Elucidating the Cognitive Deficits following Doxorubicin treatment

by

Ahmad Hamad Alhowail

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 13, 2014

Keywords: Doxorubicin, Chemobrain, long-term potentiation, Cognitive deficits, AMPA receptors

Approved by

Vishnu Suppiramaniam, Chair, Associate Professor of Drug Discovery and Development
Robert D. Arnold, Co-chair, Associate Professor of Drug Discovery and Development
Murali Dhanasekaran, Associate Professor of Drug Discovery and Development
Rajesh Amin, Assistant Professor of Drug Discovery and Development

Abstract

Chemotherapeutic drugs are effective in the treatment of various types of tumors; however, the optimal clinical effectiveness is limited due to secondary effects including cognitive impairment, also known as “chemobrain”, which refers to a phenomenon in which cancer survivors exhibit cognitive impairment following chemotherapy. The present study investigated the effects of doxorubicin on cognitive impairment through its effects on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-glutamate receptor expression and function. To achieve this aim, long-term potentiation (LTP), a cellular model of memory and the function as well as expression of AMPA receptors were assessed. Results indicate that doxorubicin treated animals showed impaired LTP and AMPA receptor channel function. In addition, AMPAR subunit GluR1, brain-derived neurotrophic factor (BDNF), and α-stargazin expression were significantly decreased, whereas GluR2 subunit expression significantly increased in doxorubicin treated animals compared to controls. Therefore, we conclude that doxorubicin induces cognitive impairment by modulating glutamatergic system.

Acknowledgments

I would like to thank my advisor, Dr. Vishnu Suppiramaniam, for his encouragement, guidance, and dedication to my project. Dr. Suppiramaniam's intelligence and kindness were the real motives for me to be interested in electrophysiology and pharmacology. I am grateful to Dr. Suppiramaniam, who imparted knowledge and critical thinking skills to me as well as resolving obstacles and problems that I have encountered during my study. My special thanks are extended to Dr. Robert Arnold for providing the doxorubicin treated animals. I also thank the rest of my committee members Dr. Muralikrishnan Dhanasekaran and Dr. Rajesh Amin for their understanding and support besides many stimulating discussions. I am in debt to those people who made this work possible with their enthusiasm, support, and help. I could not say enough to thank Dr. Manal Buabeid, Mr. Subhrajit Bhattacharya, Mr. Dwipayan Bhattacharya, and Ms. Jenna Bloemer, for their talents and skills in helping me with the experimental procedures in our lab. I would like to extend my gratefulness and appreciation to all the faculty members, staff and fellow students at the Department of Drug Discovery and Development in the Harrison School of Pharmacy.

Wholehearted thanks to my wonderful parents and my wife for their love, encouragement, inspiration, that I would not have accomplished my graduate studies without them. My parents and wife were behind all the success I have achieved in my life.

At last but not final, I thank my sponsor, Qassim University, College of Pharmacy, for granting me the scholarship to pursue my graduate studies. Also, I appreciate the cooperation and supervision of Saudi Arabia Cultural Mission in The Royal Embassy of Saudi Arabia, USA, and especially, Dr. Samar Alsaggaf, Director of the Department of Medical and Health Science, and

Dr. Abdulaziz Aleisa, my academic advisor and for their understanding and prompt response to all of my requests and questions.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ACSF	Artificial cerebrospinal fluid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
BBB	Blood-brain barrier
BSA	Bovine Serum Albumin
CA	Cornu Ammonis
CaMKII	Ca2+/calmodulin-dependent protein kinase II
DG	Dentate Gyrus
EAATs	Excitatory Amino Acid Transporters
LTP	long-term potentiation
NMDAR	N-methyl-D-aspartate receptor
PAESe	Phenyl-2-aminoethyl selenide
SAP102	Synapse-associated protein 102

CHAPTER I

Introduction and Literature Review

1.1. Introduction: Chemobrain

The term "chemobrain," also known as chemofog, is a phenomenon in which cancer patients exhibit cognitive impairment following chemotherapy. Cognitive impairment is observed in more than 75% of cancer patients exposed to chemotherapy (Jenkins et al., 2006) (Schagen et al., 1999), and it is persistent in 17-34% of cancer survivors. Although chemobrain related memory loss has been noted since 1970s, research in this field had not received considerable attention until the mid-1990s (Ahles et al., 2007). In recent years, studies have revealed causal relations between chemotherapy and memory loss, but the underlying mechanism still remain largely unfound.

1.2. Chemotherapy and Chemobrain:

Chemotherapy has been used in cancer treatment since the early 20th century (DeVita & Chu, 2008). Chemotherapy regimens aim to eliminate tumors in most cases by reducing the rate of cellular proliferation (Chabner & Roberts, 2005). Chemotherapeutic agents are divided into several groups based on dynamics such as mechanism of action and chemical structures. Alkylating agents, such as cyclophosphamide, directly damage the DNA structure. Antimetabolites, such as 5-fluorouracil (5-FU) and methotrexate, are a class of drugs that interfere with DNA and RNA synthesis. Anthracyclines, such as doxorubicin, are anti-tumor antibiotics that interfere with enzymes involved in DNA replication. Hormone therapy drugs, such as tamoxifen and somatostatin, are another class of drugs that regulate and prevent cancer proliferation by inhibiting hormone receptors or stymying cell proliferation. Anti-angiogenesis

agents, such as Platelet Factor 4-DLR drugs, inhibit blood vessel growth and are administered after chemotherapy treatment to aid remission.

Chemotherapeutic agents are effective in treatment of various types of tumors. However, the optimal clinical effectiveness is limited due to secondary effects including cognitive dysfunction. For instance, patients who had received treatment for breast cancer suffered varying degrees of cognitive dysfunction (Jenkins et al., 2006). Although the mechanism of cognitive dysfunction is unknown for most of these drugs, some chemotherapeutic agents may trigger cognitive impairment by their unique ability to access the brain by crossing the blood-brain barrier (BBB) (Janelsins et al., 2010). The BBB is primarily a physiological obstruction to insulate the brain from harmful toxins that are carried in the blood stream. However, some agents are incapable of penetrating the BBB but can still induce chemobrain (Christie et al., 2012).

The hippocampus is the site of complex and novel memory formation. It is the part of the brain and limbic system that is located in the medial temporal lobe of the cerebral cortex **(Figure 1.1)**. It is one of the most well studied structures of brain. The hippocampus is hypothesized to play a major role in memory formation and storage. This has been proven by creating lesions in the hippocampus, resulting in anterograde amnesia. The adult human brain contains approximately 100 billion neurons. Each neuron is connected to many other neurons with junctions known as synapses. During learning, synaptic communication becomes stronger, which helps to encode memories. In the contrary, impaired synaptic communication leads to deficits in learning and memory (Davis et al., 1992).

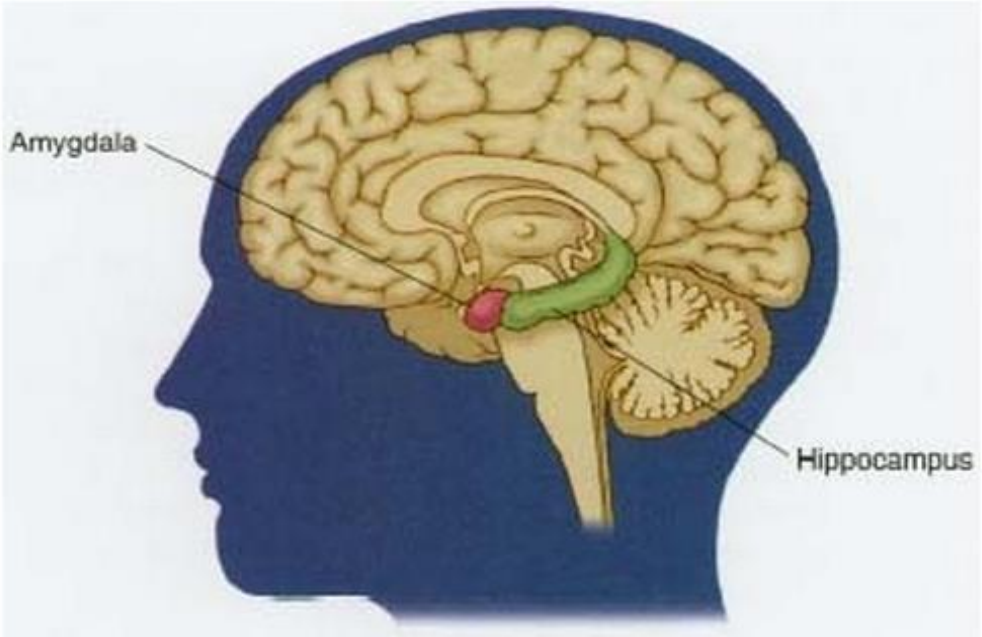


Figure 1.1: the localization of hippocampus in human brain. Adapted from *Human Diseases and Conditions*. *(n.d.)*. *Retrieved December 4, 2014, from http://www.humanillnesses.com/Behavioral-Health-Fe-Mu/Memory.html*

The hippocampus is divided into four sub-regions known as Cornus Ammonis (CA) areas **(Figure 1.2)**. CA is comprised of three sub-regions namely CA1, CA2, and CA3. Neighboring CA, there is the Dentate Gyrus (DG), main region of neurogenesis in adult brain and also important for new memory formation (Ref.). The hippocampus consists of three major pathways. The Schaffer Collaterals pathway connecting CA3 and CA1 areas, and the Mossy fiber pathway connecting CA3 area and DG are two of the tracts. The third pathway (perforant pathway) provides inputs to DG from entorhinal cortex. The major excitatory neurotransmitter in these areas is glutamate and it carries out its effects through a family of receptors known as glutamate receptors.

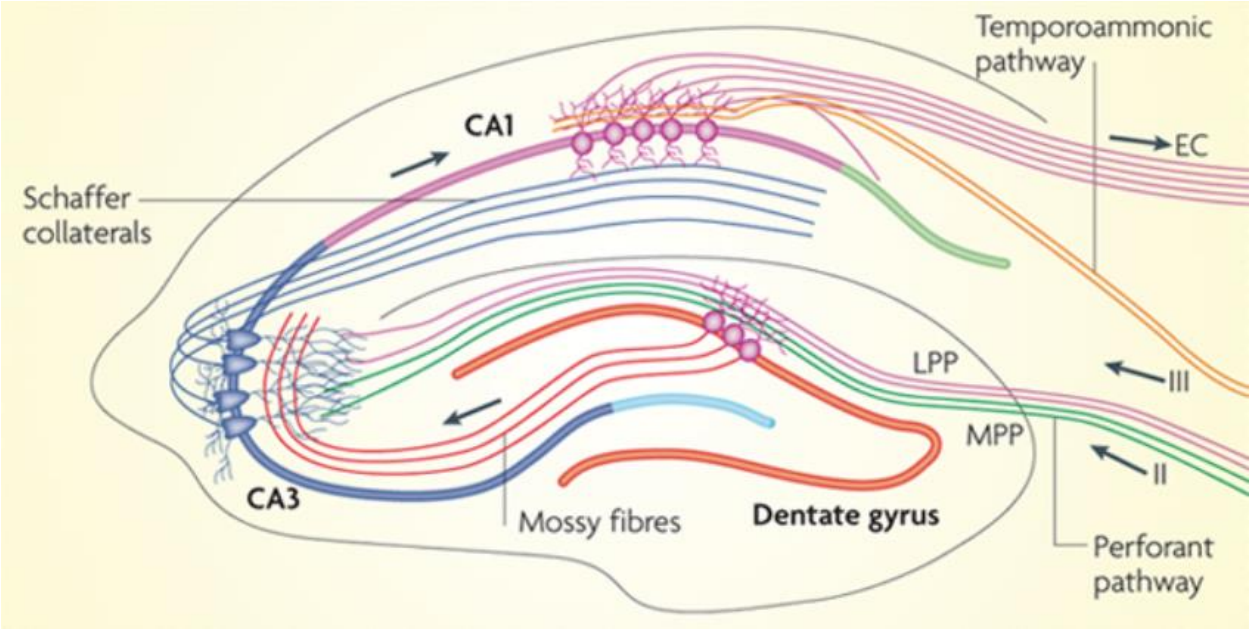


Figure 1.2: The structure of the hippocampus in the brain and the three traditional excitatory synaptic pathways. Adapted from *Wei Deng et al*., *Nature Reviews Neuroscience 11, 339-350 (May 2010)*.

1.2.1. The glutamatergic system:

The glutamatergic system is the major excitatory neurotransmitter system in the vertebrate brain. Glutamate by itself is a non-essential amino acid that does not cross the BBB. It is generally synthesized in the neurons from the readily available precursor, glutamine. Glutamate from synaptic cleft is taken by the Excitatory Amino Acid Transporters (EAATs). EAATs are located in presynaptic terminals or glial cells. Glutamate in glial cells is converted to glutamine by glutamine synthetase and then transported outside to presynaptic terminals by other transporters. Glutamine is converted to glutamate in the presynaptic neurons by glutaminase (Daikhin & Yudkoff, 2000). Finally, glutamate is packaged into the synaptic vesicles by vascular glutamate transporters (VGLUT) until the time of release (Montana et al., 2004) **(Figure 1.3)**.

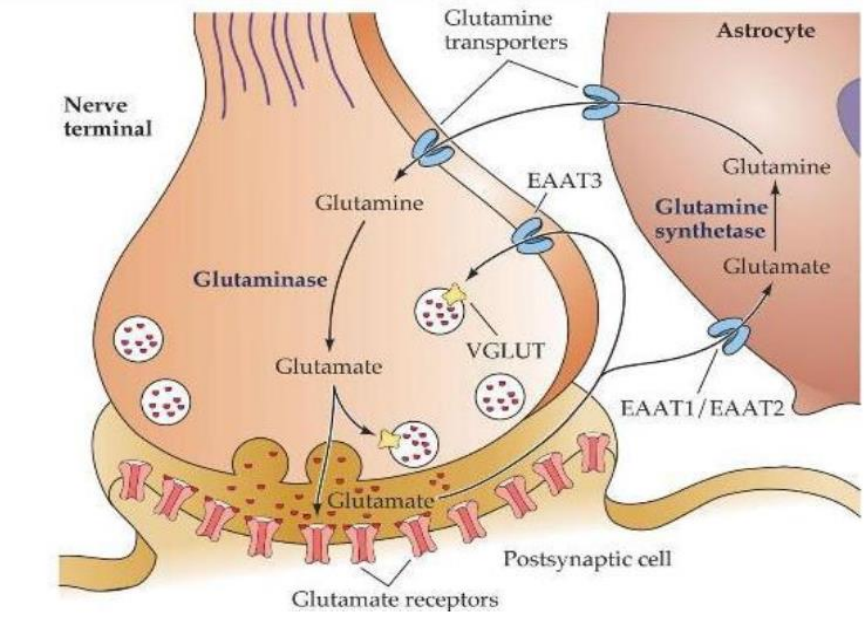


Figure 1.3: the glutamate-glutamine cycle. Adapted from *Neuroscience*, *Fifth Edition by Dale Purves*.

