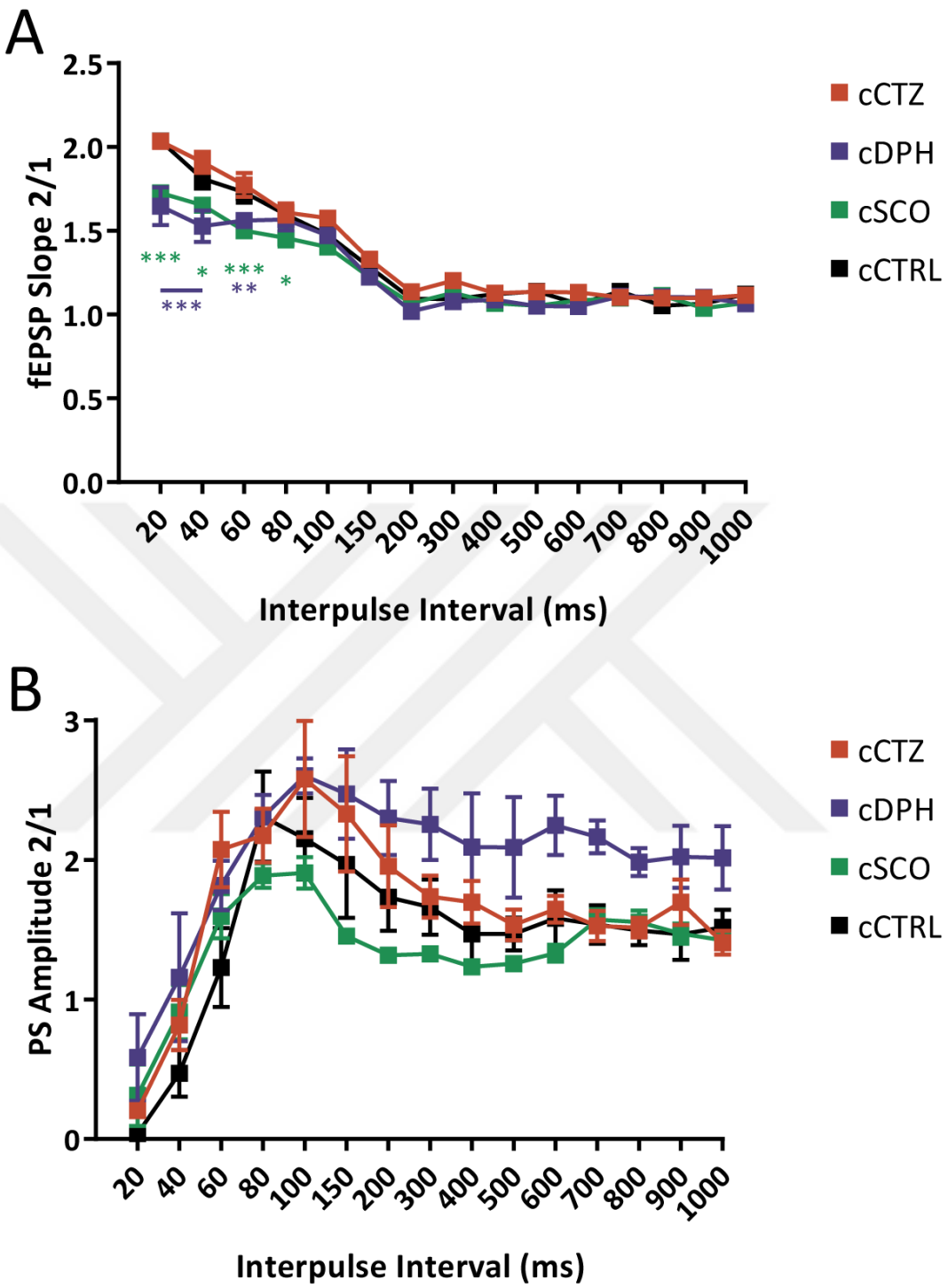

Figure 4.8. Paired-pulse recordings from CA1 strata radiatum and pyramidale of chronic treatment groups. A) While cetirizine did not cause any changes in paired-pulse responses from stratum radiatum, diphenhydramine and scopolamine continued to inhibit the facilitation in 20 to 80 ms IPI. (Two-way ANOVA treatment effect $F(3,360)=31.9$, $p \leq 0.001$; post-hoc Dunnett's multiple comparisons test) **B)** Chronic cetirizine, diphenhydramine and scopolamine treatment did not change the paired-pulse ratios in the rat CA1 stratum pyramidale. (Post-hoc Dunnett's multiple

comparisons test) (12-day chronic treatment; cCTZ: chronic cetirizine, cDPH: chronic diphenhydramine, cSCO: chronic scopolamine, cCTRL: chronic control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$; purple * CTRL vs DPH, green * CTRL vs SCO; n=7)

4.2.3. Long-Term Potentiation and Long-Term Depression Results of Chronic Treatment Groups

Chronic cetirizine administration prominently blocked LTP on the contrary of acute treatment. Following induction of LTP with HFS, EPSP slopes potentiated to the level of the controls. However, potentiation immediately started decaying and after 30 minutes, the difference between cetirizine and control became significant (Figure 4.9; $p \leq 0.05$; n=7). Chronic cetirizine group of rats could not sustain LTP and almost 2 hours after the induction, potentiation was completely vanished. Diphenhydramine followed a similar decline trend to that of the cetirizine but the slope differences with respect to controls were not significant. Chronic scopolamine treatment prevented the induction of LTP from the very beginning ($p \leq 0.001$ from 6 minutes to 51 minutes, $p \leq 0.01$ from 54 minutes to 96 minutes, $p \leq 0.05$ from 99 minutes to 126 minutes). 120 minutes after LTP induction, normalized fEPSP slope amplitudes were 110±11.2% for cetirizine, 125±15.9% for diphenhydramine, 113.9±10.8% for scopolamine and 153.6±8.6% for the control group (Two-way ANOVA treatment effect $F(3,360)=151.3$, $p \leq 0.001$; n=7).

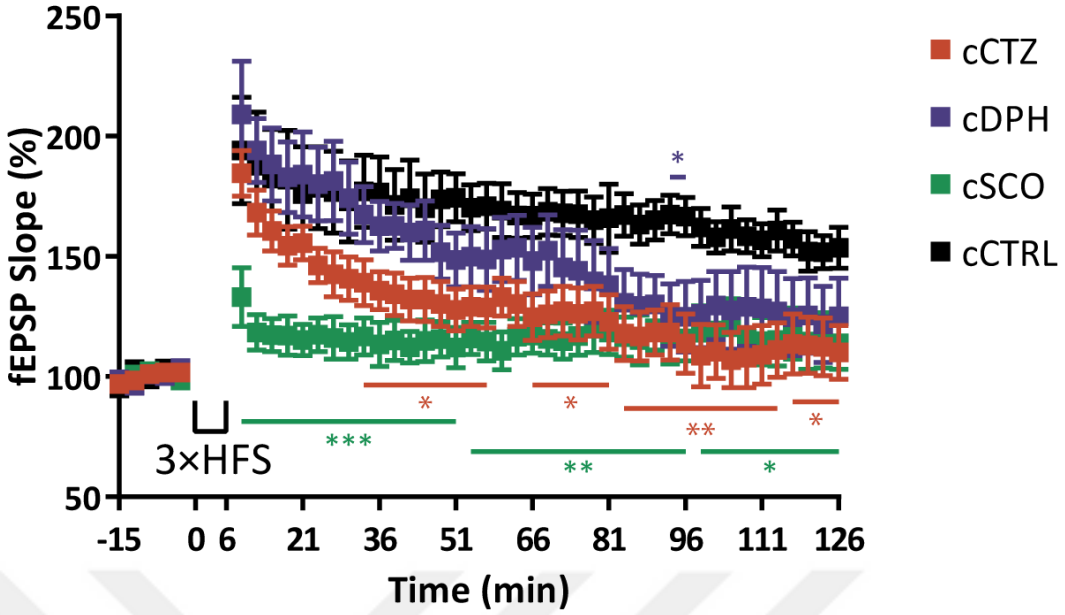

Figure 4.9. Long-term potentiation recordings from CA1 synapses for chronic treatment groups. Cetirizine treatment prevented the retention of LTP. Diphenhydramine, while visually following a similar trace to cetirizine, did not cause any statistically significant result at the end of the recordings. Scopolamine completely eliminated the LTP. (Two-way ANOVA treatment effect F(3,1080)=151.3, p≤0.001; post-hoc Dunnett's multiple comparisons test) (12-day chronic treatment; cCTZ: chronic cetirizine, cDPH: chronic diphenhydramine, cSCO: chronic scopolamine, cCTRL: chronic control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; * p≤0.05, ** p≤0.01, *** p≤0.001; red * CTRL vs CTZ, purple * CTRL vs DPH, green * CTRL vs SCO; HFS: 1 s 100 Hz high frequency stimulus trains with 3 minutes between each train; n=7)

Cetirizine and scopolamine significantly blocked the development of LTD while diphenhydramine caused no changes (Figure 4.10; Two-way ANOVA treatment effect F(3,360)=116.1, p≤0.001; n=7). The normalized fEPSP slope amplitudes at 75 minutes after the beginning of LFS were significantly higher for cetirizine and scopolamine than that of control (p≤0.05; n=7). Average amplitudes at the end of the recordings were 86.5±4.3% for cetirizine, 64±5.8% for diphenhydramine, 85.8±4% for scopolamine and 69.9±4% for the control group.

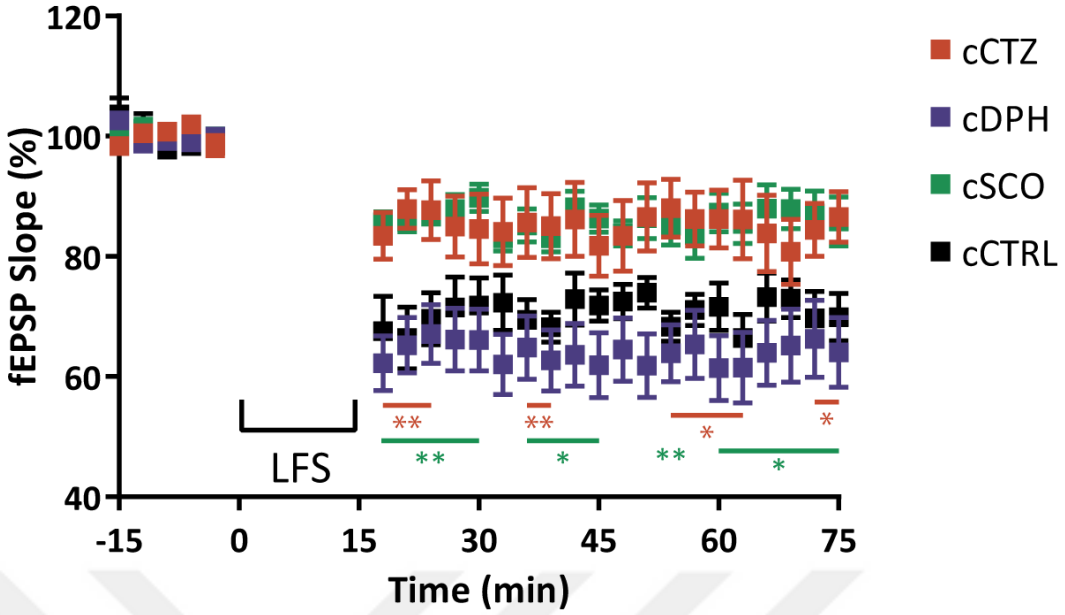

Figure 4.10. Long-term depression recordings from CA1 synapses of acute treatment groups. Both cetirizine and scopolamine significantly prevented the induction of LTD. Diphenhydramine treatment did not cause any significant alterations in LTD development. (Two-way ANOVA treatment effect F(3,600)=116.1, p≤0.001; post-hoc Dunnett's multiple comparisons test) (12-day chronic treatment; cCTZ: chronic cetirizine, cDPH: chronic diphenhydramine, cSCO: chronic scopolamine, cCTRL: chronic control; CTZ and DPH at 20 mg/kg, SCO at 2 mg/kg; * p≤0.05, ** p≤0.01; red * CTRL vs CTZ, green * CTRL vs SCO; LFS: 15 minute 1 Hz low frequency paired-pulse stimulation with IPI of 40 ms at a total of 900 paired-pulses; n=7)

4.3. Behavioral Experiments of Acute Cetirizine Treatment Groups

4.3.1. Open Field Arena Results of Acute Cetirizine Treatment Groups

Acute cetirizine treatment did not affect distance traveled in the open field arena (Figure 4.11 A). Average traveling distances were 3.3±0.4 m for acute cetirizine treatment group (n=8) and 3.1±0.9 m for the control group (n=6). Distance traveled in the center was also not significantly affected by acute cetirizine administration (Figure 4.11 B). Average distances were 0.2±0.07 m for the acute cetirizine group (n=8) and 0.3±0.1 m for the control group (n=6).

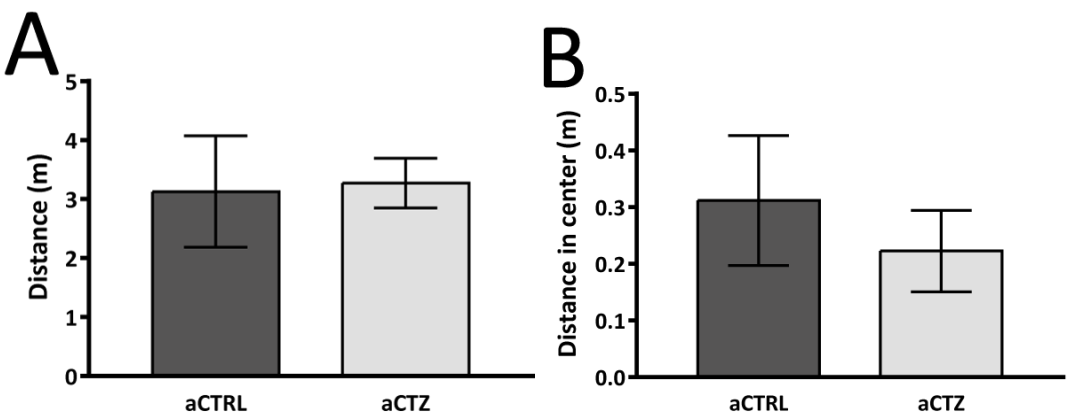

Figure 4.11. Locomotion in the open field arena for acute cetirizine treatment group. A) Acute cetirizine treatment did not cause a change in total distance traveled. **B)** Distance traveled in the center was also not affected by acute cetirizine treatment. (3-day acute treatment; aCTZ: acute cetirizine, aCTRL: acute control; CTZ at 5 mg/kg; n=6 for aCTRL and n=8 for aCTZ)

4.3.2. Elevated Plus Maze Results of Acute Cetirizine Treatment Groups

Times spent and distances traveled in open arms are given for elevated plus maze experiments; increase in duration or distance in open arms denote decreased stress in the animals. Acute treatment with cetirizine did not cause a significant change in either time spent in open arms (Figure 4.12 A; 67.9±39 s for acute cetirizine treatment group vs 137.2±46.6 s for the control group; n=8 and n=6, respectively) or distance traveled in open arms (Figure 4.12 B; 0.5±0.2 m for acute cetirizine treatment group vs 0.4±0.2 m for the control group).

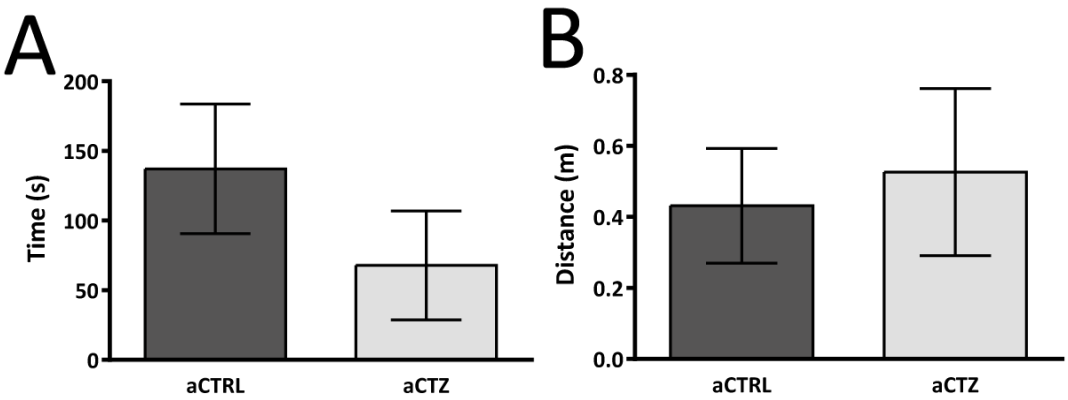

Figure 4.12. Elevated plus maze results of acute cetirizine treatment group. A) Acute cetirizine treatment did not cause a significant change in total time spent in open arms. **B)** Distance traveled in open arms was also not significantly different. (3-day acute treatment; aCTZ: acute cetirizine, aCTRL: acute control; CTZ at 5 mg/kg; n=6 for aCTRL and n=8 for aCTZ)

4.3.3. Passive Avoidance Results of Acute Cetirizine Treatment Groups

Both control and cetirizine groups got habituated to the shuttlebox and crossed to the dark chamber with similar latencies (Figure 4.13 A; 68.7±22.5 s for aCTZ and 66.4±21 s for aCTRL; n=8 and n=6, respectively). At the training phase, before receiving the shock, control group animals entered the dark chamber faster than the cetirizine group on average but this difference was not statistically significant (Figure 4.13 B; 80.6±40.4 s for aCTZ and 9±6 s for aCTRL; n=8 and n=6, respectively). The third phase is the phase where the animals are expected to remember not to enter the dark chamber, where they previously received electric shock. All control animals stayed in the light chamber while some of the animals from the cetirizine group have failed to avoid the dark chamber (Figure 4.13 C; 244.8±37.7 s for aCTZ and 300±0 s for aCTRL; n=8 and n=6, respectively) but the result was not statistically significant.

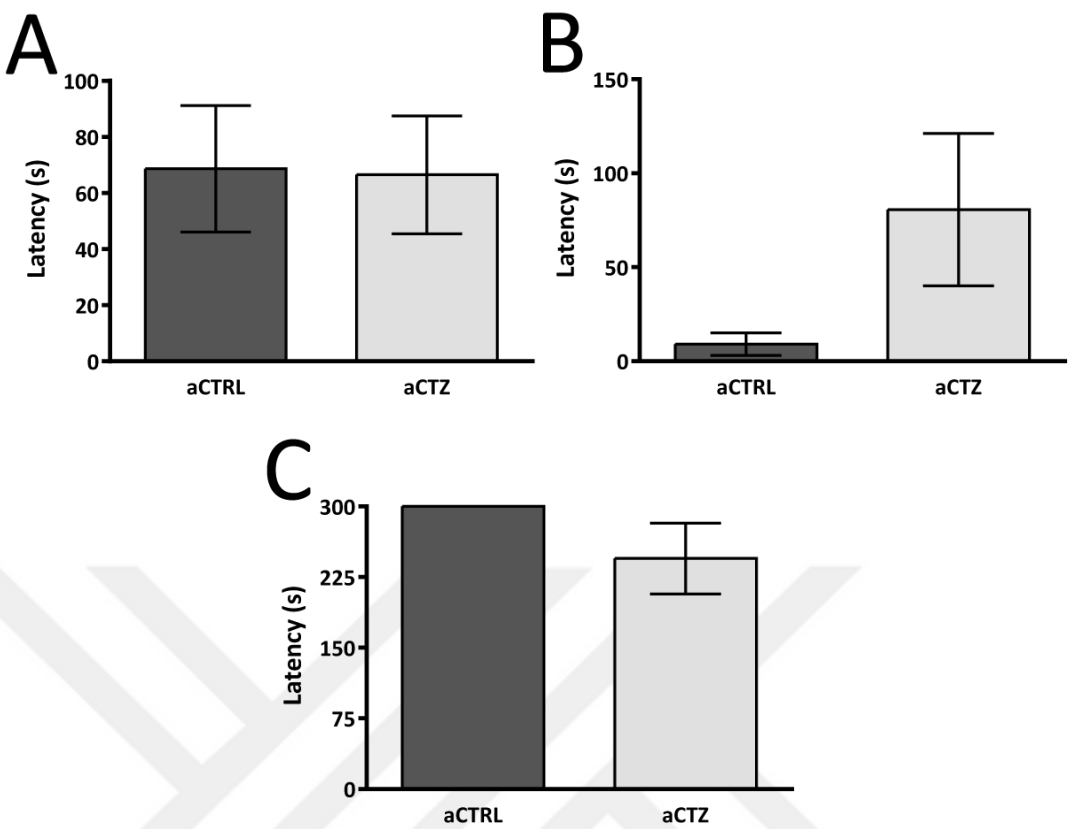

Figure 4.13. Passive avoidance results of acute cetirizine treatment group. A) Animals from both groups entered the dark chamber with similar latencies. **B)** Before receiving the shock, control group animals entered the dark compartment more readily and **C)** and after receiving the shock, they avoided the dark compartment completely but the differences were not statistically significant. (3-day acute treatment; aCTZ: acute cetirizine, aCTRL: acute control; CTZ at 5 mg/kg; n=6 for aCTRL and n=8 for aCTZ)

4.3.4. Forced Swim Test Results for Acute Cetirizine Treatment Groups

In the test phase of forced swim test, the average immobility duration of the cetirizine group—while being higher than it—was not significantly different than the average immobility duration of the control group (Figure 4.14). The averages were 49.2±12.2 s for the acute cetirizine treatment group (n=8) and 23.2±17.6 for the control group (n=6).

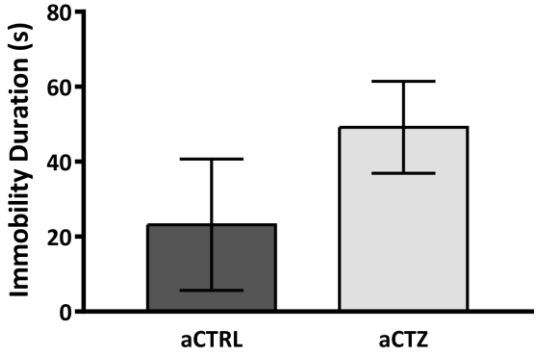

Figure 4.14. Forced swim test results for acute cetirizine treatment group. The difference in immobility duration between acute cetirizine treated animals and controls was not found to be statistically significant. (3-day acute treatment; aCTZ: acute cetirizine, aCTRL: acute control; CTZ at 5 mg/kg; n=6 for aCTRL and n=8 for aCTZ)

4.3.5. Morris Water Maze Results for Acute Cetirizine Treatment Groups

Morris water maze acquisition test was conducted over 4 days and averages for each day and each trial were calculated. Animals received acute cetirizine treatment did not display altered performance compared to control animals at any day of the experiment (Figure 4.15 A). At first day, the average latencies for reaching the platform were 81.7±7.2 s the acute cetirizine treatment group (n=8) and 66.3±8.6 s for the control group (n=6). At the last day, the latencies have improved to 18.8±3 s for the acute cetirizine treatment group (n=8) and 16.29±3.03 s for the control group (n=6). The differences between cetirizine and control groups were not statistically significant at any point of the experiments. One day after the acquisition phase, the platform was removed and probe test was performed. During the probe tests, the durations spent in target quadrants were not different for control and cetirizine groups (Figure 4.15 B; 11.8±2.5 s for acute cetirizine vs 16.4±2.8 s for control; n=8 and n=6, respectively).