Glutamatergic receptors are mainly composed of two main sub-types AMPARs and NMDARs. A third sub-type of glutamatergic receptors exist which is known as the kainate receptors. Classically, depending of ligand binding and signal transduction glutamate receptors are divided into ionotropic and metabotropic sub-classes. In our discussion, we mainly focus on ionotropic glutamate receptors **(Figure 1.4)**. AMPARs and NMDARs are the major inotropic receptors that play roles in complex and contextual learning and memory processes (Rao et al., 2007). When presynaptic neurons release glutamate into the synaptic cleft, it binds to AMPARs and elicits an influx of Na^{+} causing depolarization of the postsynaptic neurons. At resting potential (-70 mV), NMDARs are blocked by Mg^{2+}. Depolarization of the neuron caused by opening of AMPARs removes this blockade allowing influx of Ca^{2+} and Na+. Ca^{2+} is known to play an important role in downstream signaling by acting on Calcium Calmodulin-dependent Kinase II (CaMKII) (Fink & Meyer, 2002). This leads to a complex signaling cascade down stream of AMPA and NMDARs involving other kinases such as Protein Kinase A (PKA) and Protein Kinase B (PKB). This intern affects expression and trafficking of AMPARs and NMDARs leading to altered synaptic strength (Luscher et al., 2012) (**Figure 1.5**). Several studies have further suggested that changes in AMPARs or NMDARs expression or function results in memory deficits (Rao et al., 2007).

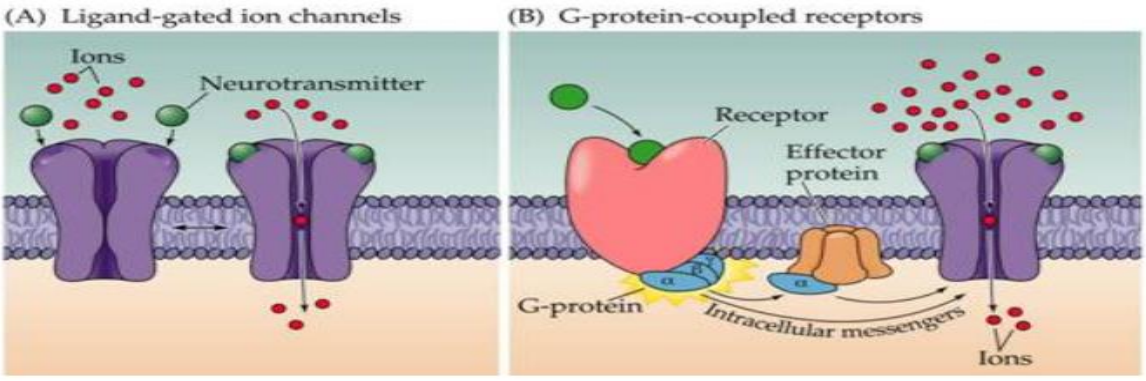


Figure 1.4: Two receptors type. Adapted from *Neuroscience, Fifth Edition by Dale Purves*.

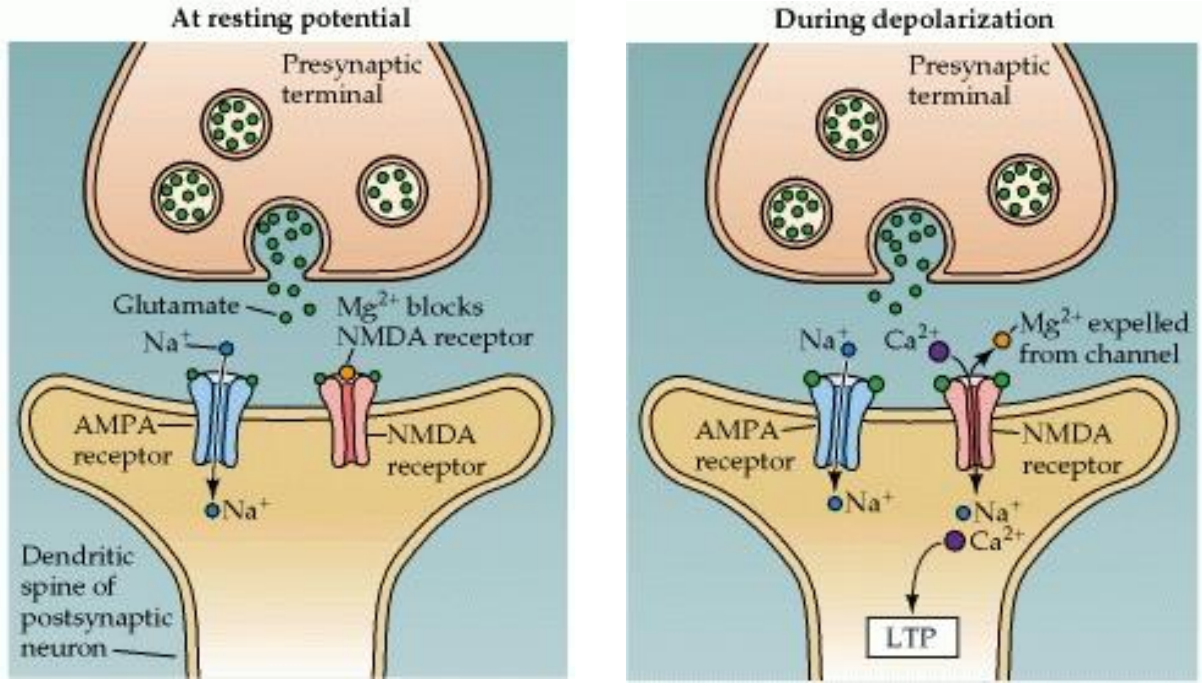


Figure 1.5: Glutamate binding to AMPARs allows Na^+ to influx causing postsynaptic membrane depolarization. Adapted from *Neuroscience*, *Fifth Edition by Dale Purves*.

1.2.2. AMPA receptor:

Action potential propagates from presynaptic neuron to postsynaptic neuron across the synapse. Glutamate released from presynaptic neurons into the synaptic cleft binds with AMPARs and NMDARs as discussed above (Dingledine, Borges, Bowie, & Traynelis, 1999). AMPARs and NMDARs are the major receptors in the postsynaptic neurons for the glutamate ligand released. AMPARs are constituted of GluA1 through GluA4 while NMDARs are made up of GluN1, GluN2A, GluN2B and other subunits.

AMPARs are also classified into flip and flop AMPARs depending on mRNA splicing. The major difference between these two isoforms is in the location of the N and C- terminus. Each AMPAR subunits has distinct functional and pharmacological contributions. Stoichiometric

combinations of GluAs and GluNs are responsible for the variety of effects mediated by AMPARs and NMDARs signaling. For instance, heteromeric channels comprising of GluA1, 3, and 4 subunits are permeable to Ca2+ entry. In contrast, homomeric GluA2 subunits prevent Ca2+ permeability of the receptor complex. This gives rise to edited and un-edited version of GluA2 AMPARs (Glutamine/ Arginine replacement also known as Q/R editing) (Dingledine et al., 1999; Hollmann et al., 1991; Hume et al., 1991) **(Figure 1.6).**

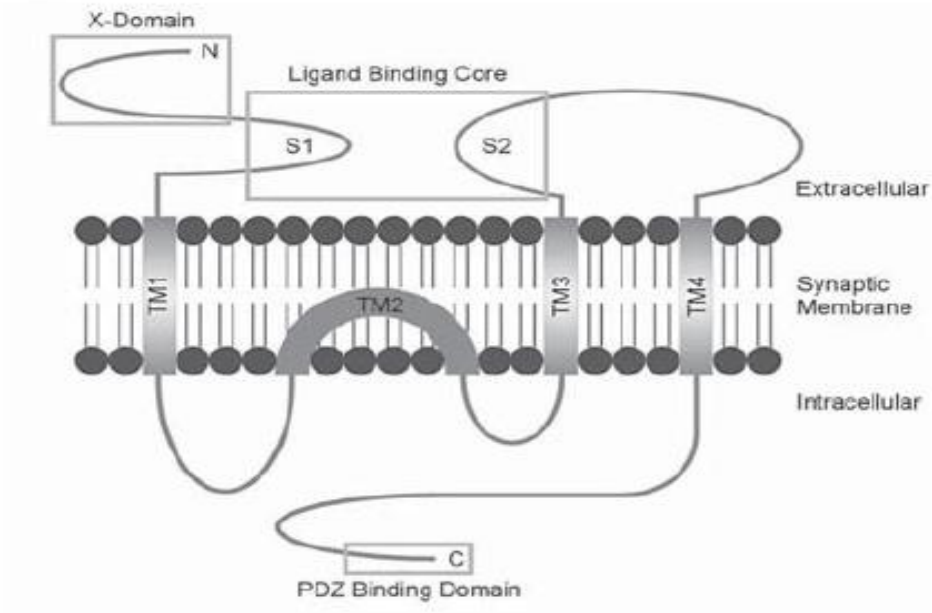


Figure 1.6: AMPA receptor structure and the different subunits inserted in transmembrane. Adapted from Jiang J., Suppiramaniam V., Wooten M. (2006). Posttranslational modifications and receptor associated proteins in AMPA receptor trafficking and synaptic plasticity. *Neurosignals* 15: 266-282.

Studies have shown that learning process enhances long lasting changes in the synaptic strength of glutamatergic synapses (Mayford et al., 2012). These changes are known as "synaptic plasticity", which is necessary for maintaining learning and memory. AMPA receptors are primary transducer of fast synaptic excitatory neurotransmission in the brain (Boehm et al.,

2005). Therefore, changes in postsynaptic AMPA receptors result in changes in synaptic plasticity and strength (Derkach et al., 2007).

In addition, NMDARs, another major player in learning and memory, play vital roles in AMPARs subunits trafficking into and out of the synapse (Collingridge et al., 2004). For example, CaMKII is a kinase molecule downstream of NMDARs. CaMKII activated by Ca^{2+} influx through NMDARs. Both phosphorylated CaMKII and protein kinase C (PKC) phosphorylate Ser^{831} residue on AMPA-GluA1 helping in trafficking of the receptors into synaptic membrane. It also increases receptor single channel conductance of the receptors discussed above. On other hand, PKA can phosphorylate Ser^{845} residue of GluR1-AMPARs resulting in increasing opening probability (Boehm et al., 2005; Bredt et al., 2003; Collingridge et al., 2004).

1.3. Pathophysiology of chemobrain:

Although the precise etiology of chemobrain is not yet elucidated, recent studies have provided some realistic insights into possible mechanisms **(Figure 1.7)**. These findings include disruption of hippocampal cell proliferation and neurogenesis (Briones & Woods, 2011; Christie et al., 2012), hormonal changes (Usuki et al., 1998), epigenetic alterations (Briones & Woods, 2011), increased oxidative stress and reactive oxygen species (ROS) production (Inagaki et al., 2007), chronic increases in inflammation (Inagaki et al., 2007), white matter disruption (Myers, Pierce, & Pazdernik, 2008) and a decrease in long-term potentiation (LTP) (G. D. Lee et al., 2006) during and after chemotherapy.

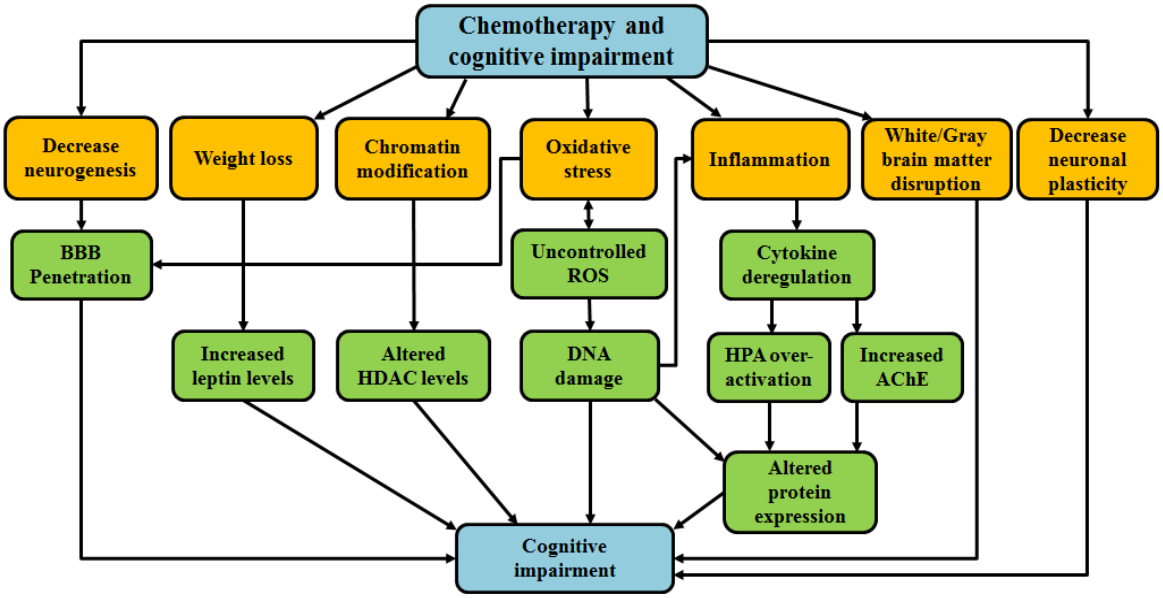


Figure 1.7: Mechanisms of chemotherapy-induced cognitive impairment

Furthermore, pharmacokinetic studies indicate that neurotoxicity from chemotherapeutic agents, such as doxorubicin, is dose dependent and in some cases is associated with BBB penetration (Al-Abd, et al., 2009; Boaziz, et al., 1991). Therefore, understanding the mechanisms that underlie chemobrain will help to develop preventive strategies and to alleviate the adverse effects of chemotherapy treatment.

1.4. Chemobrain and neurogenesis:

Numerous studies have speculated that anti-cancer drugs can reduce neurogenesis, possibly leading to cognitive impairment. Although treatment with the chemotherapeutic drugs Cyclophosphamide, Methotrexate and Fluorouracil (CMF) results in decreased hippocampal neurogenesis by 20% compared to control animals (Briones et al., 2011), the exact mechanism is not yet elucidated. It has been hypothesized that chemotherapeutic drugs that can cross the BBB cause a reduction in neurogenesis and lead to cognitive impairment. However, these drugs such as cyclophosphamide and fluorouracil produce a reduction in neural cell proliferation similar to

those unable to cross the BBB including paclitaxel and doxorubicin (Janelsins et al., 2010). In spite of the similar effects, the mechanism of how chemotherapeutic drugs that are unable to cross BBB affect cognitive function is still not known. Moreover, recent data from animal studies suggest that minute amounts of non-BBB permeable chemotherapeutic agents can indeed cross the BBB, cause cell death, and reduce cell division in the brain (Ahles & Saykin, 2007). In 1998, plasma concentration of insulin-like growth factor 1 (IGF-1) was measured in breast cancer patients undergoing chemotherapy to correlate the levels of IGF-1 and neurogenesis. The study found that IGF-1 levels in serum dropped 10% compared to before receiving treatment; however, they rapidly returned to normal levels (Peyrat et al., 1998). Multiple studies demonstrate that IGF-1 administration with chemotherapy increases neurogenesis, which may be a potential therapeutic strategy for preventing chemobrain (Janelsins et al., 2010).