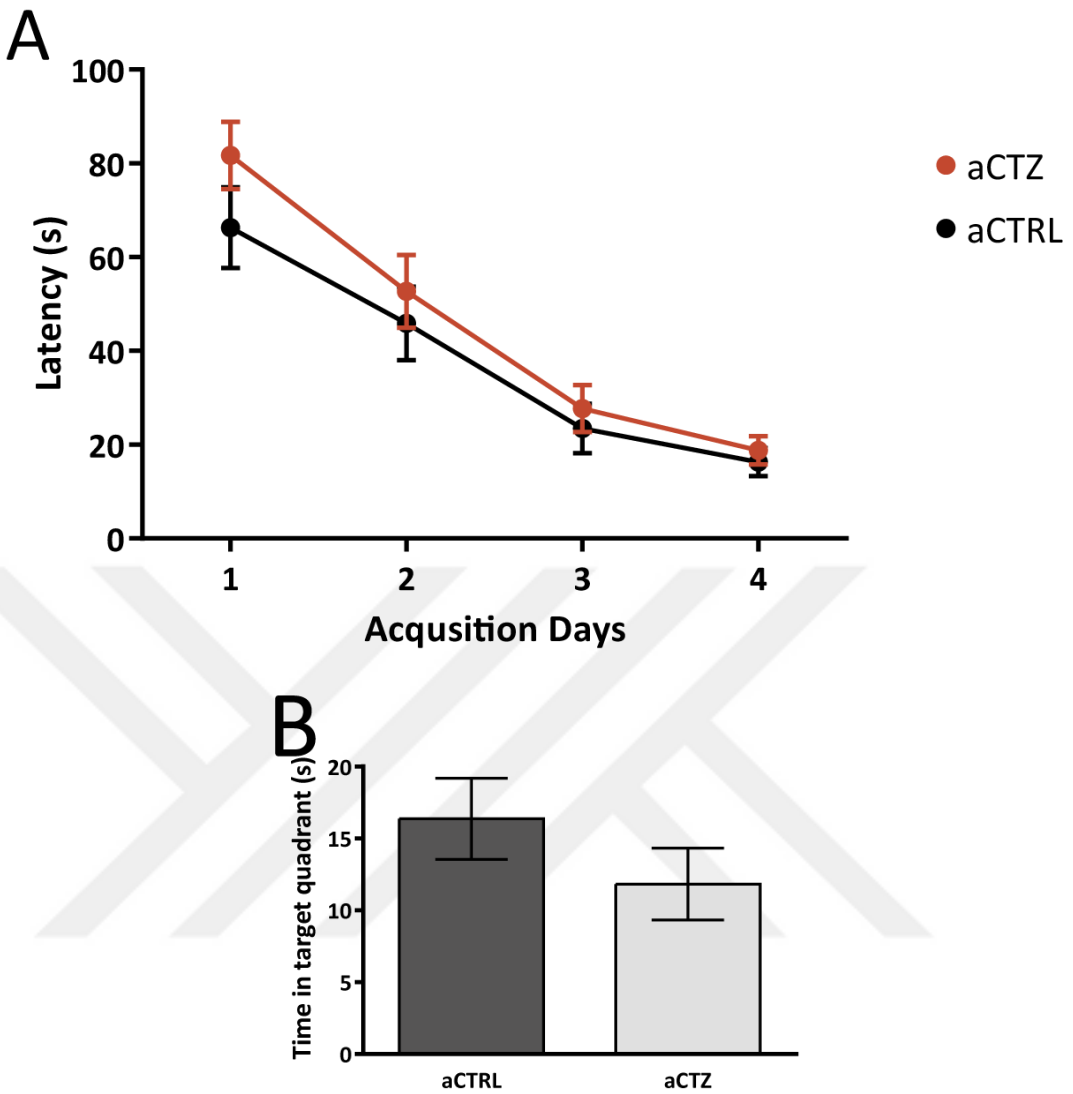

Figure 4.15. Morris water maze results of acute cetirizine treatment group. **A)** During the acquisition trials, cetirizine treatment did not alter the latency to find the platform. **B)** Probe test also did not show any difference between memory performances of the cetirizine treatment group. (4-day acute treatment; aCTZ: acute cetirizine, aCTRL: acute control; CTZ at 5 mg/kg; n=6 for aCTRL and n=8 for aCTZ)

4.4. Behavioral Experiments of Chronic Cetirizine Treatment Groups

4.4.1. Open Field Arena Results of Chronic Cetirizine Treatment Group

After 12 days of cetirizine treatment, cetirizine did not alter the average distance traveled by the animals in the arena (Figure 4.16 A). Acute cetirizine treated animals' average for total distance traveled was 23.4±4.4 m and control animals' was 26.5±4.4 s (n=9). Mean distances in the center of the arena was also not affected by

the chronic cetirizine treatment (Figure 4.16 B). The treatment group had an average of 1.6±0.3 m and the control group had an average of 1.3±0.6 s in the center of the arena (n=9).

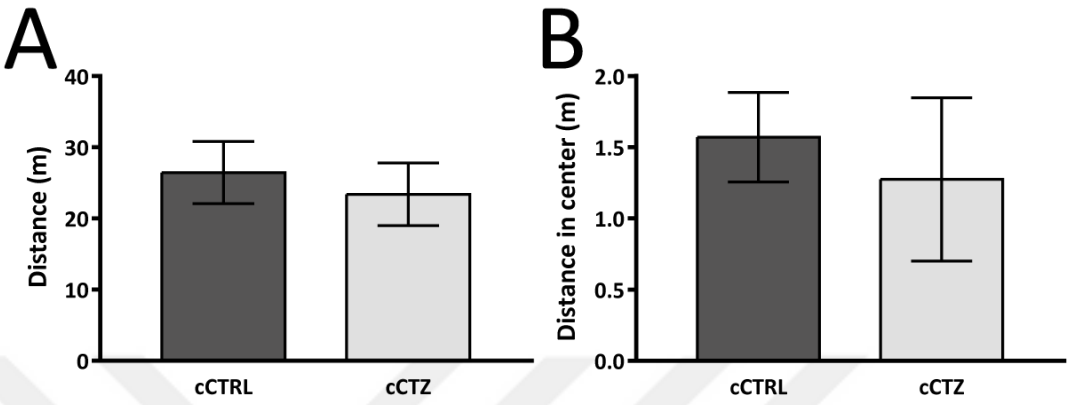

Figure 4.16. Locomotion in the open field arena for chronic cetirizine treatment group. A) Chronic cetirizine treatment did not statistically alter the total distance traveled. **B)** Distance traveled in the center area was also not affected by the cetirizine treatment. (13-day chronic treatment; cCTZ: chronic cetirizine, cCTRL: chronic control; CTZ at 5 mg/kg; n=9)

4.4.2. Elevated Plus Maze Results of Chronic Cetirizine Treatment Group

Time spent in the open arms of the elevated plus maze showed no significant change between chronic cetirizine treatment and control groups. (Figure 4.17 A; 32.4±9.6 s for cCTZ vs 30.2±11.2 s for cCTRL; n=9) The distance traveled in open arms was likewise not statistically significant between groups (Figure 4.17 B; 0.54±0.18 m for cCTZ vs 0.58±0.23 m for cCTRL; n=9).

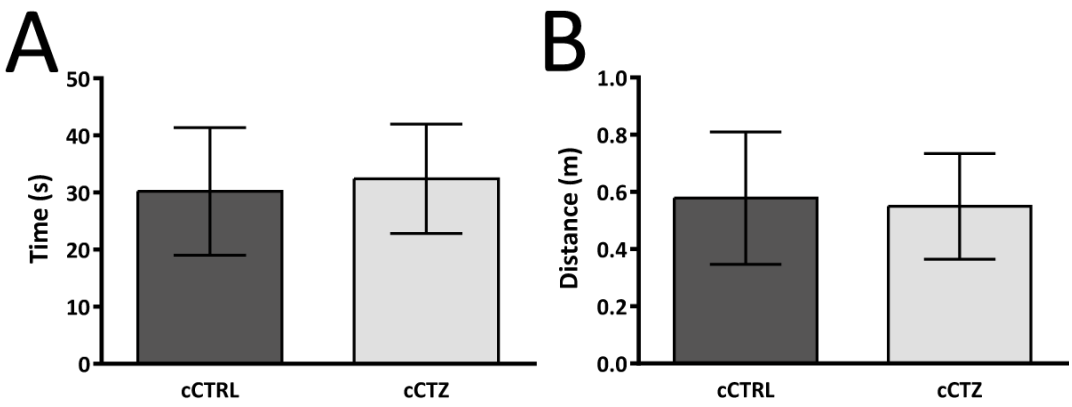

Figure 4.17. Elevated plus maze results of chronic cetirizine treatment group. A) Time spent in open arms averages were not significantly different between cetirizine and control groups. **B)** Distance traveled was also similar between groups. (14-day chronic treatment; cCTZ: chronic cetirizine, cCTRL: chronic control; CTZ at 5 mg/kg; n=9)

4.4.3. Passive Avoidance Results of Chronic Cetirizine Treatment Group

In the first phase of passive avoidance test, the latencies to cross the door to the dark chamber were similar on average (Figure 4.18 A; 120.7±35.8 s for cCTZ vs 125.9±42.5 s for cCTRL; n=9). Second phase, where the foot shock was delivered to the animals when they move to dark chamber was also similar between treatment and control groups (Figure 4.18 B; 94.9±39.7 s vs 124.4±40.6 s; n=9). After the foot shock training, none of the animals from the groups entered to the dark chamber and the experiments were cut off at 300 seconds for all animals in the test phase (Figure 4.18 C; 300±0 s for aCTZ vs 300±0 s for aCTRL; n=9).