1.5. Chemobrain, Appetite, and Cognition:

An association between cognitive impairment, weight loss, and chemotherapy has been suggested (Briones et al., 2011; Janelsins et al., 2010). Chemotherapy can enhance pro-inflammatory cytokines such as Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6) (Goldberg et al., 2010) causing several symptoms including fever, fatigue, and a decrease in appetite (Myers et al., 2008). The experiment for examining a potential role of chemotherapy-caused weight loss was conducted on healthy animals in order to avoid the effects caused by cancer itself. Animals treated with cyclophosphamide and doxorubicin weighed 8.5% and 10% less respectively compared to control animals (Briones et al., 2011). These results illustrate how chemotherapeutic agents are non-specific to tumor cells. Therefore, they cause cell apoptosis and reduced proliferation not only in malignant but also in normal cells.

Leptin, a hormone released by adipose tissue in the body that regulates food intake, has an essential impact in facilitating learning and memory (Oomura et al., 2006). Low levels of leptin lead to cognitive deficits (Farr et al., 2006). In contrast, high levels of leptin have been found to decrease cognitive function as well (Gisou et al., 2009). Cancer patients who received chemotherapy treatment (cyclophosphamide and doxorubicin) have shown increased serum levels of leptin (Usuki et al., 1998). These findings are particularly exciting as high serum leptin levels lead to decreased cognitive function and appetite, the two major side effects of chemotherapy. Since chemotherapy has been identified to increase leptin expression in addition to causing these adverse effects, a leptin-containing pathway may play a significant role in the chemobrain mechanism. Further research into why and how chemotherapy increases leptin levels could unveil much about chemobrain and lead to the discovery of potential corrective strategies.

1.6. Chemobrain and chromatin modification:

Eukaryotic DNA is tightly packaged within the nucleus by folding into a structure called chromatin. Chromatin is primarily composed of four histones that compact DNA. Enzyme modifications of histones are important in transcription and replication of DNA (Kouzarides et al., 2007). Therefore, changes in the natural state of chromatin can negatively affect how genetic code is expressed. Chromatin modification caused by chemotherapy has been suggested as a possible mechanism of chemobrain. Chemobrain may result from a decrease in hippocampal cell neurogenesis. The decreased neurogenesis was explained by an increase in chromatin acetylation by 21% and a decrease of histone deacetylase (HDAC) by 37% in CMF-treated animals compared to saline-treated animals (Briones et. al., 2011). The result was unexpected, since acetylation is known to enhance transcription by neutralizing the positive charge of histone and unwinding the DNA. Similarly, HDAC class I inhibitors are correlated with an increase in

learning and memory (Levenson et al., 2004). However, other forms of HDAC do not share this correlation. For example, certain HDAC class II inhibitors are associated with a decrease in learning and memory, and evidence was found that higher levels of HDAC4 inhibition reduced synaptic plasticity, (Kim et al., 2012) which is the ability of a synapse to change the connection and strength between two neurons (Ho et al., 2011). Briones' study measured class I and II HDAC activity totally. Since these two classes of HDAC have contradicting effects on cognitive function, a total measurement of HDAC activity fails to differentiate how chemotherapy affects each class of HDAC and, therefore, cognitive dysfunction. Measuring each class of HDAC separately would expose these effects and improve understanding of chemobrain. Further research into how chemotherapy affects HDAC class activity could not only discover this HDAC-chemotherapy relationship but also solve a key puzzle in the mechanism chemotherapy-depressed neurogenesis.

1.7. Chemobrain and oxidative stress:

The US-Food and Drug Administration has approved up to 132 chemotherapeutic drugs, and 56 of them have been reported to induce oxidative stress (Myers et al., 2008). Oxidative stress is one of several mechanisms suggested to be a cause of cognitive impairment (Fukui et al., 2002). Doxorubicin is one of the drugs under investigation for cognitive dysfunction. Doxorubicin treatment results in the generation of excess ROS (Zhang et al., 2012), which in high concentrations, acts as neurotoxic molecules leading to oxidative stress (Massaad et. al., 2011). Although some chemotherapeutic drugs are able to cross the BBB, doxorubicin does not cross the BBB in significant amounts, but it still causes cognitive deficits, so excessive ROS generation may be the cause of this cognitive dysfunction. Oxidative stress is mainly caused by increased lipid peroxidation and depletion of glutathione, an endogenous antioxidant, and

induces apoptosis of neuron cells (Subramaniam et. al., 1994). These changes consequently lead to cognitive dysfunction.

Oxidative stress up-regulates the NMDARs expression and function on cerebrovascular endothelium leading to disruption of the BBB (Betzen et al., 2009). Thus, the disruption of the BBB allows neurotoxic compounds into brain. However, one pharmacokinetic study claims that low concentrations of doxorubicin can cross the BBB during normal treatment (Al-Abd et al., 2009); however, these amounts may be insufficient to disrupt the brain function. Overall, most studies suggest that neurogenesis results from indirect extracranial changes caused by the treatment rather than the doxorubicin itself accessing the brain.

In addition to disrupting the BBB, chemotherapeutic drugs such as alkylating agents can also damage DNA directly and generate inflammation, which leads to oxidative stress and release of ROS. Uncontrolled ROS results in DNA damage (Barzilai et al., 2004) which leads to telomere shortening, impairment of the DNA repair system, and even cell apoptosis. DNA damage is also reported to promote pro-inflammatory cytokines such as IL-6, tumor necrosis factor (TNF-α) and IL-1β, which play an essential role in cognitive deficits (Krabbe et al., 2005).

1.8. Chemobrain and inflammation:

Inflammation, cytokine activity, and cognitive functions are topics of interest in revealing the mechanism of chemobrain. Multiple chemotherapeutic drugs have been reported to promote inflammation. For instance, cyclophosphamide, one of the most commonly prescribed chemotherapeutic drugs that can cross the BBB, has been noted to cause hippocampal inflammation (Seruga et al., 2008) and disrupt hippocampus-dependent memory (Yirmiya & Goshen, 2011). In 2002, Wilson et al., reported that peripheral cytokines are able to cross the

blood-brain barrier via an active transport mechanism or vagal nerve stimulation. Activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis can facilitate learning and memory by enhancing glucocorticoids, norepinephrine, epinephrine, and dopamine release (Yirmiya et al., 2011). However, over-activation of HPA axis caused by cytokines is associated with cognitive impairment (Wilson et. al., 2002).

Acetylcholine (ACh), a neurotransmitter, likewise plays a significant role in regulation of synaptic plasticity and cognitive function (Jerusalinsky et al., 1997). Consequently, pro-inflammatory cytokines such as IL-1β are reported to impair cognitive function by increasing acetylcholinesterase (AChE) levels, which results in breakdown of ACh (Yirmiya et al., 2011). In addition, multiple neurodegenerative diseases such as Alzheimer's disease, multiple sclerosis and Parkinson's disease deregulate cytokine activity (Ahles et al., 2007). Furthermore, cytokines such as IL-6 and TNF-α have been reported to induce sickness behavior that correlates with multiple symptoms including cognitive disturbance (Myers et al., 2008).

Levels of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β sharply increase in cancer patients even before receiving treatment (Goldberg et al., 2010; Sultani et al., 2012). Rising levels of circulating IL-6 and TNF-α are correlated with cognitive dysfunction (Krabbe et al., 2005). In addition, pro-inflammatory cytokines were found to promote the inflammatory response, thus increasing cytokine levels (Sultani et al., 2012). Cytokines also penetrate the BBB (Yarlagadda et al., 2009). In fact, a recent study has revealed significant amounts of communication between the systemic cytokines in both peripheral tissues and central nervous system (CNS) (Hopkins et al., 2007). For example, preclinical and clinical studies have discovered that an increase of peripheral cytokine enhances CNS cytokine release through the vagus nerve (Ahles et al., 2007), thus leading to cognitive deficits.

1.9. Chemobrain and white/grey brain matter disruption:

Magnetic resonance imaging (MRI) has been used to investigate many clinical problems such as white and grey matter size in the brain. Loss of white or grey matter regions in the brain are correlated to different diseases in the brain (Thompson et al., 2003). Changes in multiple regions of the brain after chemotherapy treatment have been reported with MRI (Inagaki et al., 2007). To correlate the cognitive impairment and exposure to chemotherapy, two groups of cancer patients were studied. The first group of patients received chemotherapy uracil, cyclophosphamide, methotrexate, and 5-fluorouracil regimen after the surgery while the other patients did not. MRIs were acquired one and three years after chemotherapy. Both gray and white regions in the brain were diminished in chemotherapy patients after one year. (Inagaki et al., 2007).

1.10. Chemobrain and neuronal plasticity:

Donald Hebb hypothesized LTP in 1949 indicating that memory formation requires reorganization of neuronal activity (Hebb, 1949). It has been reported that the synaptic strengthening lasts for few hours to days after by repetitive stimulation of the excitatory synapses (Bliss & Lomo, 1973). The hippocampus controls cognitive function by structurally reorganizing the synapse (Leuner et al., 2010). Recent studies have investigated the effect of chemotherapy on LTP. Learning and memory was evaluated in multiple periods after cyclophosphamide and 5-fluorouracil treatments. The Morris water maze and T-maze techniques were selected to evaluate spatial memory in rodents. The LTP was selected for to measure synaptic plasticity and strength (G. D. Lee et al., 2006). The rats were evaluated after 8, 29, 42 and 53 weeks of recovery from the last treatments. The performance was better and LTP was increased in both 8 and 53 weeks. On the other hand, 29 and 42 weeks after chemotherapy treatment, the behavioral performance

and LTP of treated rats were reduced compared to control rats. Although the effect of chemotherapy on LTP is temporary, these results exhibit the link between chemotherapy and decreased learning and memory as well as point to topics of further research to elucidate the intricate mechanisms of chemobrain.

1.11. Summary:

The discussion above illustrates that chemotherapy is linked with changes in brain function and animal behavior, histopathology (Briones et al., 2011; Christie et al., 2012) and biochemical parameters that indicate brain toxicity. Chemotherapy targets DNA, increases ROS generation and binds to enzymes, which can trigger pro-inflammatory cytokine release (Rodier et al., 2009). This release in turn results in further damage to DNA and its repair system and, hence, additional cytokine activities (Jaiswal et al., 2000). Therefore, more research is needed to determine the interaction between chemotherapeutic agents, DNA damage, inflammation, and cytokine deregulation for a more complete understanding of chemobrain (Ahles et al., 2007).

Cancer is an almost unavoidable specter in modern medicine that shows little sign of disappearing. Cancer treatment and chemotherapy continue to be necessary to combat and treat cancer. Although long-term effects of chemotherapy affect a smaller fraction of people who receive treatment, the effects on the brain and the quality of life are undeniable. In order to counteract chemobrain and save patients from its negative effects on the brain and body, elucidation of mechanisms of chemobrain is essential.

Furthermore, the present study has undertaken to investigate the hypothesis that doxorubicin treatment impairs cognitive function in rodents by modifying synaptic AMPAR expression and function. In these experiments, the hippocampal synaptic AMPAR function, and

expression of associated synaptic proteins as well as LTP were measured to evaluate the synaptic function in the nude mice treated with doxorubicin. Understanding of the mechanisms by which doxorubicin alters synaptic receptor expression and function can shed the light into new therapeutic approaches to prevent chemobrain related memory loss.

CHAPTER II

Materials and Methods

2.1. Doxorubicin Treatment:

Six-week-old nude mice (NCr nude; Taconic Biosciences, Inc.) were housed in pathogen free condition following NIH guidelines using a protocol approved by Auburn University (IACUC PRN: 2012-2144). Mice were treated with doxorubicin 5 doses at 5 mg/kg i.v. for 4 weeks. An equivalent amount of vehicle (saline) was administrated to all control animals.

2.2. Synaptosome preparation:

The synaptosomes, synaptic terminals isolated from neurons **(Figure 2.1)**, were isolated as previously described (Johnson et al., 1997). Nude mice (6 weeks old) were euthanized with CO2 after receiving doxorubicin treatment. Brains were immediately removed and immersed into ice-cold oxygenated Phosphate Buffered Saline (PBS) (95% O2 and 5% CO2). The hippocampus was dissected. The hippocampus was submerged in modified Krebs-Henseleit buffer (mKRBS) (118.5 mMNaCl, 4.7 mMKCl, 1.18 mM MgSO4, 2.5 mM CaCl2, 1.18 mM $KH2PO_4$, 24.9 mM $NaHCO_3$, 10 mM dextrose, 10mMadenosinedeaminase) (70 ul/0.5g) and homogenized with a hand held and Potter homogenizer (10 strokes). Samples were maintained at 4°C on ice. Protease inhibitors (0.01 mg/ml leupeptin, 0.005 mg/ml pepstatin A, 0.10 mg/ml aprotinin, 5 mM Benzamide) were added to the homogenized buffer to diminish proteolysis. The buffer was oxygenated with 95% O_2 and 5% CO_2. Then the pH was adjusted to 7.4. Homogenates were loaded by syringe into two types of filters. Nylon filters, which have 100 μm diameter pores (obtained from BD Falcon, Bedford, MA) pre-wetted with 150 μl of mKRBS. The other filter is a low-protein binding filter (Millex-SV; Millipore Corp., Bedford, MA), which has 5 μm diameter pores. Finally, the filtered homogenates were collected and centrifuged at

1000gs for 15 minutes at 4°C. The supernatant was removed; the pellet (Synaptosome) was re-suspended with 20 µl of mKRBS and stored in -80°C until use (Parameshwaran et al., 2012).

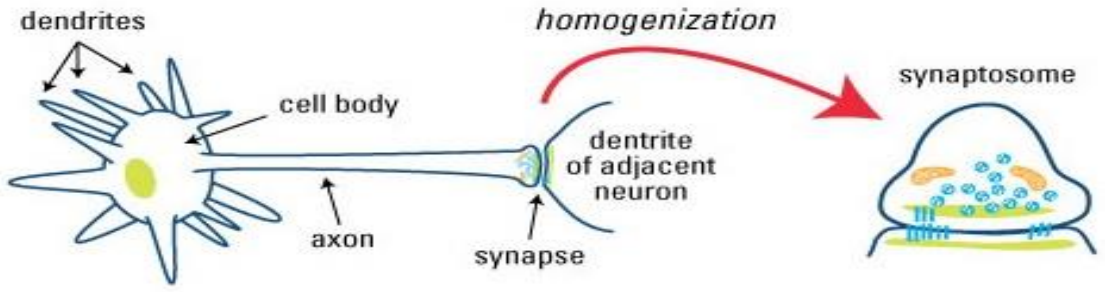


Figure 2.1: Synaptic terminals isolated from neurons. Adapted from *Hai-Yan Wu et al*., *Method to isolate functional synaptosomes*. *(2012)*.

2.3. Preparations of acute hippocampal slices:

Animals were euthanized with CO_2 and then decapitated for removal of the brain. The brain was washed with oxygenated cutting solution in order to remove the blood. A vibratome Series 1000 tissue sectioning system (Technical products international Inc., St. Louis, MO,USA) was used with a oxygenated cutting solution (NaCl 85, KCl 2.5, $MgSO_4$ 4.0, $CaCl_2$ 0.5, NaH_2PO_4 1.25, $NaHCO_3$ 25, glucose 25, sucrose 75, kynurenic acid 2.0, ascorbate 0.5) to slice the brain (350 micrometers). The slices were preserved in a holding chamber, submerged in oxygenated-ACSF (artificial cerebral spinal fluid) for 1 hour at 30◦C, before starting the LTP recording.

2.4. Extracellular field recordings:

Slices were transferred into a submerge-type recording chamber, under the microscope (Nikon SMZ 745T microscope), held between two nylon nets. This submersion chamber is

continuously perfused with oxygenated ACSF (31°C) with a flow rate of 2–3 ml/minute. A platinum bipolar electrode was placed on the CA3 region of the hippocampus and a glass microelectrode, 1.5 mm outer diameter (World Precision Instruments, Sarasota, Florida), was pulled with a micropipette puller (Narishigie scientific instruments Lab, Tokyo) and filled with ACSF solution approximately 200µl. It has been placed on the stratum radiatum in the CA1 region of hippocampus to record field excitatory postsynaptic potentials (fEPSPs) from the Schaffer collateral pathway. Two electrodes were inserted in the middle of the stratum radiatum with the Model 4D Digital Stimulus Isolation Amplifier (SIU) stimulating the CA3 region (0.33Hz) for 10 minutes monitoring basal synaptic transmission. LTP was induced using theta burst stimulation (TBS). Four TBS was applied with an inter-TBS interval of 20 s. Each TBS consists of 10 bursts delivered at 5Hz, each burst containing 4, 0.2 ms, pulses at 100Hz. Field potentials were recorded using LTP software with Axoclamp 2B (Axon Instruments, Foster City, CA) and analyzed using WinLtp software (Anderson et al., 2001).

2.5. Single channel recording:

Preparation of the artificial lipid bilayer membrane is performed by drying out the chloroform including 1,2 diphytanoyl-sn-glycero-3-phosphocholine (Avanti Polar-Lipids Inc., Alabaster, AL) with nitrogen and then dissolving the precipitate with anhydrous hexane at 1 mg/ml concentration (Sigma-Aldrich Co., Milwaukee, WI). The glass electrode (1.5 millimeter diameter, 100 MΩ) was pulled to create a pipette with 1 µm diameter and then filled with intercellular fluids (ICF), which contains 110 mM KCl, 4 mM NaCl, 2 mM NaHCO3, 1 mM MgCl2, 0.1 mM $CaCl_2$, and 2 mM 3-N-Morpholino propanesulfonic acid with adjusted pH of 7.4. The pulled pipette was then placed with a reference electrode and immersed inside the extracellular fluid (ECF) in a microbeaker that contains contains 125 mM NaCl, 5 mM KCl, 1.25

mM $NaH2PO_4$, and 5 mM Tris HCl, and adjusted pH of 7.4 (Parameshwaran et al., 2012). Then 5 µl of the phospholipid was added to the ECF, which spreads out to form lipid monolayers on the top of the ECF. The tip of the pipette, containing ICF, was dipped twice to generate the phospholipid bilayer **(Figure 2.2)**. Then synaptosomes were added into the microbeaker for the receptors to incorporate into the artificial bilayer and glutamate was then added to activate the receptors (Suppiramaniam et al., 2006).

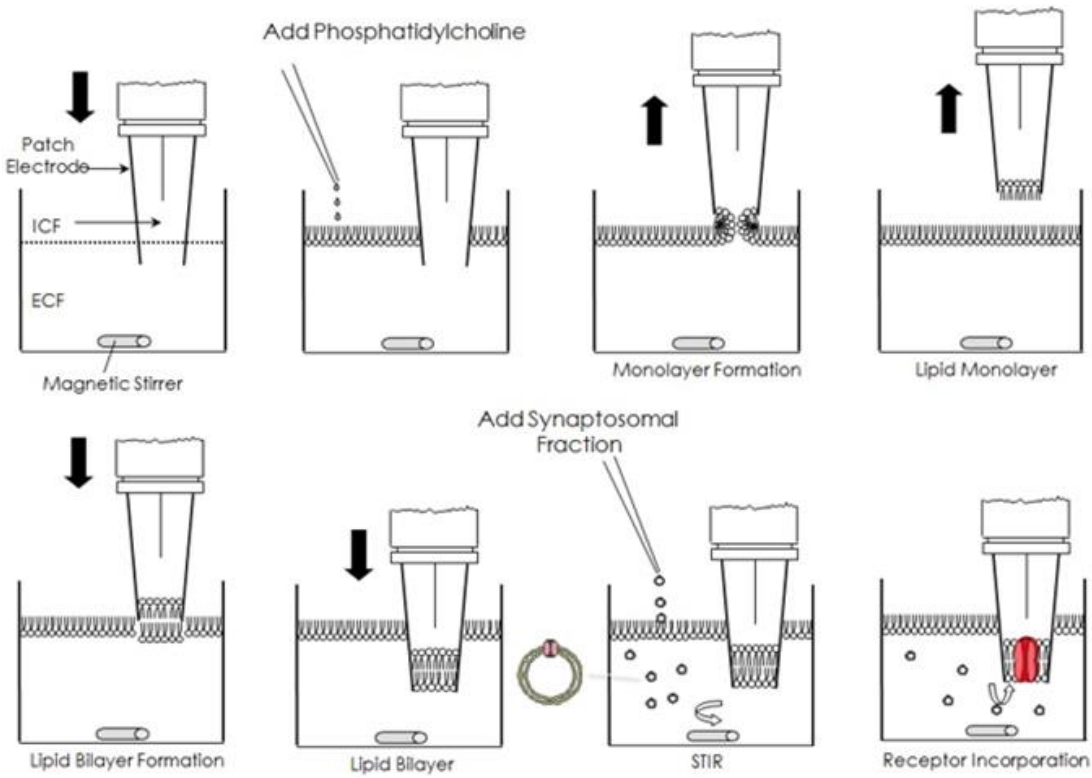


Figure 2.2: Preparation of artificial lipid bilayer membrane. Adapted from *Suppiramaniam et al., Methods Enzymol. (2006)*.

The synaptosomes were prepared as described elsewhere (Johnson et al., 1997). The synaptosomal suspensions were submerged into ECF containing the artificial bilayer membrane and glutamate is delivered. The voltage was applied and the current was recorded after successful reconstitution of synaptosomes into lipid bilayer.

2.6. Western Blot Analysis:

Hippocampi from doxorubicin treated animals and vehicle were lysed with lysis buffer (obtained from Cell signaling 10x). The sample was mixed thoroughly with 2x Laemmli buffer and was loaded into 10% SDS-page gel. The proteins were transferred to PVDF membranes (Immobilon-p Millipore, Germany), and blocked 5% non-fat dry milk for 1 hour in Tris Buffered Saline (TBS) containing 0.01% Tween 20. Membranes were washed with TBST and incubated with anti-AMPA-GluR1 (1:1000, Cell Signaling Technology, Danvers, Massachusetts), anti-AMPA-GluR2 (1:1000, Alomone Labs products, Jerusalem, Israel.), anti-PSD-95 (1:1000, Cell Signaling Technology, Danvers, Massachusetts), anti-SAP102 (1:1000, Cell Signaling Technology, Danvers, Massachusetts), anti-CaMKII (1:1000, Cell Signaling Technology, Danvers, Massachusetts), anti-synaptophysin (1:1000, Cell Signaling Technology, Danvers, Massachusetts), anti-α-stargazin (1:1000, EMD Millipore, Germany), anti-BDNF (1:1000, Santa Cruz biotechnology, Paso Robles, CA), anti-pro-BDNF (1:1000, Santa Cruz biotechnology, Paso Robles, CA), or anti-beta actin (1:1000, Cell Signaling Technology, Danvers, Massachusetts) overnight at 4°C. Then, membranes were probed with secondary anti-rabbit antibody (1:10000) that is conjugated with fluorophore DyLight 550 at room temperature for 4 hours. Then, the membranes were scanned to be visualized by utilizing FLA-5100 imager with the 532 nM green laser and the LPG filter set (Fujifilm Inc., Tokyo, Japan). These scans were performed using excitation at λ_{532} nm and emission at λ_{570} nm. Finally, the densities of these bands were compared over β-actin for both doxorubicin treated animals and control.

2.7. GluR1 and CaMKII Phosphorylation assay:

To study phosphorylation of GluR1 (pGluR1 at-Ser^{831} and at -Ser^{845}) and phospho-CaMKII at T286 residue, western blot analysis was used to measure the quantity of phosphorylated proteins. Briefly, 4 weeks after treatment with doxorubicin, animals were euthanized and the brain was dissected to collect the tissue of the hippocampus. The hippocampi were homogenized and sonicated in lysis buffer. The sample was solubilized in the lysis buffer and mixed with Laemmli buffer by 1:1 ratio. Afterward the sample was resolved on 10% SDS-page gel. Proteins were transferred onto PVDF membrane (Immobilon-p Millipore, Germany) and then blocked with 5% BSA. pGluR1, total GluR1, pCaMKII, and total CaMKII and were detected by rabbit (anti-pGluR1-Ser^{831}, EMD Millipore, Germany), anti-pCaMKII, anti-GluR1, and anti-CaMKII antibodies (1:1000, Cell Signaling Technology, Danvers, Massachusetts) respectively. Blots were exposed with secondary anti-rabbit antibody (1:10000) that is conjugated with fluorophore DyLight 550 at room temperature for 4 hours. The membranes were visualized by FLA-5100 imager with the 532 nM green laser and the LPG filter set (Fujifilm Inc., Tokyo, Japan). These scans were performed using excitation at λ_{532} nm and emission at λ_{570} nm. The results were normalized according to proteins that are loaded and compared the ratio of pGluR1-Ser831, pGluR1-Ser845 and pCaMKII over total expressions of total GluR1 and total CaMKII respectively.

2.8. Statistics:

Data from in vitro study was analyzed utilizing one-way ANOVA with Tukey post hoc test to analyze electrophysiological data and fEPSP data (treat-animals& control) and unpaired t-test to analyze biochemical data. All experiments were repeated for n=6 animals and a p value of less than 0.05 were considered significant.

CHAPTER III

Results

The numbers of postsynaptic AMPARs subunits at the synapse are recognized as important feature of long-term synaptic changes and long-term potentiation. Doxorubicin treatment has shown to alter the level of AMPAR composition at the synapse, which leads to changes in synaptic strength.

3.1. Doxorubicin treatment impairs LTP:

The alterations in GluR1, GluR2 subunits expression show that doxorubicin has direct effects on synaptic function. By measuring extracellular field recordings, we examined the effects of doxorubicin treatment on glutamatergic transmission in the Schaeffer Collateral pathway of the hippocampus. Our results indicate there is a significant decrease in the LTP in the doxorubicin treated mice. **Figure 3.1** illustrates the difference between slice inductions and LTP. The doxorubicin-treated samples reached induction of approximately 15-20% increase from the baseline whereas the saline-treated slices reached approximately 70-80% increase from the baseline. In addition, the effects of doxorubicin treatment on within-train facilitation were assessed by measuring the fEPSP amplitudes of multiple responses and the results were normalized to the amplitude of the first response of each train.

3.2. Effects of doxorubicin treatment on single channel properties of synaptosomal AMPA receptors:

Single-channel recordings show that doxorubicin treatment alters kinetic properties of synaptic AMPARs. We used synaptosomes isolated from mice hippocampi, and we used cocktail antagonists to block all receptors except AMPA receptors. **Figure 3.2** represents a comparison of

the AMPAR channel properties of the doxorubicin and saline-treated animals. The amplitude histograms constructed for the sample traces show a significant decrease in frequency of channel opening as indicated by the area under the open peak in doxorubicin treatment. Similarly, the opening probability of AMPARs has decrease 82 % in doxorubicin treated animals compared to controls. In addition, the dwell-open time showed a marked decrease 72 % in experimental group compared to controls, whereas dwell closed times and increased 50 % in doxorubicin treated animals compared to controls.

3.3. Doxorubicin treatment results in altered expression of synaptic GluR1, GulR2 subunits, BDNF, and α-stargazin in the hippocampus:

Our results illustrate that doxorubicin treatment produced a robust increase in the levels of GluR2, which is postsynaptic AMPAR subunit that regulates calcium influx, in the hippocampal homogenate and synaptosomes indicating that less calcium will influx into the cells. In contrast, our results revealed a decrease in GluR1, BDNF, and α-stargazin levels in doxorubicin treated mice **(Figure 3.3)**. The number of postsynaptic AMPAR subunits at the synapse is recognized as an important feature of LTP. The results of western blot is also illustrate multiple protein expressions related to AMPARs trafficking and function following doxorubicin treatment, which may lead to changes in synaptic strength.

3.4. Doxorubicin treatment alters the phosphorylation of AMPAR subunits in the hippocampus:

The two major phosphorylation sites in the GluR1 subunit that have an impact on synaptic plasticity are Ser^{831} and Ser^{845} residues. These phosphorylation sites are occurring in the C-terminus of GluR1. The potential of doxorubicin to regulate hippocampal AMPARs subunit phosphorylation was the first to be explored in our study. As it is shown in **Figure 3.4**, doxorubicin treatment induces GluR1-Ser^{831}phosphorylation. On the other hand, doxorubicin treatment was found to downregulate the phosphorylation of GluR1 subunit in Ser^{845} residue, which is necessary in synaptic plasticity and memory function.