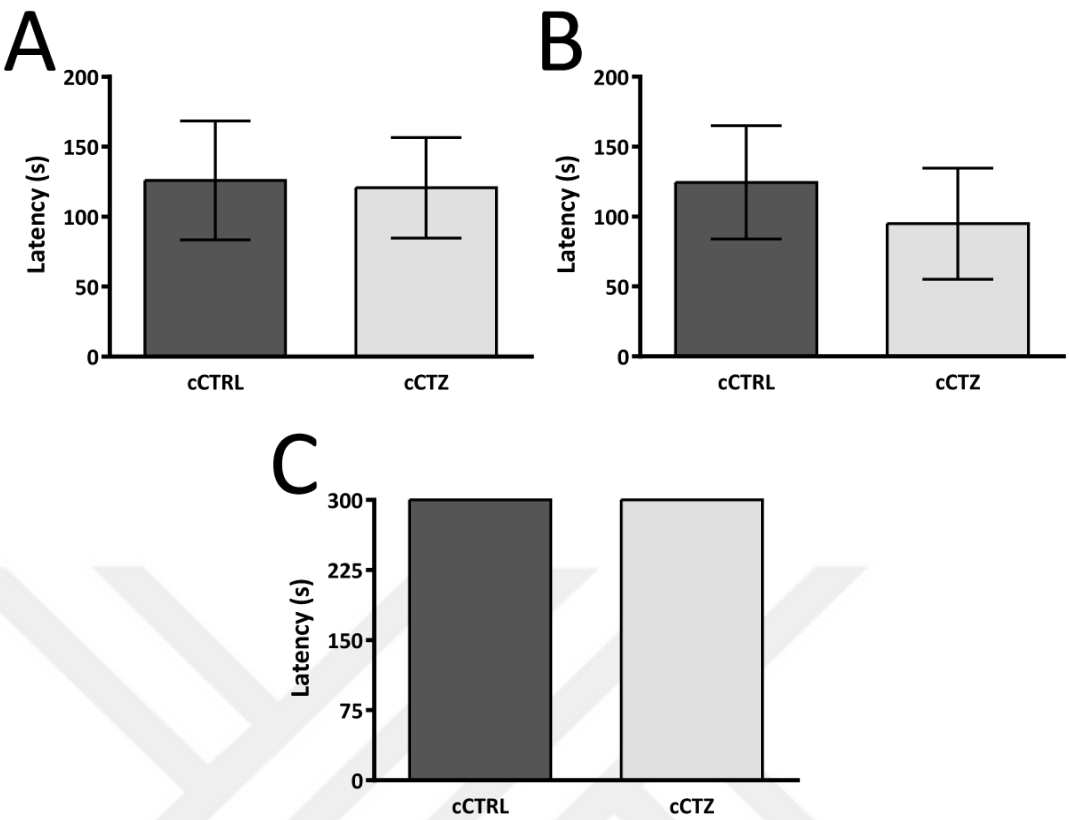

Figure 4.18. Passive avoidance results of chronic cetirizine treatment group. A) Latency averages in habituation or **B)** Training phases were not significantly different between groups. **C)** After the training, none of the animals entered the dark chamber, showing no decrease in learning and memory capacity of the animals between the groups. (11-day chronic treatment; cCTZ: chronic cetirizine, cCTRL: chronic control; CTZ at 5 mg/kg; n=9)

4.4.4. Forced Swim Test Results for Chronic Cetirizine Treatment Group

The average immobility durations for the groups were 40.8±8.4 s for the chronic cetirizine treatment group and 36.7±7.3 s for the chronic control group (n=9). These averages were not statistically significantly different (Figure 4.19).

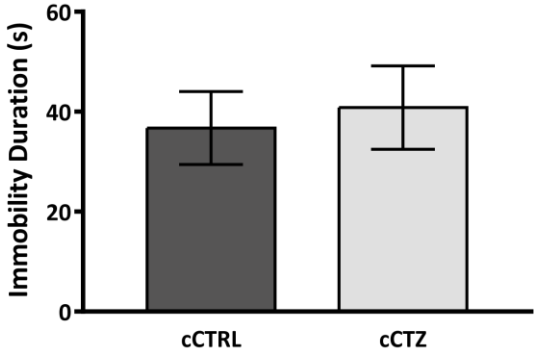

Figure 4.19. Forced swim test results for chronic cetirizine treatment group. The immobile floating duration averages between the groups were not significantly different and was at about 40 s for both groups. (12-day chronic treatment; cCTZ: chronic cetirizine, cCTRL: chronic control; CTZ at 5 mg/kg; n=9)

4.4.5. Morris Water Maze Results for Chronic Cetirizine Treatment Group

Acquisition phase was started at the 11th day of drug injections and continued until the 15th day. Obtained trace of average latencies to reach the platform showed no difference in the learning and memory performance between the treatment and control groups (Figure 4.20). At the first day, the average latency for the chronic cetirizine group was 74±7.7 s and for the control group 75.2±7.9 s (n=9). At the last day, the latencies were 18±4.3 s for the chronic cetirizine treatment group and 18±3.5 s for the control group (n=9). Throughout the experiments, the differences between the groups were not significantly different.

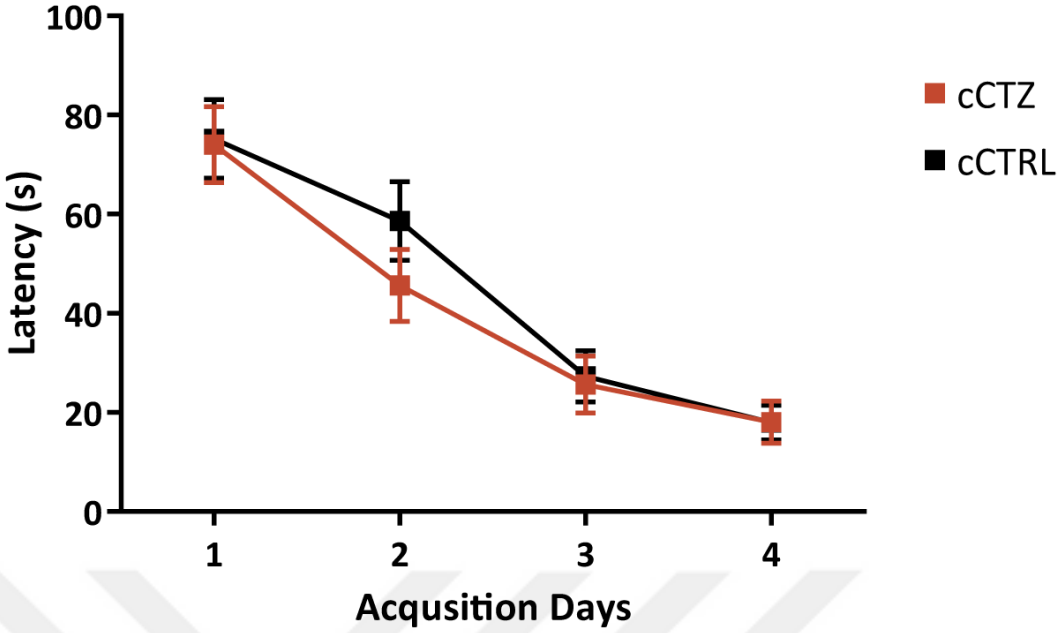

Figure 4.20. Morris water maze results for the chronic treatment group. Both groups showed similar performances in average for this learning and memory task. The average differences were not statistically significant. (14-day chronic treatment; cCTZ: chronic cetirizine, cCTRL: chronic control; CTZ at 5 mg/kg; * p≤0.05, ** p≤0.01, *** p≤0.001; n=9)

4.4.6. Morris Water Maze Reversal Learning Results for the Chronic Treatment Group

After the completion of the acquisition phase of Morris water maze, a reversal paradigm is used to assess relearning. After the platform was placed in a new quadrant, the animals were expected to learn and remember the new placement of the platform over a 3-day acquisition phase. In this paradigm, cetirizine group animals seemed to show better performance in the 2nd and 3rd days but statistically these differences were not significant (Figure 4.21 A). First day averages of the groups were 31.3±6.1 s for the cetirizine and 28.1±4.6 s for the control group (n=9). At the second day, cetirizine group animals performed slightly better with an average of 13.4±2.4 s against control group's 20±4.1 s (n=9). The last day performances of cetirizine group animals were again slighly better with 8.6±1.3 s against control group average of 13.8±2.6 s (n=9).

After the reversal learning was completed, a probe test was conducted. The average for the chronic cetirizine group was 27.4±2.4 s and the average for the control group was 24.8±2 s (n=9). The difference between these results was not statistically significant (Figure 4.21 B).

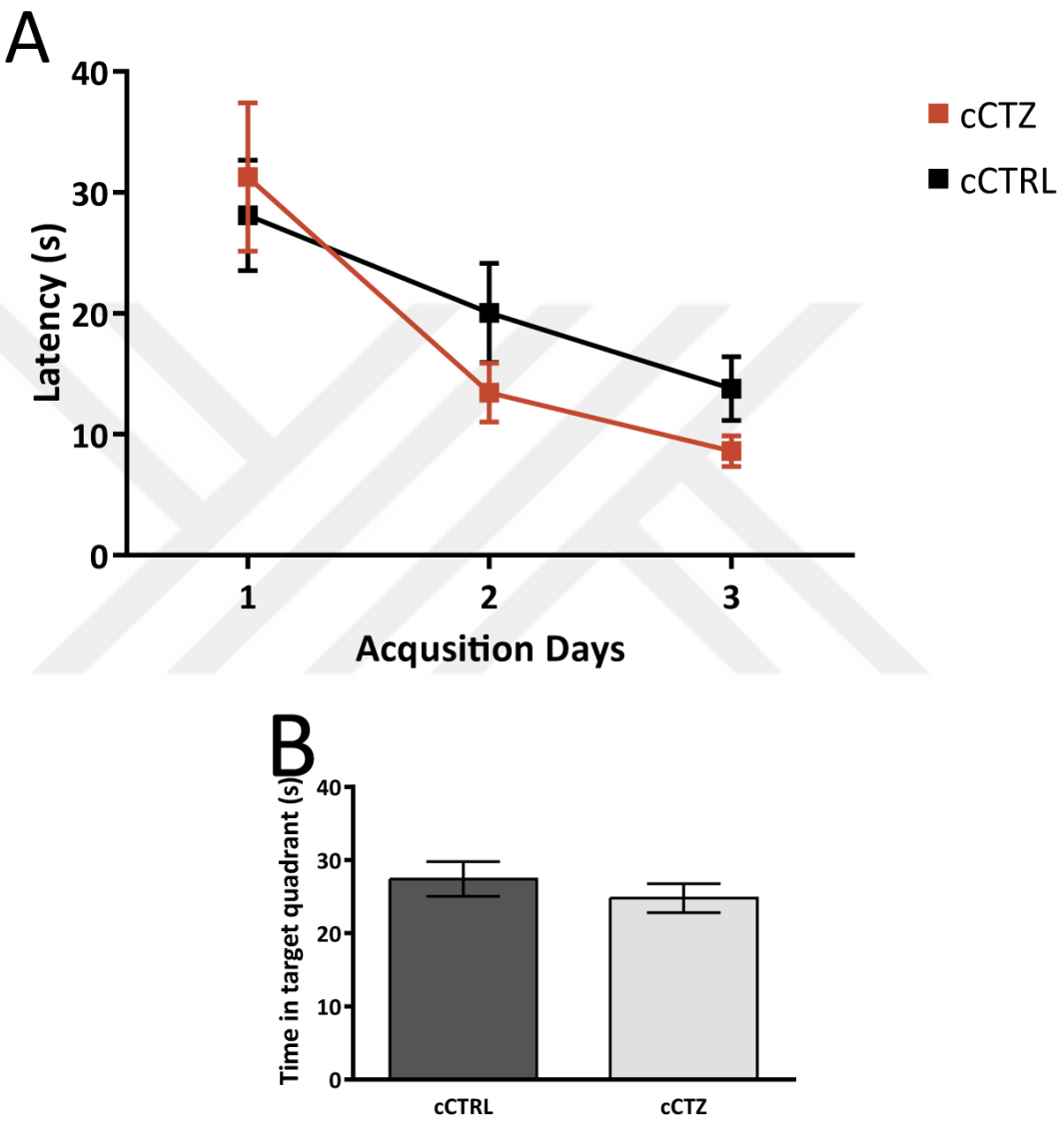

Figure 4.21. Morris water maze reversal learning results for the chronic treatment groups. A) Acquisition experiments revealed no statistical significance between the relearning performances of the groups. **B)** Probe trial after the relearning showed similar durations spent in the target quadrant for the groups. (18-day chronic treatment; cCTZ: chronic cetirizine, cCTRL: chronic control; CTZ at 5 mg/kg; n=9)

4.5. Acute Cetirizine REM Deprivation Experiments

4.5.1. Input/Output Results from Strata Radiatum and Pyramidale of REM Sleep Deprivation Groups

REM sleep deprivation caused a marked decrease in maximal fEPSP slope in the stratum radiatum (Figure 4.22 A). Acute cetirizine treatment partially ameliorated this effect, completely returning the maximal slope to control group's levels at 13 V to 15 V. (At 15 V, $p \leq 0.01$ between REMD and other two groups; n=6.) fEPSP slope amplitudes at maximal stimulation strength were 2.6±2.3 mV/ms for REM sleep deprivation, 3.6±0.2 mV/ms for REM sleep deprivation with acute cetirizine treatment and 3.7±0.4 mV/ms for the control group (Two-way ANOVA treatment effect $F(2,225)=45.17$, $p \leq 0.001$; n=6). The I/O curve was also shifted to the right by REM sleep deprivation. The difference between REMD group and the control group was statistically significant starting from 5 V ($p \leq 0.05$ at 5 V and between 11 V to 14 V; $p \leq 0.01$ from 6 V to 10 V and at 15 V; n=6).

Normalization of the fEPSP slopes using maximal fEPSP affirmed the right shift of the REM sleep deprivation group was significant (Figure 4.22 B; $p \leq 0.05$ at 5 V and 7 V, $p \leq 0.01$ at 6 V; n=6). Acute cetirizine treatment during REM sleep deprivation shifted the I/O curve slightly to the left but this result was not statistically significant. The stimulus strengths that produced the response closest to 50% of the maximum were 7 V for the control group, 8 V for REMD+aCTZ group and 9 V for REMD group (Two-way ANOVA treatment effect $F(2,225)=20.95$, $p \leq 0.001$; n=6).

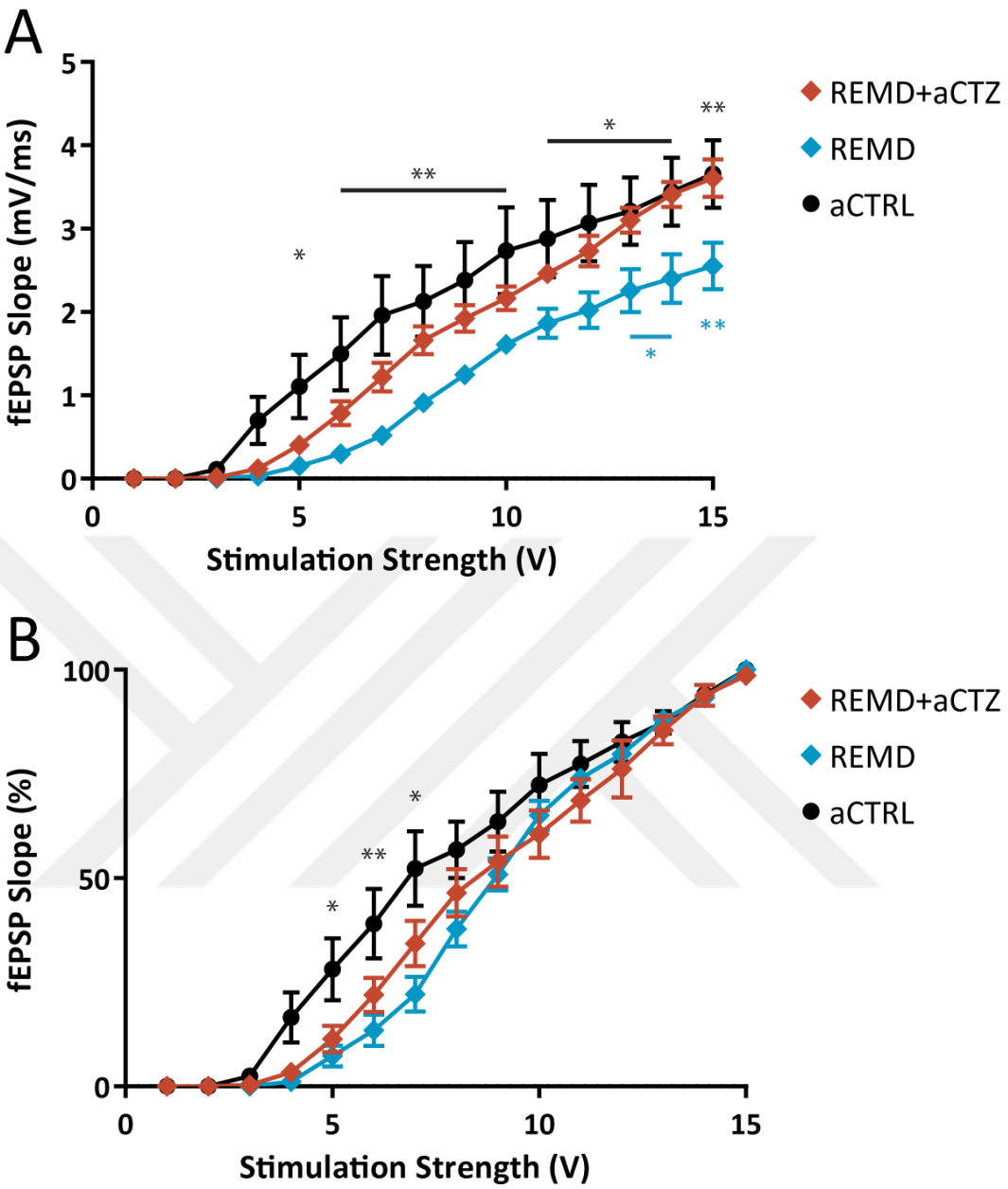

Figure 4.22. The effects of cetirizine administration during in REM sleep deprivation on the I/O curves from CA1 stratum radiatum. A) REM sleep deprivation significantly decreased the maximum fEPSP slope and shifted the I/O curve to the right. Cetirizine treatment raised the maximal fEPSP to the control group levels and decreased the rightward shift. (Two-way ANOVA treatment effect $F(2,225)=45.17$, $p\leq0.001$; post-hoc Sidak's multiple comparisons test) **B)** Normalized curves displayed the right shift in the REMD group. (Two-way ANOVA treatment effect $F(2,225)=20.95$, $p\leq0.001$; post-hoc Sidak's multiple comparisons test) (72-hour REM sleep deprivation, 4-day acute treatment; REMD: REM sleep deprivation, REMD+aCTZ: REM sleep deprivation with acute cetirizine, aCTZ: acute cetirizine; CTZ at 5 mg/kg; * $p\leq0.05$, ** $p\leq0.01$; black * CTRL vs REMD, blue * REMD vs REMD+CTZ; n=6)

Stratum pyramidale I/O results showed a shift to the right for the REM sleep deprivation group (Figure 4.23 A; $p \leq 0.05$ for 8 V and 10 V, $p \leq 0.01$ for 9 V; n=6). In contrast to the radiatum data, cetirizine-treated REMD and REMD groups' curves overlapped ($p \leq 0.01$ from 8 V to 10 V; n=6). Neither REM sleep deprivation nor the treatment significantly altered the maximum PS amplitudes. At maximum stimulation strength, PS amplitude averages were 13.7±1.6 mV for REM sleep deprivation, 13.4±0.6 mV for REM sleep deprivation with cetirizine treatment and 14.1±0.6 mV for the control group (Two-way ANOVA treatment effect $F(2,225)=29.8$, $p \leq 0.001$; n=6). Normalized I/O graph showed similar results (Figure 4.23 B; $p \leq 0.05$ at 8 V and 9 V between REMD and control groups; $p \leq 0.05$ at 8 V and 10 V, $p \leq 0.01$ at 9 V between REMD with cetirizine treatment and control groups; n=6). Stimulation strengths that evoked PS amplitudes closest to half-maximal amplitudes were 12 V for REM sleep deprivation and cetirizine-treated REM sleep deprivation groups and 9 V for the control group. (Two-way ANOVA treatment effect $F(2,225)=25.8$, $p \leq 0.001$; n=6).

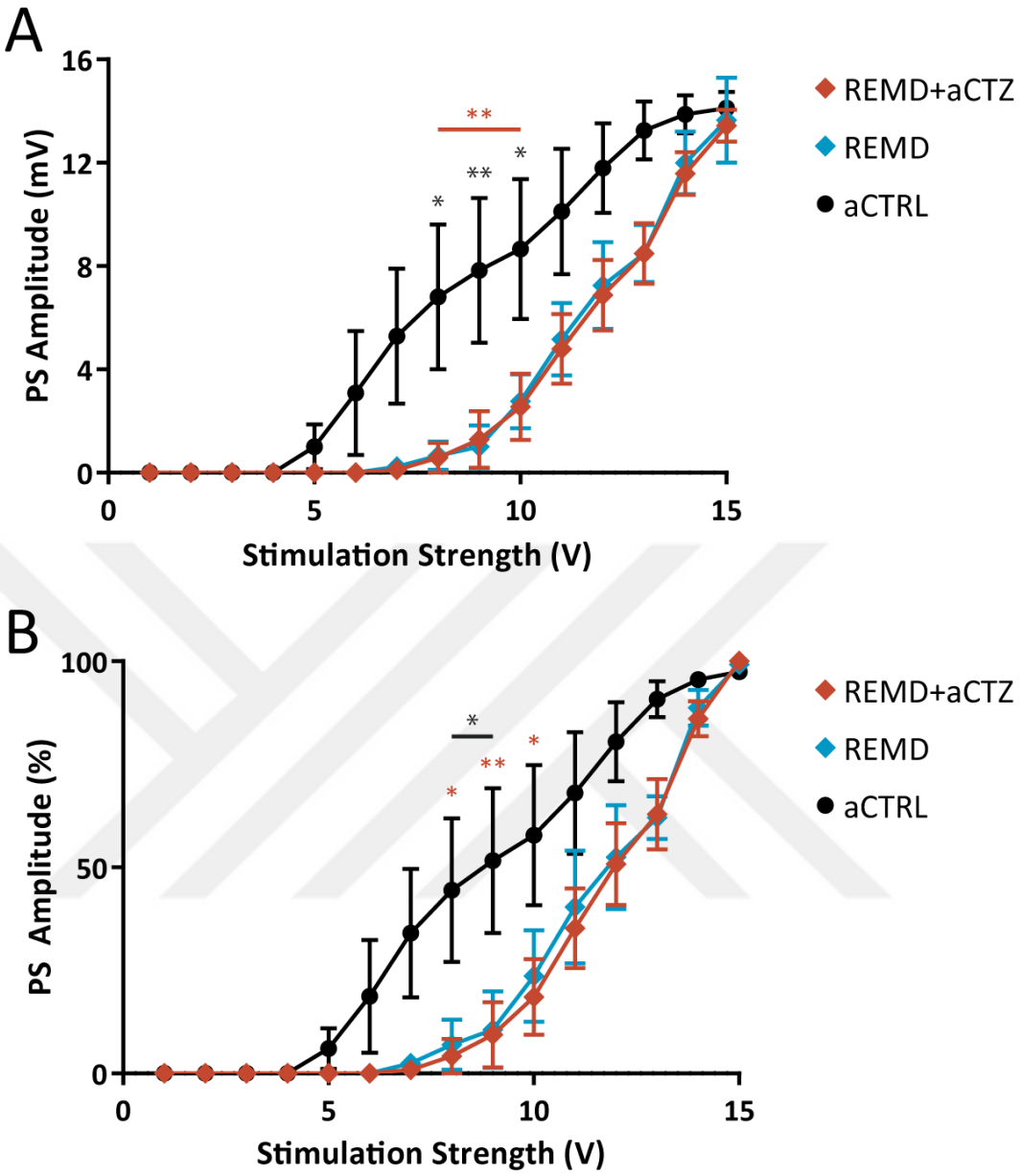

Figure 4.23. The effects of cetirizine administration during REM sleep deprivation on the I/O curves from rat CA1 stratum pyramidale. A) REM deprivation significantly shifted the I/O curve to the right and acute cetirizine treatment did not reverse this decline in excitability. Maximum PS amplitudes were unchanged. (Two-way ANOVA treatment effect $F(2,225)=29.8$, $p \leq 0.001$; post-hoc Sidak's multiple comparisons test) **B)** Normalized PS amplitude graph displayed similar results. (Two-way ANOVA treatment effect $F(2,225)=25.8$, $p \leq 0.001$; post-hoc Sidak's multiple comparisons test) (72-hour REM sleep deprivation, 4-day acute treatment; REMD: REM sleep deprivation, REMD+aCTZ: REM sleep deprivation with acute cetirizine, aCTZ: acute cetirizine; CTZ at 5 mg/kg; * $p \leq 0.05$, ** $p \leq 0.01$; black * CTRL vs REMD, blue * CTRL vs REMD+CTZ; n=6)