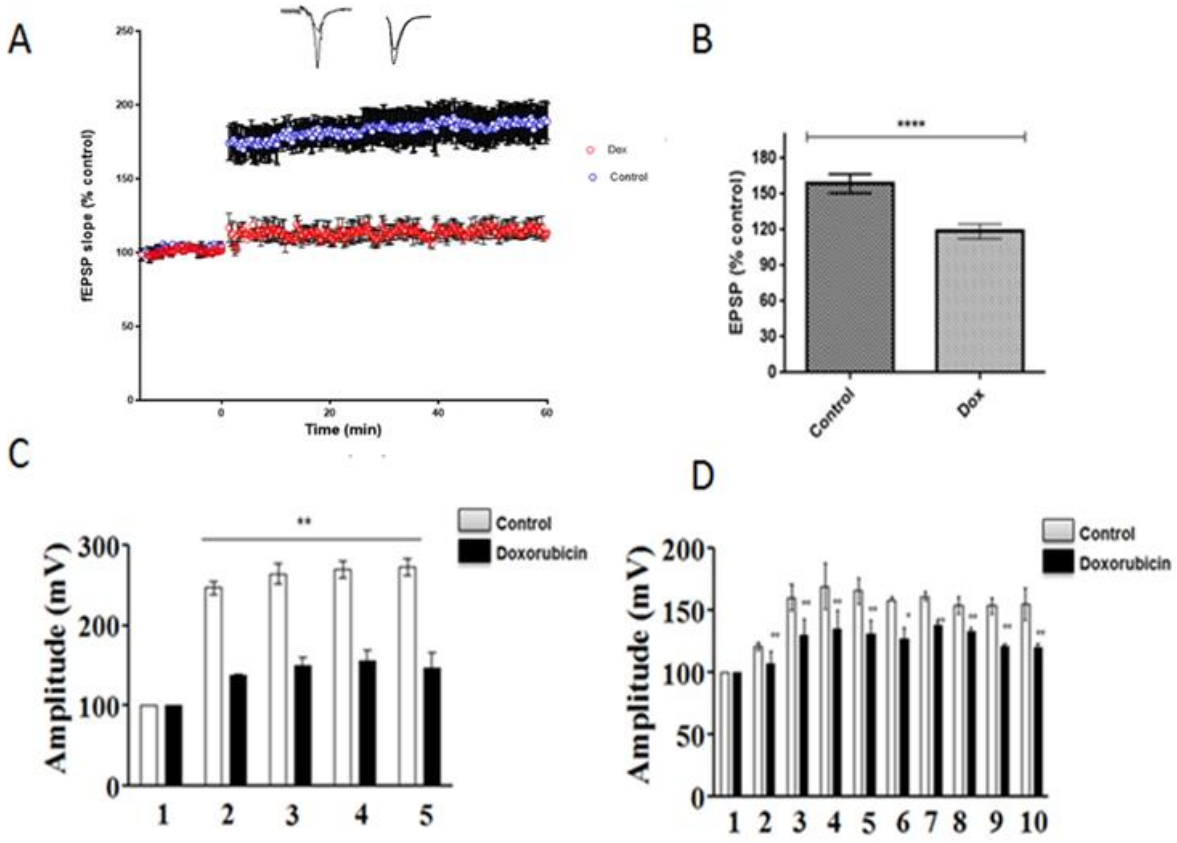
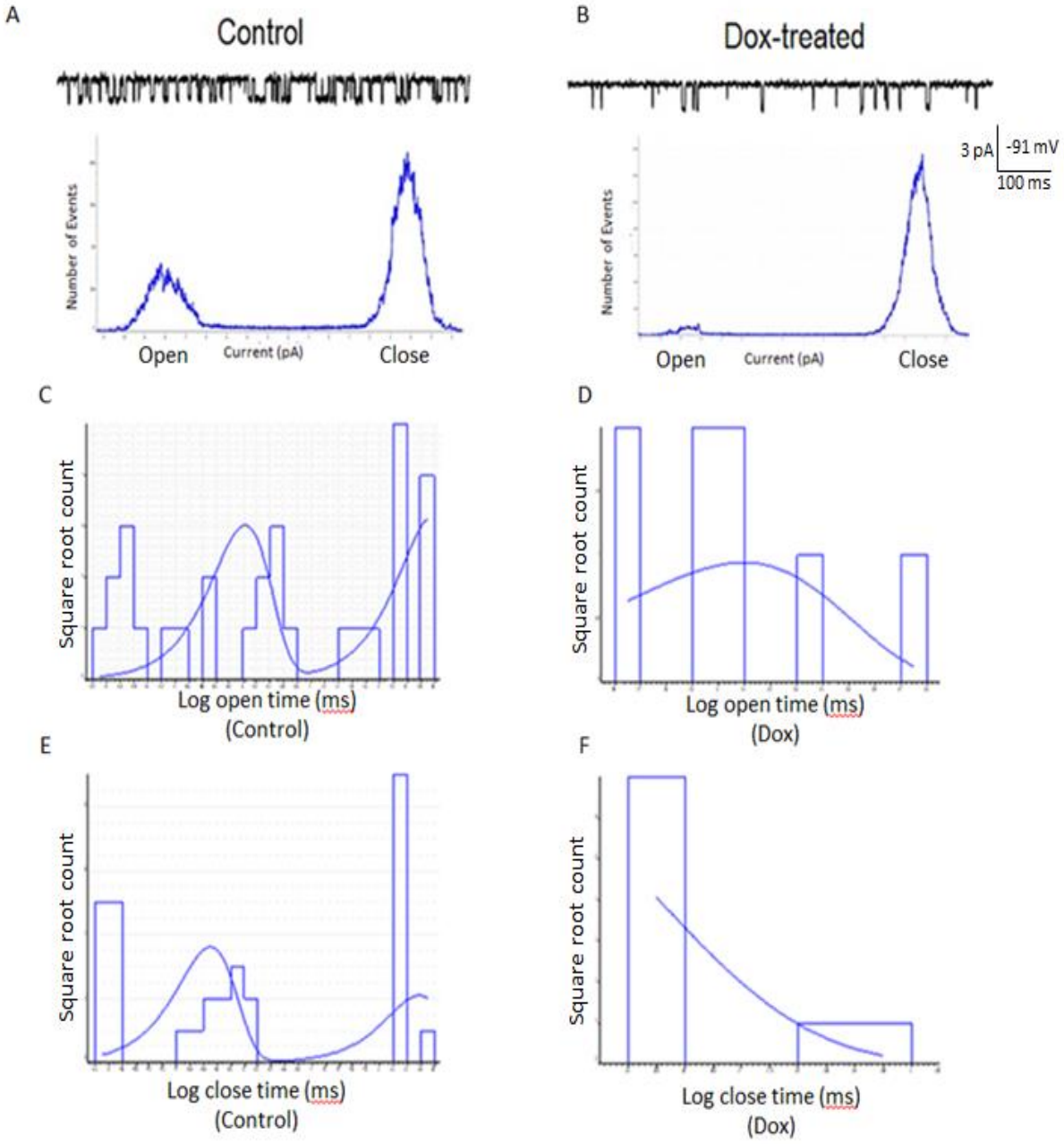


Figure 3.1: Recording of LTP of Schaffer collateral in CA1 area synapses. This data shown is the field excitatory postsynaptic potentials (fEPSP) of hippocampi of doxorubicin and saline-treated rodents. (A) LTP was induced by theta burst stimulation (TBS) and measured at 1 hour after TBS. Blue, Synaptic responses recorded indicate that LTP was higher and prolonged after the high- frequency stimulus in both induction and maintenance stage (fEPSP). Red, Synaptic responses recorded indicate that LTP was decreased in the hippocampus of doxorubicin treated mice compared to control mice (Blue). This potentiation of synaptic transmission produced in control mice was 70-80%, while the amplitude of fEPSPs produced by doxorubicin animals was 15-20%. (B) Bar chart illustrating the significant reduction of fEPSP responses in doxorubicin acute slices compared to controls. (C) Comparison of amplitudes of fEPSP responses within train and within successive trains. The amplitudes of fEPSP were normalized to

the amplitude of the first response. Within-train facilitation shows significant decrease in doxorubicin treated slices (D) Tetanic facilitation has occurred with each successive train in control slices but not in the doxorubicin treated ($p < 0.01$; n=6).


A
Control
Number of Events
Open
Current (pA)
Close
B
Dox-treated
3 pA
-91 mV
100 ms
Number of Events
Open
Current (pA)
Close
C
Square root count
Log open time (ms)
(Control)
D
Square root count
Log open time (ms)
(Dox)
E
Square root count
Log close time (ms)
(Control)
F
Square root count
Log close time (ms)
(Dox)

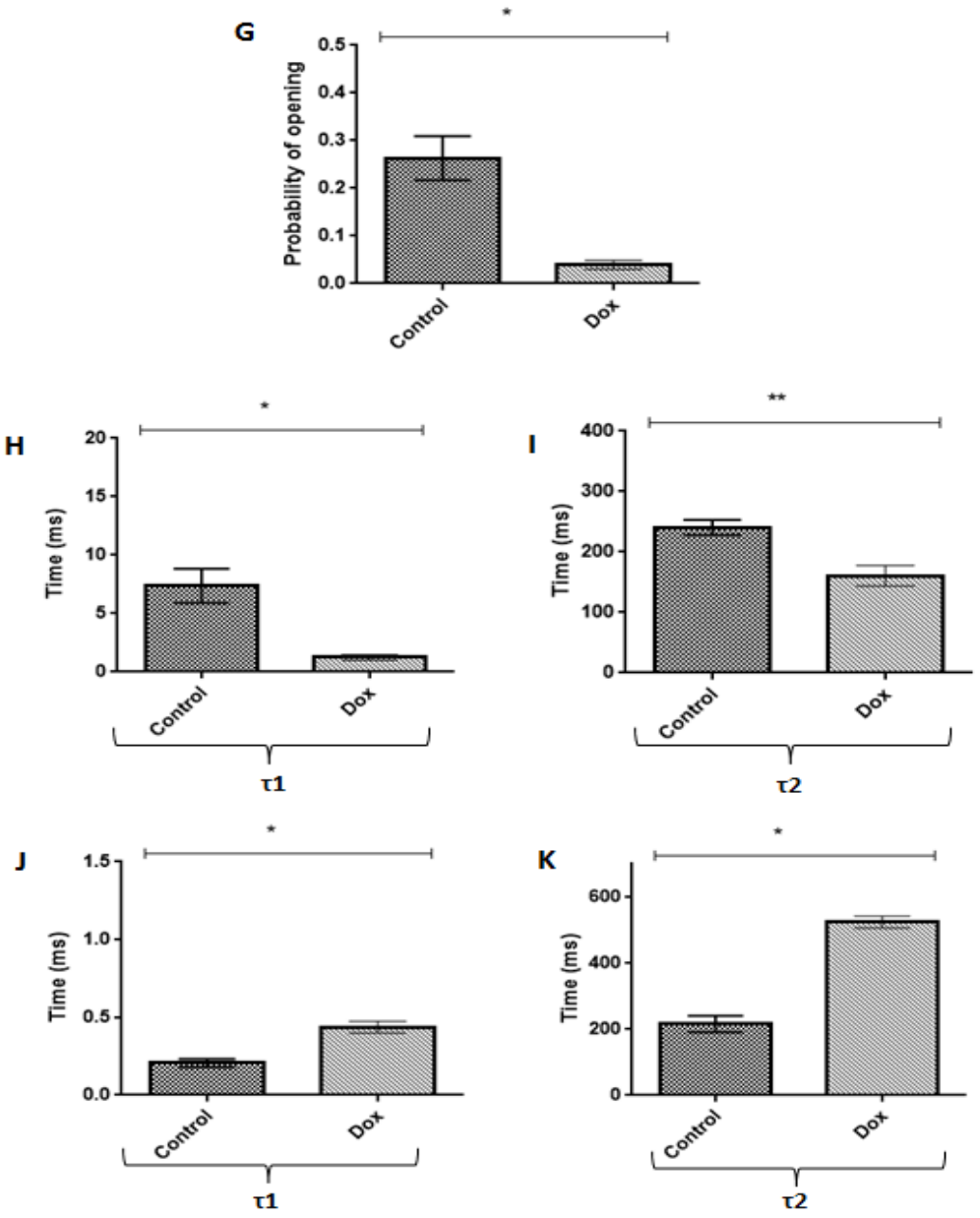

G
*
Probability of opening
0.5
0.4
0.3
0.2
0.1
0.0
Control
Dox
H
*
Time (ms)
20
15
10
5
0
Control
Dox
τ1
I
**
Time (ms)
400
300
200
100
0
Control
Dox
τ2
J
*
Time (ms)
1.5
1.0
0.5
0.0
Control
Dox
τ1
K
*
Time (ms)
600
400
200
0
Control
Dox
τ2

Figure 3.2: Doxorubicin treatment altered the single channel properties of hippocampal synaptic AMPA receptors. (A-B) Amplitude histograms display two distinct peaks for close and open states. Channel open peak is higher in the control than doxorubicin treated. Demonstrative traces revealed that each of the histograms illustrate reduced channel activity. (C-D) histogram represents of the log-transformed time of AMPA receptors opening in hippocampi of control and doxorubicin treated mice respectively. (E-F) show histogram of close time for control and doxorubicin treated mice. G) Bar chart illustrating the significant reduction of AMPA receptors channel open probability in Doxorubicin synaptosomes. (H, I) Bar plot showing significantly decreased open time $\tau 1$ and $\tau 2$ in Doxorubicin synaptosomes. (J, K) Bar chart showing significantly prolonged close time component ($\tau 2$). Values are expressed as mean ± SEM from 6 animals per group $p < 0.05$, $p < 0.01$ two-tailed, unpaired Student's *t*-test.

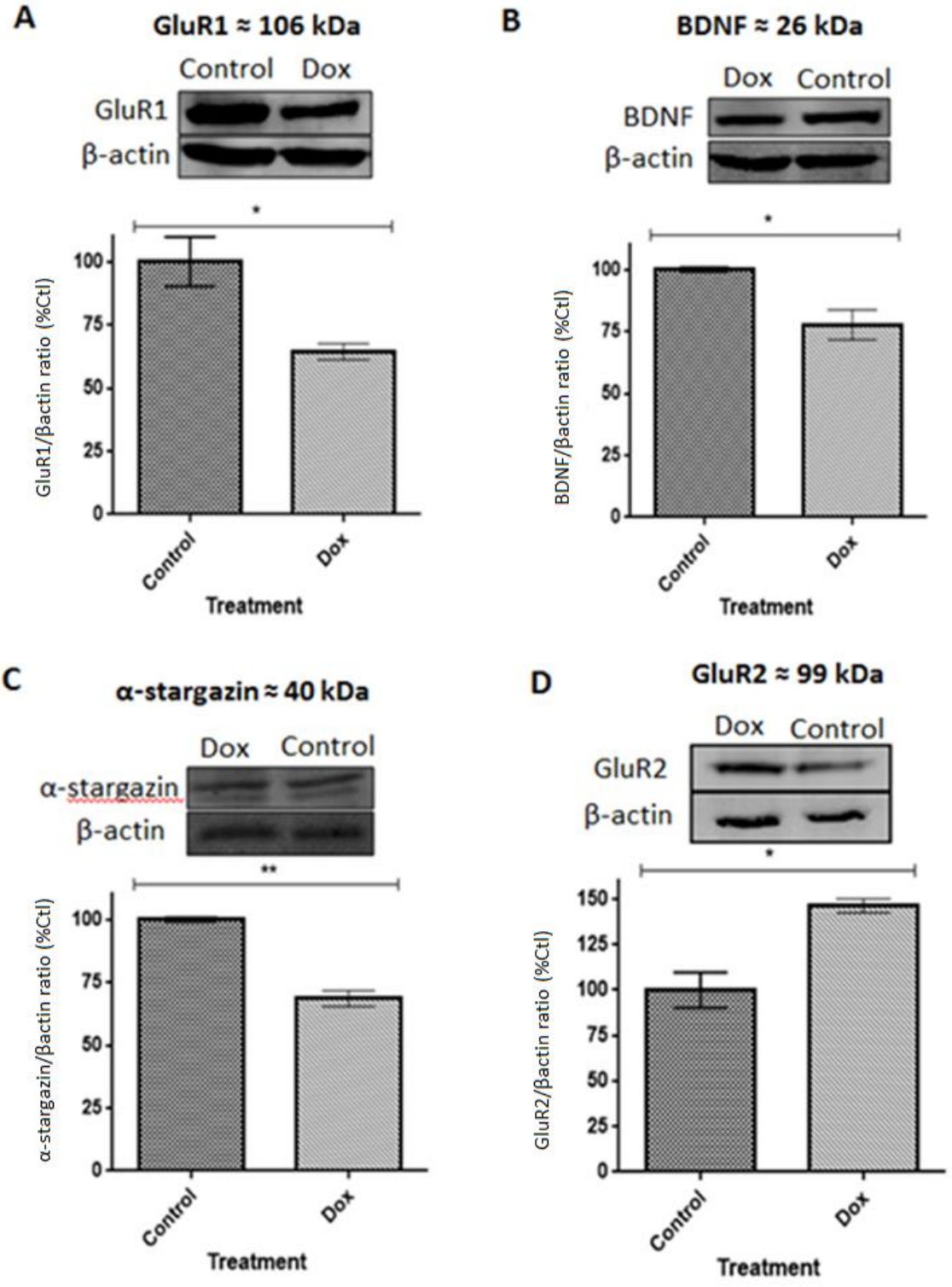
Table 1:

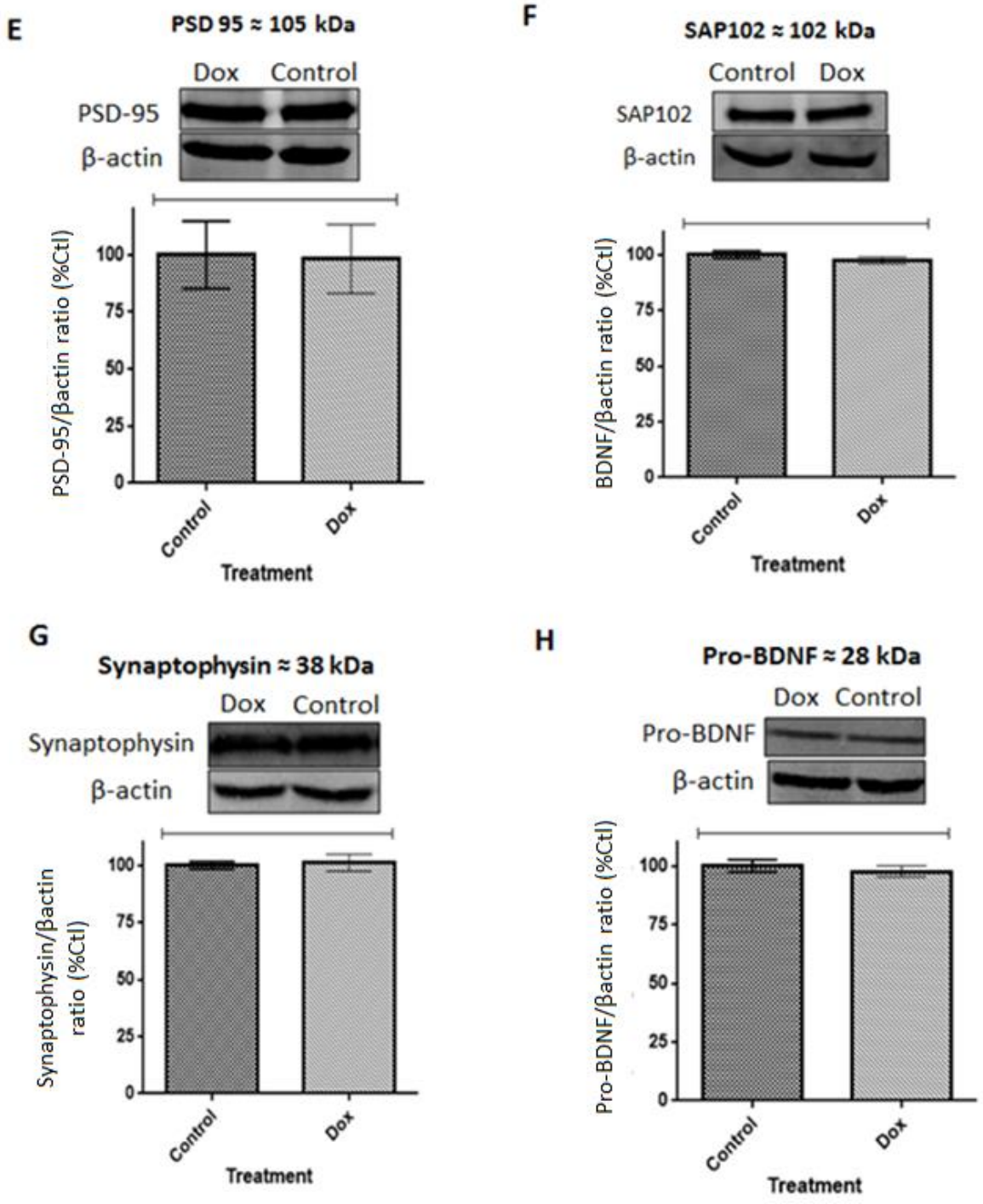
Single channel properties of synaptic AMPA receptors in the control and doxorubicin treated mice.

	Control (-/+ SEM)	Doxorubicin (-/+ SEM)
Open time (ms)		
τO1	7. 36 ± 1.46	1.23 ± 0. 23*
τO2	240.7 ± 12.35	160.93 ± 17.01**
Close time (ms)		
τC1	0.21 ± 0.026	0.43 ± 0.037*
τC2	216.15 ± 24.97	524.02± 18.23*

* $p < 0.05$, **$p < 0.01$ versus control by two tailed student's t test

The data were expressed as the mean of three independent experiments for the control and doxorubicin treated.


A
GluR1 ≈ 106 kDa
Control
Dox
GluR1
β-actin
*
GluR1/βactin ratio (%Ctl)
100
75
50
25
0
Control
Dox
Treatment
B
BDNF ≈ 26 kDa
Dox
Control
BDNF
β-actin
*
BDNF/βactin ratio (%Ctl)
100
75
50
25
0
Control
Dox
Treatment
C
α-stargazin ≈ 40 kDa
Dox
Control
α-stargazin
β-actin
**
α-stargazin/βactin ratio (%Ctl)
100
75
50
25
0
Control
Dox
Treatment
D
GluR2 ≈ 99 kDa
Dox
Control
GluR2
β-actin
*
GluR2/βactin ratio (%Ctl)
150
125
100
75
50
25
0
Control
Dox
Treatment


E
PSD 95 ≈ 105 kDa
Dox
Control
PSD-95
β-actin
PSD-95/βactin ratio (%Ctl)
100
75
50
25
0
Control
Dox
Treatment
F
SAP102 ≈ 102 kDa
Control
Dox
SAP102
β-actin
BDNF/βactin ratio (%Ctl)
100
75
50
25
0
Control
Dox
Treatment
G
Synaptophysin ≈ 38 kDa
Dox
Control
Synaptophysin
β-actin
Synaptophysin/βactin ratio (%Ctl)
100
75
50
25
0
Control
Dox
Treatment
H
Pro-BDNF ≈ 28 kDa
Dox
Control
Pro-BDNF
β-actin
Pro-BDNF/βactin ratio (%Ctl)
100
75
50
25
0
Control
Dox
Treatment

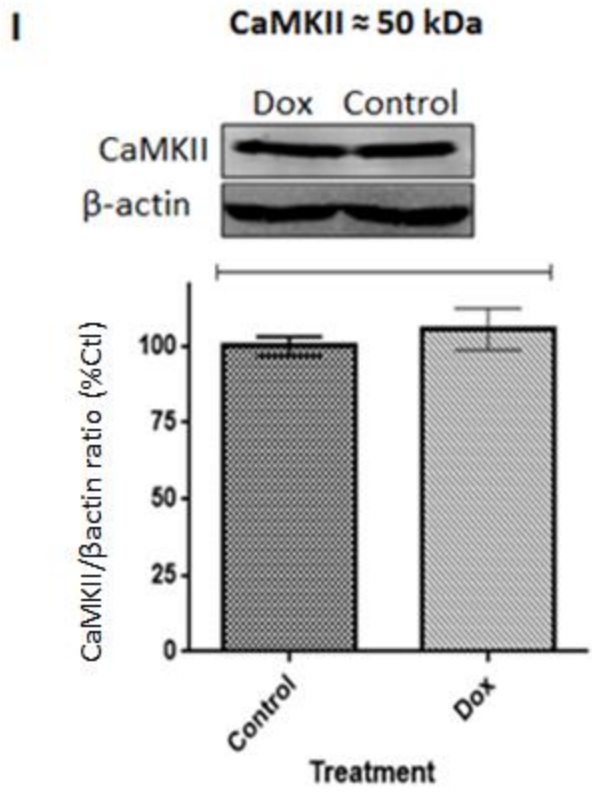


Figure 3.3: Effect of doxorubicin treatment on regulation of hippocampal postsynaptic markers (GluR1, GluR2, PSD-95, SAP102, synaptophysin, α-stargazin, pro-BDNF, BDNF, and CaMKII) in mice. Western blot analysis shows that AMPA-GluR1 subunit, BDNF, and α-stargazin expression levels were downregulated following doxorubicin treatment (A, B, C). The levels of GluR2 expression was increased in respond to doxorubicin treatment (D). In figure (E, F, G, H, and I), the levels of PSD-95, SAP102, synaptophysin, pro-BDNF, and CaMKII expressions in both doxorubicin treated mice and the controls are not significantly different. Protein levels were lysate from whole hippocampus in both control and doxorubicin treated animals. Protein levels were normalized to β-actin and stated as a percentage of the control group which was set as 100%.

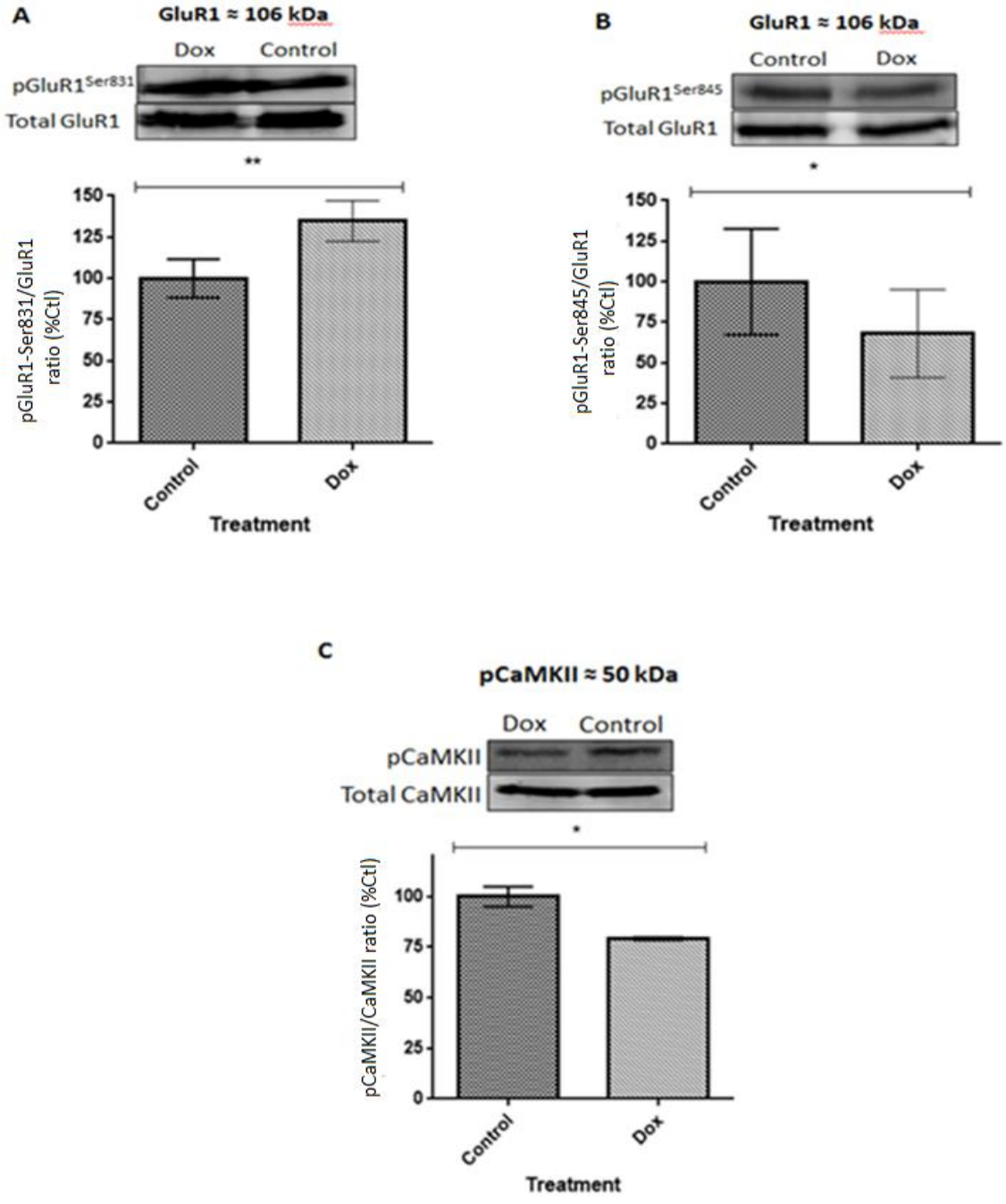

A
GluR1 ≈ 106 kDa
Dox
Control
pGluR1Ser831
Total GluR1
**
pGluR1-Ser831/GluR1 ratio (%Ctl)
150
125
100
75
50
25
0
Control
Dox
Treatment
B
GluR1 ≈ 106 kDa
Control
Dox
pGluR1Ser845
Total GluR1
*
pGluR1-Ser845/GluR1 ratio (%Ctl)
150
125
100
75
50
25
0
Control
Dox
Treatment
C
pCaMKII ≈ 50 kDa
Dox
Control
pCaMKII
Total CaMKII
*
pCaMKII/CaMKII ratio (%Ctl)
100
75
50
25
0
Control
Dox
Treatment

Figure 3.4: GluR1 phosphorylation in Ser831 and Ser845 residues and phosphorylated CaMKII. (A-B-C) experiments were performed as described in material and methods. (A) Represents the pGluR1-Ser^{831} in control and doxorubicin treated animals. Representative image and result of pGluR1-Ser^{831} percentage indicate that a statistical significance difference from basal control and doxorubicin treated animals with $p<0.05$. (B) Represents the GluR1-Ser^{845} for both control and doxorubicin treated. (C) Representing the CaMKII phosphorylation and displaying decrease in doxorubicin treated animals compared to the control. The image and the result percentage show a statistical significance difference between control and doxorubicin treated animals *$p<0.05$, **$p<0.01$.