4.5.2. Paired-Pulse Stimulation Results from Strata Radiatum and Pyramidale of REM Sleep Deprivation Groups

Paired-pulse experiments in stratum radiatum demonstrated a significant decrease of the facilitation seen in shorter IPIs by REM sleep deprivation (Figure 4.24 A; $p \leq 0.001$ at 20 ms and 40 ms intervals, $p \leq 0.01$ at 60 ms interval; n=6). Treating REM sleep deprived animals with cetirizine throughout the deprivation period reverted this decrease ($p \leq 0.001$ for 20 ms and $p \leq 0.05$ for 40 ms; n=6). Ratios of second fEPSP slope to first one at 20 ms were 1.4±0.2 for REM sleep deprivation group, 1.9±0.1 for REM sleep deprivation with cetirizine treatment group and 2.1±0.04 for the control group. After 60 ms, traces for the groups slowly converged and overlapped at 200 ms (Two-way ANOVA treatment effect $F(2,225)=15.8$, $p \leq 0.001$; n=6).

Stratum pyramidale paired-pulse experiments yielded no significant change between REM sleep deprivation with or without cetirizine treatment groups and control group in post-hoc analyses (Figure 4.24 B). The ratios at 150 ms IPI were 1.1±0.14 for REM sleep deprivation, 1.3±0.04 for REM sleep deprivation with cetirizine treatment and 1.4±0.04 for the control group (Two-way ANOVA treatment effect $F(2,225)=16.5$, $p \leq 0.001$; n=6).

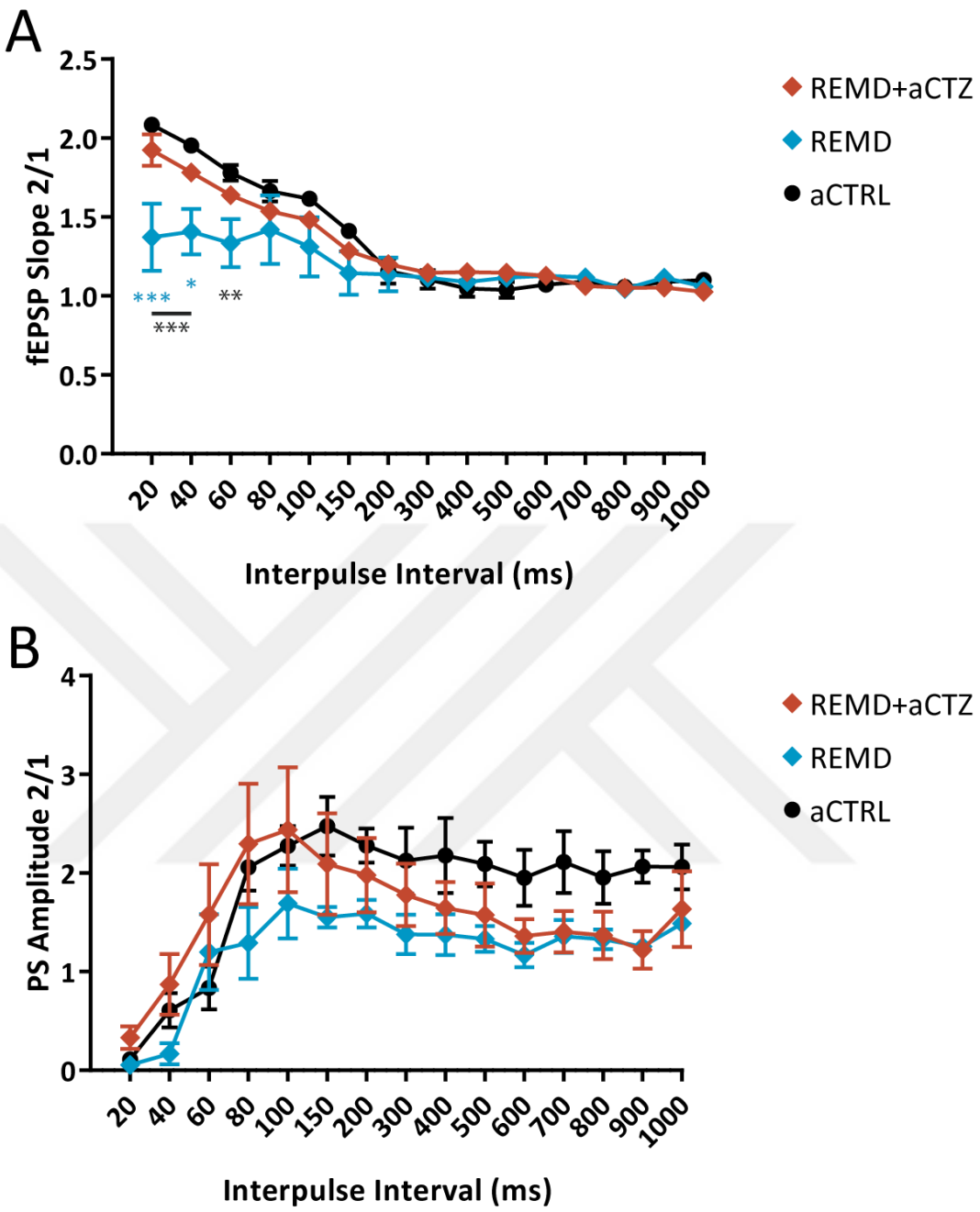

Figure 4.24. Paired-pulse recordings from CA1 strata radiatum and pyramidale of REM sleep deprivation groups. A) REM deprivation decreased paired-pulse facilitation in the shorter IPI durations in stratum radiatum. Cetirizine treatment restored the facilitation to control levels. (Two-way ANOVA treatment effect $F(2,225)=15.83$, $p \leq 0.001$; post-hoc Sidak's multiple comparisons test) **B)** Paired-pulse recordings in stratum pyramidale elicited no statistically significant change between the groups. (Post-hoc Sidak's multiple comparisons test) (72-hour REM sleep deprivation, 4-day acute treatment; REMD: REM sleep deprivation, REMD+aCTZ: REM sleep deprivation with acute cetirizine, aCTZ: acute cetirizine; CTZ at 5 mg/kg; *

p≤0.05, ** p≤0.01, *** p≤0.001; black * CTRL vs REMD, blue * REMD vs REMD+CTZ; n=6)

4.5.3. Long-Term Potentiation and Long-Term Depression Results of REM Sleep Deprivation Groups

REM sleep deprivation attenuated both induction and maintenance of LTP compared to that of the control group (Figure 4.25; p≤0.05 at 6 minutes and 126 minutes; n=6). Cetirizine almost completely restored REMD-induced blockade of LTP (p≤0.05 at 126 minutes; n=6). The normalized fEPSP slopes at the end of LTP recordings were 101.5±1.5% for the REM sleep deprivation group, 152.7±21.4% for the cetirizine treated REM sleep deprivation group and 163.6±15.8% for the control group (two-way ANOVA treatment effect F(2,675)=215, p≤0.001; n=6).

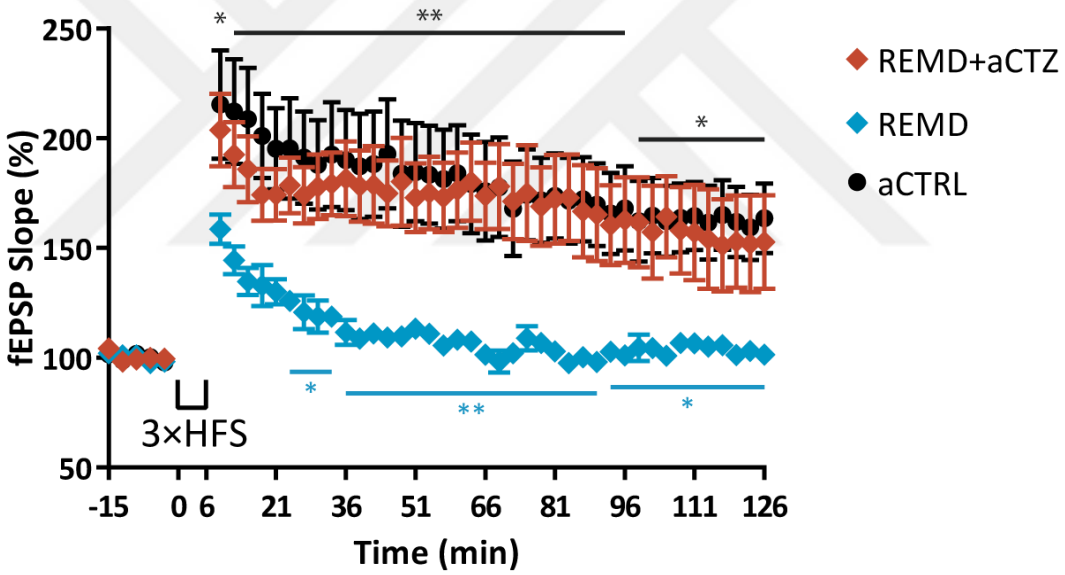

Figure 4.25. Long-term potentiation recordings from CA1 synapses for REM sleep deprivation groups. REM sleep deprivation suppressed induction of the LTP. Normalized fEPSP slopes for REMD were significantly below those of the controls during the entire recording periods after the high frequency stimuli. (Two-way ANOVA treatment effect F(2,675)=215, p≤0.001; post-hoc Tukey's multiple comparisons test) (72-hour REM sleep deprivation, 4-day acute treatment; REMD: REM sleep deprivation, REMD+aCTZ: REM sleep deprivation with acute cetirizine, aCTZ: acute cetirizine; CTZ at 5 mg/kg; * p≤0.05, ** p≤0.01; black * CTRL vs REMD, blue * REMD vs REMD+CTZ; HFS: 1 s 100 Hz high frequency stimulus trains with 3 minutes between each train; n=6)

Half of the animals in REM sleep deprivation were able to develop LTD as a result of LFS but the other half of the group failed to elicit the same response. Cetirizine treatment of REM sleep deprived animals further decreased the LTD induction but the results were not statistically significant (Figure 4.26). At the 75th minute, average normalized fEPSP slopes were 68.5±16.3% for REM sleep deprivation, 88.4±5.1 for REM sleep deprivation with cetirizine treatment and 65±6.4% for the control group (Two-way ANOVA treatment effect $F(2,375)=19.19$, $p \leq 0.001$; n=6).