CHAPTER IV

Discussion

In the present study, we determined the effects of doxorubicin treatment on hippocampal glutamatergic systems. Our results indicate that glutamatergic synaptic transmission is affected by doxorubicin treatment, which is important for triggering learning and memory processes. This study, to the best of my knowledge, is the first to demonstrate the role of glutamate receptors in chemobrain.

AMPARs consist of four major subunits: GluR1-4. The two subunits that play significant roles in learning and memory are GluR1 and GluR2, and they are different in their permeability to calcium. GluR1 subunits are known to be permeable to calcium, whereas, edited version of GluR2 subunits are impermeable to calcium (Geiger et al., 1995). Calcium influx into the postsynaptic neuron is necessary for the LTP initiation through activation of CaMKII. The doxorubicin-treated mice have increased whole cell expression of the calcium impermeable GluR2 subunit and lower quantities of the calcium permeable GluR1 subunit than the control mice. Therefore, decreased calcium influx into the neurons possibly contributed to decreased LTP.

Results from the present study demonstrated that CaMKII expression is not effected following doxorubicin treatment and it is implicated to play an important role in LTP (Lisman et al., 2012). However, phosphorylation of CaMKII is decreased in the hippocampi of doxorubicin-treated mice compared to the control indicating that CaMKII is less active in doxorubicin treated mice. CaMKII autophosphorylation may be decreased as a result of decreased Ca^{2+} influx (Meng et al., 2003). GluR1-Ser^{831}, which is phosphorylated by both CaMKII and PKC, was

demonstrated in this study to be up-regulated. This up-regulation is likely not due to CaMKII phosphorylation, but possibly due to phosphorylation by PKC.

Postsynaptic density protein-95 (PSD-95) is a scaffolding protein that is responsible for stabilizing AMPARs and NMDARs to the synaptic membrane (Beique et al., 2006). In the present study, our results illustrate that PSD-95 is not significantly altered in the doxorubicin-treated mice compare to control. This suggests that AMPARs subunits alteration is not due to PSD-95 but could be due to altered DNA transcription or mRNA translation, which we intend to examine in the follow-up studies. Indeed, the electrophysiological data suggest that the doxorubicin-treated mice exhibited significantly impaired LTP compared to the control mice. Deficits in LTP in doxorubicin treated slices could be due to altered axonal depolarization, presynaptic release or postsynaptic mechanisms. Thus, the postsynaptic fEPSP amplitudes and slops were assessed. The result indicated that there is a significant modification in the postsynaptic neurons response. The decrease in LTP incited by doxorubicin treatment may serve as an explanation for cognitive deficits in chemobrain and could help identify a possible mechanism for the disorder.

In addition to causing decreased calcium to influx into the cells, doxorubicin also appears to limit the functionality of post-synaptic AMPARs. Conductance and opening probability influence current passing through ion channels (Mortensen et al., 2007). Therefore, a decrease in conductance or open probability will reduce the mean current amplitude of the single channel receptors. Our single-channel data illustrates that doxorubicin decreased open probability of AMPAR compared to their untreated counterparts. In addition, a decrease in single channel conductance of synaptic AMPAR was observed in synaptosomes extracted from doxorubicin treated rodents. Limiting the opening probability and conductance of the postsynaptic glutamate

receptors would decrease postsynaptic potentials and reduce the synapse ability to undergo LTP leading to cognitive dysfunction (Bredt et al., 2003). The decrease in opening events suggests that doxorubicin or its metabolites may antagonize or increase the desensitization of AMPARs reducing postsynaptic currents.

Synapse-associated protein 102 (SAP102) belongs to the membrane-associated guanylate kinase (MAGUK) protein family (Chen et al., 2012). SAP102 is identified to play a vital role in synaptic regulation and plasticity (Elias et al., 2008). SAP102 is known to participate in NMDAR binding particularly interacting with the carboxy-terminal domain of the NMDAR subunit 2B (NR2B) (Chen et al., 2012). When SAP102 interacts with NR2B, it will facilitate AMPARs withdrawal from the postsynaptic membrane (Murata et al., 2013). Thus, SAP102, NMDARs, and AMPARs function together to intermediate synaptic plasticity and signal transduction. Our study has carried out experiments to explore the differences in expression levels of SAP102 between control and doxorubicin treated animals. The results revealed that there is no significant difference in expression levels, and SAP102 has no direct relation to the cognitive deficits that caused by doxorubicin treatment.

Stargazin is a transmembrane protein that plays a crucial role in learning and memory by regulation of synaptic AMPARs. Stargazin interacts with AMPARs and facilitates AMPARs trafficking resulting in surface expression, which can increase AMPARs responsiveness to glutamate (Deng et al., 2006). Stargazin is required for LTP regulation and synaptic plasticity (Matsuda et al., 2013). Stargazin is found to regulate AMPARs channel properties by increasing opening probability (Tomita et al., 2005). Interestingly, our results show that stargazin is downregulated following doxorubicin treatment, might explain alteration in AMPAR expression and phosphorylation.

Synaptophysin is a glycoprotein that is expressed in presynaptic neurons (Sze et al., 1997). Synaptophysin is transmembrane that plays important role in memory function. It binds to the synaptobrevin, the SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors) protein in the synaptic vesicle, which is essential for exocytosis (Reisinger et al., 2004). Electrophysiological recording of LTP from CA1 region in synaptophysin and synaptogyrin knockout mice revealed severe reduction in LTP maintenance. due to insufficient induction (Janz et al., 1999). Therefore, we investigated the expression levels of synaptophysin. Our results indicated that the expression levels of synaptophysin is not significantly changed, so we conclude that the fEPSP amplitude decrease in doxorubicin treated mice was not a due to altered expression of synaptophysin.

Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophin family and is identified to be a major regulator of synaptic transmission and synaptic plasticity in the hippocampus (Huang & Reichardt, 2001; McAllister, Katz, & Lo, 1999). BDNF expression regulates development and processes of hippocampal function (Lu & Chow, 1999). Increased BDNF expression enhances signaling, neurogenesis, and electrophysiological activity (Xu et al., 2000). BDNF is the mature form that cleaved from its precursor pro-BDNF, and they are implicated to play an opposite effects in terms of long-term potentiation and long-term depression. BDNF facilitates hippocampal synaptic potentiation through TrkB activation. However, pro-BDNF activates p75NTR facilitating long term depression (Woo et al., 2005). The present data show that pro-BDNF expression following doxorubicin treatment is not altered. In contrast, BDNF expression is revealed to be down-regulated in doxorubicin treated mice compared to controls and this alteration may be the cause of synaptic weakening after doxorubicin treatment.

Synaptic plasticity including LTP requires phosphorylation of particular proteins, to be induced and maintained for several hours to days (Klann et al., 2004). Previous studies have demonstrated that GluR1 subunit of AMPA receptors phosphorylation was modified during LTP. Gene knockin of sites of phosphorylation has revealed deficits in spatial learning task (Lee et al., 2003). The two major GluR1 phosphorylated sites are in residues Ser^{831} and Ser^{845} (Lee et al., 2010; Snyder et al., 2000; Wang et al., 2005). Therefore, in our study, we hypothesized that GluR1 phosphorylation patterns would be reduced in doxorubicin treated animals. Thus, we have investigated these two patterns of phosphorylation. Our results indicate increased phosphorylation of Ser^{831} residue in treated animals than the controls, however, phosphorylation of Ser^{845} residue was decreased. To strengthen our results, another study found that Ser^{831} gene knockin revealed normal LTP, but Ser^{845} gene knockin showed impaired LTP (Lee et al., 2010).

In summary, our electrophysiological and biochemical results support the previous asserted hypothesis that doxorubicin leads to cognitive dysfunction. In addition, the present study has probed the mechanism by uncovering a possible link between chemobrain and alterations in the cellular machinery responsible for LTP and learning and memory. Using the current results, further research can attempt to elucidate the effects of doxorubicin on AMPA and NMDA receptors expression, trafficking, function and their downstream signaling in order to pinpoint the mechanisms of chemobrain.

CHAPTER IV

Conclusion and Prospective

Cancer remains an important public health concern because it is widely spread in all ages, and genders. It is reported to be the second leading cause of death. It causes death, and an estimated 1600 people lose their lives to cancer each day (American Cancer Society, 2013). Doxorubicin is an anthracyclines antibiotic that is utilized to treat cancer based on its ability to intercalate DNA and interrupt the process of DNA replication (Munger et al., 1988), inhibiting topoisomerase II (Burden et al., 1998) as well as formation of ROS that causes cytotoxicity (Feinstein et al., 1993). Interestingly, doxorubicin cannot cross the blood brain barrier; however, studies show cognitive impairment following doxorubicin treatment. Therefore, cellular and molecular mechanism of cognitive deficits associated with doxorubicin treatment remains unknown.

5.1. General outcomes:

This study has explored the effects of doxorubicin on biochemical and physiological changes associated with hippocampal learning and memory, specifically evaluating glutamate receptor expression and function. Nude mice were selected for our experiments to diminish immune response that may influence inflammation and thus cognitive dysfunction. The study has used a variety of electrophysiological, biochemical and molecular biology techniques to elucidate the molecular mechanisms of memory loss in doxorubicin treated mice.

The first part of the study demonstrated the direct effect between the synaptic plasticity and doxorubicin treatment. Assessment of long-term potentiation and synaptic plasticity were carried out utilizing field potential measurements. This study demonstrated that synaptic

transmission is impaired in doxorubicin treated animals compared to control after high-frequency electrical stimulation in CA3-CA1 regions of hippocampus.

The second part of this study was the direct measurement of single channel properties of synaptic AMPARs. Synaptosomal isolation method and tip-dip bilayer technique has been utilized to directly measure the single channel properties of synaptic AMPARs in a controlled environment. This study presented evidence that synaptic AMPARs displayed certain changes in channel properties. These properties include smaller single channel conductance, open time distribution, probability of openings and larger closed time distribution in doxorubicin treated animals compared to controls.

The third part of the study utilized biochemical techniques to complement the electrophysiology findings. Western blot analysis was used to investigate the alterations in certain synaptic protein expression to correlate the result with altered synaptic plasticity measures. Our findings also indicated that phosphorylation patterns of certain AMPAR subunits required for synaptic delivery and function were altered after doxorubicin treatment.

Outcomes of this study help to understand the relationship between doxorubicin treatment and alteration in glutamatergic systems and how it impairs memory encoding. The findings of this study could contribute to the development of new strategies to prevent cancer survivors from experiencing the side effects of chemotherapy, specifically cognitive deficits. Our findings shreduction in long-term potentiation in doxorubicin treated mice compared to control animals. Similarly, single channel assessment presented specific kinetic properties of synaptic AMPARs activity (conductance, amplitudes, opening and closing time), which were reduced in doxorubicin animals as well. These results indicate that changes in the expression and function

of AMPARs and associated synaptic proteins lead to altered synaptic transmission and plasticity required for learning and memory in doxorubicin treated mice.

5.2. Future directions:

As stated in the introduction of Chapter 2, the development of new techniques to explore the mechanisms of cognitive deficits in chemobrain and the therapeutic interventions is an ongoing endeavor. Doxorubicin treatment is known to cause, excessive ROS generation, and altered synaptic transmission leading to cognitive deficits. Phenyl-2-aminoethyl selenide (PAESe) is an antioxidant that has been found to attenuate the cardiotoxicity induced by doxorubicin (Kang et al., 2011). In addition, co-administration of PAESe with doxorubicin resulted in rescue of long-term potentiation and synaptic plasticity in nude mice (Alhowail et al., 2014). Therefore, designing studies exploring the effects of PAESe in chemobrain is of utmost importance.

Another direction of investigation could be aimed at the NMDARs expression, function and signaling at the synapses. The NMDARs have been well studied in term of learning and memory in the hippocampus (Rezvani et al., 2006). GluN1 (a subunit of NMDAR) knockout mice have been shown to have spatial memory impairment in Morris water maze experiments (Tsien et al., 1996). Several other reports also indicate the role of NMDARs in synaptic plasticity. Thus, reduction in either expression or activity of NMDARs will influence learning and memory processes. Therefore, it is reasonable presume that changes in NMDARs expression/function could be another factor that might contribute to cognitive deficits associated with chemobrain.

In conclusion, my findings deliver new evidence on the potential molecular mechanisms of cognitive dysfunction in cancer survivors following chemotherapy. Our results show that

altered AMPARs expression and function leads to deficits in excitatory glutamatergic neurotransmission resulting in impaired hippocampal synaptic plasticity mechanisms associated with learning and memory. Future studies should be directed in exploring the molecular and cellular mechanisms by which PAESe ameliorates synaptic deficits and cognitive impairment caused by chemotherapy.

Acknowledgements:

We gratefully thank Dr. Robert Arnold for providing us the treated animals.

References

Ahles, T. A., & Saykin, A. J. (2007). Candidate mechanisms for chemotherapy-induced cognitive changes. Nature Reviews Cancer, 7(3), 192-201. doi: Doi 10.1038/Nrc2073

Al-Abd, A. M., Kim, N. H., Song, S. C., Lee, S. J., & Kuh, H. J. (2009). A simple HPLC method for doxorubicin in plasma and tissues of nude mice. Arch Pharm Res, 32(4), 605-611. doi: 10.1007/s12272-009-1417-5

Arnsten, A. F., & Goldman-Rakic, P. S. (1985). Alpha 2-adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. Science, 230(4731), 1273-1276.

Anderson, W. W., & Collingridge, G. L. (2001). The LTP Program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. J Neurosci Methods, 108(1), 71-83. doi: Doi 10.1016/S0165-0270(01)00374-0

Barzilai, A., & Yamamoto, K. (2004). DNA damage responses to oxidative stress. DNA Repair (Amst), 3(8-9), 1109-1115. doi: 10.1016/j.dnarep.2004.03.002

Beique, J. C., Lin, D. T., Kang, M. G., Aizawa, H., Takamiya, K., & Huganir, R. L. (2006). Synapse-specific regulation of AMPA receptor function by PSD-95. Proc Natl Acad Sci U S A, 103(51), 19535-19540. doi: 10.1073/pnas.0608492103

Betzen, C., White, R., Zehendner, C. M., Pietrowski, E., Bender, B., Luhmann, H. J., & Kuhlmann, C. R. (2009). Oxidative stress upregulates the NMDA receptor on cerebrovascular endothelium. Free Radic Biol Med, 47(8), 1212-1220. doi: 10.1016/j.freeradbiomed.2009.07.034

Beversdorf, D. Q., White, D. M., Chever, D. C., Hughes, J. D., & Bornstein, R. A. (2002). Central beta-adrenergic modulation of cognitive flexibility. Neuroreport, 13(18), 2505-2507. doi: 10.1097/01.wnr.0000048923.00321.a7

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature, 361(6407), 31-39. doi: 10.1038/361031a0

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol, 232(2), 331-356.