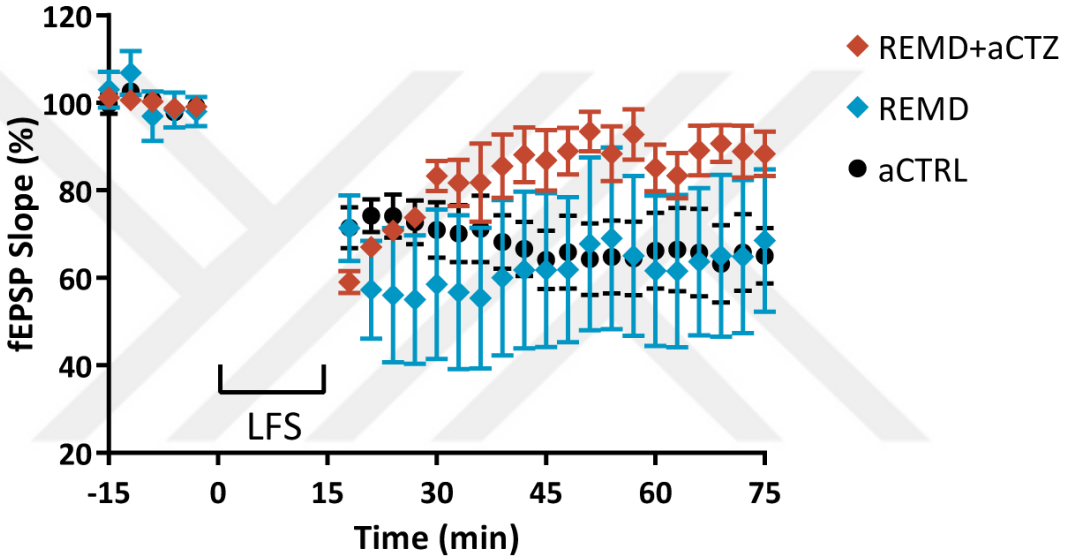

Figure 4.26. Long-term depression recordings from CA1 synapses of REM sleep deprivation groups. REM sleep deprivation failed to consistently elicit an LTD response. Cetirizine treatment of REM sleep deprivation group caused a further deterioration in LTD induction but the results were not statistically significant among groups. (Post-hoc Tukey's multiple comparisons test) (72-hour REM sleep deprivation, 4-day acute treatment; REMD: REM sleep deprivation, REMD+aCTZ: REM sleep deprivation with acute cetirizine, aCTZ: acute cetirizine; CTZ at 5 mg/kg; LFS: 15 minute 1 Hz low frequency paired-pulse stimulation with IPI of 40 ms at a total of 900 paired-pulses; n=6)

5. DISCUSSION

This study has demonstrated that: i) Acute treatment with cetirizine caused a minor leftward shift of I/O curves in stratum pyramidale. ii) Both cetirizine and diphenhydramine acute treatment reduced the facilitation in IPIs in the stratum radiatum and in the stratum pyramidale. iii) Acute cetirizine completely blocked the LTD while diphenhydramine had no effect. iv) Cetirizine acute treatment did not cause a change in locomotion, anxiety or mood. v) Acute cetirizine also did not alter the performance in learning and memory tasks. vi) Chronic cetirizine treatment increased excitability in stratum pyramidale. vii) Chronic diphenhydramine treatment reduced the facilitation in the stratum radiatum. viii) Chronic cetirizine treatment prevented the development of LTP. ix) LTD induction was blocked by chronic cetirizine administration. x) Chronic cetirizine treatment did not cause a significant change in locomotion, anxiety or mood behavioral tests. xi) Chronic cetirizine also failed to elicit a change in behavioral learning and memory tasks. xii) REM sleep deprivation significantly reduced excitability in both strata radiatum and pyramidale while reducing the maximal fEPSP slope in stratum radiatum. Acute cetirizine reverted fEPSP slope reduction in stratum radiatum but failed to restore the excitability in stratum pyramidale. xiii) Acute cetirizine treatment restored the reduction of PP facilitation caused by REM sleep deprivation. xiv) REM sleep deprivation abolished LTP and acute cetirizine treatment during the REM deprivation completely restored this effect. xv) REM sleep deprivation caused partial failure of LTD induction. Cetirizine treatment during REM sleep deprivation increased exacerbated LTD failure but the result was not statistically significant.

5.1. Acute Cetirizine but Not Diphenhydramine Treatment Decreased Firing Threshold of CA1 Pyramidal Neurons

Acute treatment with cetirizine did not alter the I/O curve in the stratum radiatum but caused a small leftward shift in stratum pyramidale, denoting an increase in neural excitability without a change in synaptic strength. This shows that

the increase in firing is likely caused by the reduction of firing threshold in CA1 neurons.

Diphenhydramine, another H1 antagonist, did not elicit any change in either CA3-CA1 synaptic transmission or CA1 pyramidal neuronal excitability, therefore the results obtained from the cetirizine group is unlikely to be caused by the H1 receptors.

Acute scopolamine, a muscarinic receptor antagonist, increased CA3-CA1 synaptic transmission or CA1 pyramidal neuronal excitability. Maximal fEPSP slopes were also increased by scopolamine in stratum radiatum. These results show that strong and selective muscarinic activity inhibition caused an increase in the synaptic strength and consequently facilitated postsynaptic neuronal firing. Diphenhydramine was unable to induce such a response.

5.2. Acute Treatment with All Drugs Tested Decreased PP Facilitation in Both Strata Radiatum and Pyramidale

In stratum radiatum, PP stimulations caused facilitation in the range of 20 to 150 ms IPIs in the control group and after 150 ms, neither facilitation nor depression is observed. Acute treatment with the drugs altered the short-term plasticity by reducing the facilitation. There are several reasons that might result in this alteration. First, an increase in the release probability may reduce the fEPSP2/1 ratio towards 1 through eliciting the near-maximal response in both first and second stimuli. Second, presynaptic autoreceptor activation causing a reduction in release can cause this decrease (87). Lastly, inhibitory systems including interneurons that are activated through stimulation via Schaffer collaterals can activate to reduce the fEPSP curve in the second stimulus.

In stratum pyramidale, PP stimulations initially cause a depression phase lasting up to 60 ms that is followed by a facilitation phase in the control animals. The initial depression is mainly caused by the GABAergic inhibitory interneuron recruitment that is activated through Schaffer collateral signaling. Postsynaptic

inhibitory $GABA_A$ receptors in GABAergic synapses are mainly found in the pyramidal neuron bodies and are activated shortly after a stimulus reaches the CA3-CA1 synapses. These receptors are Cl^- channels and hyperpolarize/clamp the cells to increase firing thresholds. In longer IPIs however, self-modulatory presynaptic G_i protein-coupled $GABA_B$ receptors in GABAergic cells activate to prevent further GABA release therefore reducing the GABAergic inhibition of the pyramidal cells (88).

Acute treatment with all three drugs tested did not affect the $GABA_A$-dominated depression phase but almost completely blocked the facilitation phase indicating that they do not bind to $GABA_A$ receptors or do not alter the initial GABA release. In facilitation phase however, they all prevent the increase in second population spike probably via blocking the facilitative changes in pyramidal cells as PS2/1 ratio reaches 1. Even though H1 receptor activity is shown to hyperpolarize pyramidal cells in hippocampus, H1 receptor activation also increases intracellular Ca^{++} concentration and PKC. Intracellular Ca^{++} facilitates depolarization of neurons through activation on membrane Na^+-Ca^{++} exchanger (53). H1 receptors also positively modulate the NMDA receptor activity (32).

These central functions of H1 receptor might explain the inhibition of synaptic facilitation in CA1 with H1 antagonists. Scopolamine treatment producing a very similar result can be explained by similar downstream G-protein messengers; hippocampus is dominated by M1 receptors which are $G_{q/11}$-coupled like histamine H1 receptor (89).

5.3. Acute Cetirizine Treatment Blocked LTD but Not LTP

Neither cetirizine nor diphenhydramine affected the development of LTP. Scopolamine has prevented the potentiation as was shown before (90).

LTD recordings showed a major effect for cetirizine; after 4 days of treatment, LTD was completely inhibited for the cetirizine group. Two distinct forms of LTD mechanisms coexist in CA1 pyramidal cells; NMDAR-dependent and mGluR-dependent. Both of these mechanisms are associated with reduced postsynaptic

membrane concentration of AMPARs (91,92). Furthermore, GSK3β is demonstrated to mediate an interaction between NMDAR-dependent LTP and NMDAR-dependent LTD. GSK3β inhibitors were shown to block the induction of LTD. LTP increases PI3K-AKT activity which in turn inhibits GSK3β and results in reduced LTD induction (93). It is also known that H1 activation positively modulates NMDAR and PI3K-AKT pathway (32). These mechanisms indicate H1 activation results in a decrease in LTD.

While this conclusion seems to contradict with the results of this study where cetirizine is found to block induction of LTD, there are a few caveats. First, most of the aforementioned interactions were shown in hippocampal slice experiments with external applications of histamine, histamine agonists or other mediators. This prevents the observation of the effects of histaminergic tonus and its modulation. Furthermore, antagonism of one receptor in an agonistic network between ligands, receptors and messengers might not result in the antagonism of the end result as there are many mechanisms that activate the same downstream pathways. Last, and probably most importantly, the LTP-blocking effect of cetirizine is likely not through the H1 histamine receptors but another mechanism as diphenhydramine failed to elicit a similar effect in LTD.

5.4. Chronic Cetirizine and Diphenhydramine Treatment Did Not Alter the CA1 Synaptic Transmission Properties

Chronic treatment with cetirizine and diphenhydramine caused no significant changes in I/O curves in either strata radiatum or pyramidale. Scopolamine's facilitating effects on synaptic transmission and postsynaptic neuronal recruitment was lost with a longer duration of treatment. This might be explained with a compensatory change in the number of muscarinic receptors.

In stratum radiatum, PP facilitation phase was still inhibited by scopolamine and diphenhydramine up to 80 ms but not cetirizine. The inhibition was not as prominent as it was in acute treatment groups where there was a significant inhibition up to 150 ms. Scopolamine is a muscarinic antagonist and

diphenhydramine effectively inhibits muscarinic receptors while cetirizine is a weak inhibitor at these sites (94). Therefore, it can be construed that chronic treatment caused tolerance or change in number of receptors especially in the cell soma as a result of chronic muscarinic blockage. PP curves of drug groups in stratum pyramidale were not statistically different than the control group, supporting the idea of a change in receptor activity or number.

5.5. Chronic Cetirizine and Scopolamine Treatment Inhibited Long-Term Plasticity

Chronic treatment with cetirizine inhibited the induction of LTP unlike acute treatment where it had no effects in the maintenance of LTP. While diphenhydramine average slopes were not significantly different than the control group, the LTP curve for the diphenhydramine was still in decline at the end of 120 minutes of post-induction. Chronic scopolamine treatment still prevented LTP induction.

H1 receptor is known to increase intracellular Ca^{++} levels and PKC activity which are secondary messengers required for the induction of LTP (53). H1 receptor knock-out mice was shown to have impaired place and reversal learning and impaired long-term memory retention performance. H1 deficiency also causes reduced neurogenesis (95). Chronic centrally active H1 antagonist use can have similar effects. Even though cetirizine does not penetrate the blood brain barrier as well as the first generation antihistamines, its R- enantiomer is less readily pumped out of the central nervous system by the P-glycoprotein and is less selective for the H1 receptor. Furthermore, cetirizine is also shown to cause sedation and affect the learning and memory processes in humans (54,96). Therefore, it is possible that long-term antihistamine administration results in disruptive effects on synaptic plasticity.

After chronic treatment, LTD was still inhibited by cetirizine similarly to the acute treatment. The mechanism involving the blockage of LTD with cetirizine did not develop a tolerance when the treatment duration was increased. Scopolamine chronic treatment was also able to significantly suppress LTD induction. To our

knowledge, this effect of scopolamine was also not previously reported in the literature.

5.6. Behavioral Experiments with 5 Mg/Kg Cetirizine Did Not Elicit Any Significant Effects.

Throughout an acute or a chronic cetirizine treatment at 5 mg/kg, animals were examined with several behavioral paradigms. Open field arena measured locomotion and anxiety through the distance travelled in the center. Elevated plus maze was used as a measurement of stress and anxiety. Forced swim test was used to assess antidepressant effects. Passive avoidance response was used to test amygdala-associated hippocampal spatial learning and memory while Morris water maze was used to test frontal cortex-associated hippocampal spatial learning and memory. Morris water maze reversal learning paradigm was used as a behavioral assay of LTD (85). Neither acute or chronic treatment with cetirizine yielded significant results in behavioral experiments.

5.7. Cetirizine Improved Synaptic Transmission Disrupted by REMD in Stratum Radiatum but Not in Stratum Pyramidale

REM sleep deprivation is known to inhibit electrophysiological parameters and prevent induction of LTP (83). During this study, 72 hours of REM sleep deprivation decreased both synaptic strength and neuronal recruitment suggested by the rightward shifts in both strata radiatum and pyramidale I/O curves. Cetirizine was able to reverse this effect in stratum radiatum but not in stratum pyramidale.

REM sleep deprivation also inhibited the short-term facilitation in stratum radiatum in facilitation phase of PP recordings. Cetirizine treatment was able to undo this effect.

5.8. Cetirizine Prevented Inhibition of LTP by REM Sleep Deprivation

REM sleep deprivation inhibited LTP and cetirizine prevented REM sleep deprivation-induced LTP inhibition. Histamine release and tonus have a sensitive balance in the central nervous system regulated by both several other neurotransmitters and its own signaling through H3 receptors. Histamine concentration also shows circadian rhythmicity with peak concentrations observed during wakefulness and lowest concentrations observed during REM sleep (41,97). REM sleep deprivation upregulates hypothalamic histidine decarboxylase concentration and activity, preventing the circadian downregulation of histaminergic tonus in the brain. This causes an increase in cerebral histamine concentration during REM sleep deprivation (98). In this study, daily cetirizine administration throughout the REM sleep deprivation period may have reduced the H1 histaminergic activity to a more physiological level, therefore prevented the disruptions seen in I/O, PP and LTP recordings. This preventive effect of cetirizine shows that H1 receptor activity plays a major role in REM sleep deprivation-mediated inhibition of hippocampal plasticity.

In this study, REM sleep deprivation resulted in a decrease in LTD in half of the animals while not causing any changes in LTD induction in the others. However, when REM sleep deprived animals were treated with CTZ, LTD could not be induced in any of the animals but this result failed to yield statistical significance. In literature, several articles reported facilitation of LTD (99,100) and others reported inhibition of LTD after sleep deprivation (101,102). These differences might be explained by the use of different sleep deprivation models, different sleep deprivation times and durations, different electrophysiological protocols, different LTD induction parameters, different species or subspecies used in the experiments and use of *in vivo* or *in vitro* methods in these studies (102,103). The conflicting results in the literature is directly observed in our study results. In this study, inconsistence of LTD induction in REM sleep deprivation group yielded a high variance. Also, even though

it was statistically not significant, cetirizine-treated REM deprivation group had reduced LTD at the end of the recordings compared to the control group averages.

5.9. Conclusion

This study showed that central nervous system histaminergic tonus is affected by short term centrally active antihistamine use regardless of generation. This in turn affects important higher brain functions such as learning and memory. While it is known that chronic use provides a tolerance for sedative side effects of these drugs, other central disruptions can still continue, or even worsen. However, in sleep deprived individuals with already disrupted central histamine cycles, the use of antihistamines can provide both somnolence through the sedative side effects and reduction of abnormally high histaminergic tonus due to sleep deprivation.

The major shortcoming of the study is the lack of treatment groups in some protocols. The effects of scopolamine and diphenhydramine should also be examined in REM sleep deprivation as well as in behavioral experiments. Although both acute and chronic electrophysiology experiments with 20 mg/kg cetirizine were completed, electrophysiological effects of cetirizine at 5 mg/kg was not investigated. Furthermore, the possible effects of cetirizine on behavioral experiments at 20 mg/kg dose should be observed. Further experimental models can also be helpful in assessing the effects of antihistamines such as molecular methods to evaluate enzyme and receptor levels in different groups and new behavioral methods to assess hippocampal spatial learning and memory with applicable reversal paradigms (104).

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APPENDICES

Appendix 1: Hayvan Deneyleri Yerel Etik Kurulu Kararı

T.C.
HACETTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu

Sayı : 52338575 – 79

HAYVAN DENEYLERİ YEREL ETİK KURUL KARARI

TOPLANTI TARİHİ	:	20.06.2017 (SALI)
TOPLANTI SAYISI	:	2017/06
DOSYA KAYIT NUMARASI	:	2015/99
KARAR NUMARASI	:	2015/99 -06
ARAŞTIRMA YÜRÜTÜCÜSÜ	:	Prof. Dr. M. Yıldırım SARA
HAYVAN DENEYLERİNDEN SORUMLU ARAŞTIRMACI	:	Prof. Dr. M. Yıldırım SARA, Dr. Sadık Taşkın TAŞ, Barış ALTEN
YARDIMCI ARAŞTIRMACILAR	:	Uzm. Ecz. Arwa Al BAYRAKDAR, Dr. Metin YEŞİLTEPE
ONAYLANAN HAYVAN TÜRÜ ve SAYISI	:	***102 Adet Wistar Sıçan (3 Ay)***

Kurulumuzun 15.12.2015 tarihli toplantısında onaylanmış olan 2015/99 kayıt numaralı ***"Antihistaminik Sıçan REM Deprivasyonu Modelinde Öğrenme ve Belleğe Etkileri"*** başlıklı projeniz için vermiş olduğunuz 24.05.2017 tarihli başlık değişikliği ve araştırmacı revizyonu dilekçeniz, Kurulumuzun 20.06.2017 tarihli toplantısında değerlendirilmiş olup, Uzm. Biyolog Canan KURŞUNGÖZ' ün projeden ayrılması ve Arwa Al BERKDAR ve Dr. Metin YEŞİLTEPE' nin yardımcı araştırmacı olarak projeye dahil edilmesi ve projenin isminin ***"Antihistaminik İlaçların Akut ve Kronik Kullanımında Öğrenme-Bellek ve Uykusuzluk Üzerine Etkileri*** olarak değiştirilmesi **uygun bulunmuş** ve kayıtlarımıza eklenmiştir. Kurul üyesi Prof. Dr. M. Yıldırım SARA çıkar çatışması nedeni ile tartışma ve oylamaya katılmamıştır.

Prof. Dr. Sema ÇALIŞ
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2. Education

Graduate: 2011 – 2018, Hacettepe University Institute of Medical Sciences Medical Pharmacology PhD Program

Undergraduate: 2003 – 2010, Hacettepe University Faculty of Medicine Doctor of Medicine Program (English Language)

3. Occupational Experience

Researcher: 2011 – 2018, Scientific research experience in Hacettepe University Department of Medical Pharmacology Laboratories

4. Scientific Activities

4.1. Research articles in international journals

2017: Aksoz BE, Ucar G, Tas ST, Aksoz E, Yelekci K, Erikci A, Sara Y, Iskit, AB. New hMAO-A Inhibitors with Potential Antidepressant Activity: Design, Synthesis, Biological Screening and Evaluation of Pharmacological Activity. Comb Chem High Throughput Screen. 2017;20.

2017: Kursungoz C, Taş ST, Sargon MF, Sara Y, Ortaç B. Toxicity of internalized laser generated pure silver nanoparticles to the isolated rat hippocampus cells. Toxicol Ind Health. 2017 Jul;33(7):555-563.

2015: Cil O, Esteva-Font C, Tas ST, Su T, Lee S, Anderson MO, Ertunc M, Verkman AS. Salt-sparing diuretic action of a water-soluble urea analog inhibitor of urea transporters UT-A and UT-B in rats. Kidney Int. 2015 Aug;88(2):311-20.

2015: Soyer T, Kalkisim S, Yalcin S, Muderrisoglu A, Tas ST, Tanyel FC, Ertunc M, Sara Y. The effects of acute tension increase on rat esophageal muscle contractions: An in vitro study. J Pediatr Surg. 2015 Oct;50(10):1691-4.

2013: Yalcin S, Ertunc M, Ardicli B, Kabakus IM, Tas ST, Sara Y, Onur R, Karnak I. Ureterovesical junction obstruction causes increment in smooth muscle contractility, and cholinergic and adrenergic activity in distal ureter of rabbits. J Pediatr Surg. 2013;48(9):1954-1961.

4.2. Poster Presentations

2017: Alten B, Yesiltepe M, Al-Bayrakdar A, Tas ST, Kursungoz C, Martinez A, Sara Y. TDZD-8 attenuates insulin resistance in high fructose corn syrup fed adolescent rats. 85th European Atherosclerosis Society Congress, Prague, Czech Republic.

2016: Tas ST, Yesiltepe M, Sara Y. Diphenhydramine did not improve sleep deprivation-induced cognitive impairment. 7th European Congress of Pharmacology, Istanbul, Turkey.

2016: Tas ST, Kursungoz C, Alten B, Ortac B, Sargon MF, Sara Y. Three months of laser-generated nanoparticle exposure did not alter

cognition and mood in rats. 2016 International Nanotoxicology Congress, Boston, MA, USA.

2016: Kursungoz C, Tas ST, Alten B, Sargon MF, Ortac B, Sara Y. Localization and distribution of nanoparticles generated during real-time laser material processing in rats. 2016 International Nanotoxicology Congress, Boston, MA, USA.

2013: Tas ST, Kabakus IM, Sara Y. REM uykusu bozuklugunda bellek ve duygu durum degisiklikleri uzerine kronik essitalopram tedavisinin etkileri (The effects of chronic escitalopram treatment on memory and mood changes in REM sleep impairment). 11. Ulusal Sinirbilim Kongresi (11th National Neuroscience Congress), Izmir, Turkey.

2013: Kabakus IM, Tas ST, Sara Y. Kronik essitalopram tedavisi REM yoksunlugunda gelisen uzun donem noroplastisite bozuklugunu engeller (Chronic escitalopram treatment prevents the neuroplasticity impairment that develops in REM deprivation). 11. Ulusal Sinirbilim Kongresi (11th National Neuroscience Congress), Izmir, Turkey.

4.3. Projects

2017 – ... Sara Y (project owner), Tas ST (researcher), Alten B (researcher), Al Bayrakdar A (Researcher), Yesiltepe M (researcher). Antihistaminik ilaçların akut ve kronik kullanımında ögrenme – bellek ve uykusuzluk üzerine etkileri (The effects of antihistaminic drugs on learning – memory and insomnia in acute and chronic use). Hacettepe University Scientific Research Projects Coordination Unit, Project No: THD-2017-16553.

2015 – …: Gumusel B (project owner), Ozkan MH (researcher), Tas ST (researcher). Joint curriculum development in health technology assessment. Erasmus+ KA2 Strategic Partnership Project.

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