Boehm, J., & Malinow, R. (2005). AMPA receptor phosphorylation during synaptic plasticity. Biochem Soc Trans, 33(Pt 6), 1354-1356. doi: 10.1042/BST20051354

Bredt, D. S., & Nicoll, R. A. (2003). AMPA receptor trafficking at excitatory synapses. Neuron, 40(2), 361-379. doi: Doi 10.1016/S0896-6273(03)00640-8

Briones, T. L., & Woods, J. (2011). Chemotherapy-induced cognitive impairment is associated with decreases in cell proliferation and histone modifications. BMC Neurosci, 12, 124. doi: 10.1186/1471-2202-12-124

Boaziz, C., Breau, J. L., Morere, J. F., & Israel, L. (1991). [The blood-brain barrier: implications for chemotherapy in brain tumors]. Pathol Biol (Paris), 39(8), 789-795.

Burden, D. A., & Osheroff, N. (1998). Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme. Biochim Biophys Acta, 1400(1-3), 139-154.

Chabner, B. A., & Roberts, T. G., Jr. (2005). Timeline: Chemotherapy and the war on cancer. Nature Reviews Cancer, 5(1), 65-72. doi: 10.1038/nrc1529

Chen, B. S., Gray, J. A., Sanz-Clemente, A., Wei, Z., Thomas, E. V., Nicoll, R. A., & Roche, K. W. (2012). SAP102 mediates synaptic clearance of NMDA receptors. Cell Rep, 2(5), 1120-1128. doi: 10.1016/j.celrep.2012.09.024

Christie, L. A., Acharya, M. M., Parihar, V. K., Nguyen, A., Martirosian, V., & Limoli, C. L. (2012). Impaired cognitive function and hippocampal neurogenesis following cancer chemotherapy. Clin Cancer Res, 18(7), 1954-1965. doi: 10.1158/1078-0432.CCR-11-2000

Collingridge, G. L., Isaac, J. T., & Wang, Y. T. (2004). Receptor trafficking and synaptic plasticity. Nat Rev Neurosci, 5(12), 952-962. doi: 10.1038/nrn1556

Daikhin, Y., & Yudkoff, M. (2000). Compartmentation of brain glutamate metabolism in neurons and glia. J Nutr, 130(4S Suppl), 1026S-1031S.

Davis, S., Butcher, S. P., & Morris, R. G. (1992). The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. J Neurosci, 12(1), 21-34.

Deng, F., Price, M. G., Davis, C. F., Mori, M., & Burgess, D. L. (2006). Stargazin and other transmembrane AMPA receptor regulating proteins interact with synaptic scaffolding protein MAGI-2 in brain. J Neurosci, 26(30), 7875-7884. doi: 10.1523/JNEUROSCI.1851-06.2006

Derkach, V. A., Oh, M. C., Guire, E. S., & Soderling, T. R. (2007). Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat Rev Neurosci, 8(2), 101-113. doi: 10.1038/nrn2055

DeVita, V. T., Jr., & Chu, E. (2008). A history of cancer chemotherapy. Cancer Res, 68(21), 8643-8653. doi: 10.1158/0008-5472.CAN-07-6611

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. Pharmacol Rev, 51(1), 7-61.

Elias, G. M., Elias, L. A., Apostolides, P. F., Kriegstein, A. R., & Nicoll, R. A. (2008). Differential trafficking of AMPA and NMDA receptors by SAP102 and PSD-95 underlies synapse development. Proc Natl Acad Sci U S A, 105(52), 20953-20958. doi: 10.1073/pnas.0811025106

Everitt, B. J., & Robbins, T. W. (1997). Central cholinergic systems and cognition. Annu Rev Psychol, 48, 649-684. doi: 10.1146/annurev.psych.48.1.649

Farr, S. A., Banks, W. A., & Morley, J. E. (2006). Effects of leptin on memory processing. Peptides, 27(6), 1420-1425. doi: 10.1016/j.peptides.2005.10.006

Feinstein, E., Canaani, E., & Weiner, L. M. (1993). Dependence of nucleic acid degradation on in situ free-radical production by adriamycin. Biochemistry, 32(48), 13156-13161.

Fremouw, T., Fessler, C. L., Ferguson, R. J., & Burguete, Y. (2012). Preserved learning and memory in mice following chemotherapy: 5-Fluorouracil and doxorubicin single agent treatment, doxorubicin-cyclophosphamide combination treatment. Behav Brain Res, 226(1), 154-162. doi: 10.1016/j.bbr.2011.09.013

Fink, C. C., & Meyer, T. (2002). Molecular mechanisms of CaMKII activation in neuronal plasticity. Curr Opin Neurobiol, 12(3), 293-299.

Fonnum, F. (1984). Glutamate: a neurotransmitter in mammalian brain. Journal of Neurochemistry, 42(1), 1-11.

Fukui, K., Omoi, N. O., Hayasaka, T., Shinnkai, T., Suzuki, S., Abe, K., & Urano, S. (2002). Cognitive impairment of rats caused by oxidative stress and aging, and its prevention by vitamin E. Ann N Y Acad Sci, 959, 275-284.

Geiger, J. R., Melcher, T., Koh, D. S., Sakmann, B., Seeburg, P. H., Jonas, P., & Monyer, H. (1995). Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron, 15(1), 193-204.

Gisou, M., Soheila, R., & Nasser, N. (2009). Evaluation of the effect of intrahippocampal injection of leptin on spatial memory. African Journal of Pharmacy and Pharmacology, 3(9), 443-448.

Goldberg, J. E., & Schwertfeger, K. L. (2010). Proinflammatory cytokines in breast cancer: mechanisms of action and potential targets for therapeutics. Curr Drug Targets, 11(9), 1133-1146.

Hebb, D. O. The organization of behavior. New York: Wiley; 1949

Ho, V. M., Lee, J. A., & Martin, K. C. (2011). The cell biology of synaptic plasticity. Science, 334(6056), 623-628. doi: 10.1126/science.1209236

Hollmann, M., Hartley, M., & Heinemann, S. (1991). Ca2+ permeability of KA-AMPA--gated glutamate receptor channels depends on subunit composition. Science, 252(5007), 851-853.

Hopkins, S. J. (2007). Central nervous system recognition of peripheral inflammation: a neural, hormonal collaboration. Acta Biomed, 78 Suppl 1, 231-247.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci, 24, 677-736. doi: 10.1146/annurev.neuro.24.1.677

Hume, R. I., Dingledine, R., & Heinemann, S. F. (1991). Identification of a site in glutamate receptor subunits that controls calcium permeability. Science, 253(5023), 1028-1031.

Inagaki, M., Yoshikawa, E., Matsuoka, Y., Sugawara, Y., Nakano, T., Akechi, T., . . . Uchitomi, Y. (2007). Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. Cancer, 109(1), 146-156. doi: 10.1002/cncr.22368

Jaiswal, M., LaRusso, N. F., Burgart, L. J., & Gores, G. J. (2000). Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. Cancer Res, 60(1), 184-190.

Janelsins, M. C., Roscoe, J. A., Berg, M. J., Thompson, B. D., Gallagher, M. J., Morrow, G. R., . . . Gross, R. A. (2010). IGF-1 partially restores chemotherapy-induced reductions in neural cell proliferation in adult C57BL/6 mice. Cancer Invest, 28(5), 544-553. doi: 10.3109/07357900903405942

Janz, R., Sudhof, T. C., Hammer, R. E., Unni, V., Siegelbaum, S. A., & Bolshakov, V. Y. (1999). Essential roles in synaptic plasticity for synaptogyrin I and synaptophysin I. Neuron, 24(3), 687-700.

Jenkins, V., Shilling, V., Deutsch, G., Bloomfield, D., Morris, R., Allan, S., . . . Winstanley, J. (2006). A 3-year prospective study of the effects of adjuvant treatments on cognition in women with early stage breast cancer. Br J Cancer, 94(6), 828-834. doi: 10.1038/sj.bjc.6603029

Jerusalinsky, D., Kornisiuk, E., & Izquierdo, I. (1997). Cholinergic neurotransmission and synaptic plasticity concerning memory processing. Neurochem Res, 22(4), 507-515.

Kang, J. Y., Costyn, L. J., Nagy, T., Cowan, E. A., Oldham, C. D., May, S. W., & Arnold, R. D. (2011). The antioxidant phenylaminoethyl selenide reduces doxorubicin-induced cardiotoxicity in a xenograft model of human prostate cancer. Arch Biochem Biophys, 515(1-2), 112-119. doi: 10.1016/j.abb.2011.08.008

Kim, M. S., Akhtar, M. W., Adachi, M., Mahgoub, M., Bassel-Duby, R., Kavalali, E. T., . . . Monteggia, L. M. (2012). An essential role for histone deacetylase 4 in synaptic plasticity and memory formation. J Neurosci, 32(32), 10879-10886. doi: 10.1523/JNEUROSCI.2089-12.2012

Klann, E., & Dever, T. E. (2004). Biochemical mechanisms for translational regulation in synaptic plasticity. Nat Rev Neurosci, 5(12), 931-942. doi: 10.1038/nrn1557

Koike, M., Tsukada, S., Tsuzuki, K., Kijima, H., & Ozawa, S. (2000). Regulation of kinetic properties of GluR2 AMPA receptor channels by alternative splicing. J Neurosci, 20(6), 2166-2174.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell, 128(4), 693-705. doi: DOI 10.1016/j.cell.2007.02.005

Krabbe, K. S., Reichenberg, A., Yirmiya, R., Smed, A., Pedersen, B. K., & Bruunsgaard, H. (2005). Low-dose endotoxemia and human neuropsychological functions. Brain Behav Immun, 19(5), 453-460. doi: 10.1016/j.bbi.2005.04.010

Lee, G. D., Longo, D. L., Wang, Y., Rifkind, J. M., Abdul-Raman, L., Mamczarz, J. A., . . . Ingram, D. K. (2006). Transient improvement in cognitive function and synaptic plasticity in rats following cancer chemotherapy. Clinical Cancer Research, 12(1), 198-205. doi: Doi 10.1158/1078-0432.Ccr-05-1286

Lee, H. K., Takamiya, K., Han, J. S., Man, H. Y., Kim, C. H., Rumbaugh, G., . . . Huganir, R. L. (2003). Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell, 112(5), 631-643. doi: Doi 10.1016/S0092-8674(03)00122-3

Lee, H. K., Takamiya, K., He, K., Song, L., & Huganir, R. L. (2010). Specific roles of AMPA receptor subunit GluR1 (GluR1) phosphorylation sites in regulating synaptic plasticity in the CA1 region of hippocampus. J Neurophysiol, 103(1), 479-489. doi: 10.1152/jn.00835.2009

Leuner, B., & Gould, E. (2010). Structural plasticity and hippocampal function. Annu Rev Psychol, 61, 111-140, C111-113. doi: 10.1146/annurev.psych.093008.100359

Levenson, J. M., O'Riordan, K. J., Brown, K. D., Trinh, M. A., Molfese, D. L., & Sweatt, J. D. (2004). Regulation of histone acetylation during memory formation in the hippocampus. J Biol Chem, 279(39), 40545-40559. doi: 10.1074/jbc.M402229200

Lisman, J., Yasuda, R., & Raghavachari, S. (2012). Mechanisms of CaMKII action in long-term potentiation. Nat Rev Neurosci, 13(3), 169-182. doi: 10.1038/nrn3192

Lu, B., & Chow, A. (1999). Neurotrophins and hippocampal synaptic transmission and plasticity. J Neurosci Res, 58(1), 76-87.

Luscher, C., & Malenka, R. C. (2012). NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb Perspect Biol*, *4*(6). doi: 10.1101/cshperspect.a005710

Massaad, C. A., & Klann, E. (2011). Reactive oxygen species in the regulation of synaptic plasticity and memory. Antioxid Redox Signal, 14(10), 2013-2054. doi: 10.1089/ars.2010.3208

Matsuda, S., Kakegawa, W., Budisantoso, T., Nomura, T., Kohda, K., & Yuzaki, M. (2013). Stargazin regulates AMPA receptor trafficking through adaptor protein complexes during long-term depression. Nature Communications, 4. doi: Artn 2759

Mayford, M., Siegelbaum, S. A., & Kandel, E. R. (2012). Synapses and memory storage. *Cold Spring Harb Perspect Biol, 4*(6). doi: 10.1101/cshperspect.a005751

Melnikova, V. O., Dobroff, A. S., Zigler, M., Villares, G. J., Braeuer, R. R., Wang, H., . . . Bar-Eli, M. (2010). CREB inhibits AP-2alpha expression to regulate the malignant phenotype of melanoma. PLoS One, 5(8), e12452. doi: 10.1371/journal.pone.0012452

Meng, F., Guo, J., Zhang, Q., Song, B., & Zhang, G. (2003). Autophosphorylated calcium/calmodulin-dependent protein kinase II alpha (CaMKII alpha) reversibly targets to and phosphorylates N-methyl-D-aspartate receptor subunit 2B (NR2B) in cerebral ischemia and reperfusion in hippocampus of rats. Brain Res, 967(1-2), 161-169.

Montana, V., Ni, Y., Sunjara, V., Hua, X., & Parpura, V. (2004). Vesicular glutamate transporter-dependent glutamate release from astrocytes. J Neurosci, 24(11), 2633-2642. doi: 10.1523/JNEUROSCI.3770-03.2004

Mortensen, M., & Smart, T. G. (2007). Single-channel recording of ligand-gated ion channels. Nat Protoc, 2(11), 2826-2841. doi: 10.1038/nprot.2007.403

Murata, Y., & Constantine-Paton, M. (2013). Postsynaptic density scaffold SAP102 regulates cortical synapse development through EphB and PAK signaling pathway. J Neurosci, 33(11), 5040-5052. doi: 10.1523/JNEUROSCI.2896-12.2013

Munger, C., Ellis, A., Woods, K., Randolph, J., Yanovich, S., & Gewirtz, D. (1988). Evidence for inhibition of growth related to compromised DNA synthesis in the interaction of daunorubicin with H-35 rat hepatoma. Cancer Res, 48(9), 2404-2411.

Myers, J. S., Pierce, J., & Pazdernik, T. (2008). Neurotoxicology of chemotherapy in relation to cytokine release, the blood-brain barrier, and cognitive impairment. Oncol Nurs Forum, 35(6), 916-920. doi: 10.1188/08.ONF.916-920

Oomura, Y., Hori, N., Shiraishi, T., Fukunaga, K., Takeda, H., Tsuji, M., . . . Sasaki, K. (2006). Leptin facilitates learning and memory performance and enhances hippocampal CA1 long-term potentiation and CaMK II phosphorylation in rats. Peptides, 27(11), 2738-2749. doi: 10.1016/j.peptides.2006.07.001

Parameshwaran, K., Buabeid, M. A., Karuppagounder, S. S., Uthayathas, S., Thiruchelvam, K., Shonesy, B., . . . Suppiramaniam, V. (2012). Developmental nicotine exposure induced alterations in behavior and glutamate receptor function in hippocampus. Cell Mol Life Sci, 69(5), 829-841. doi: 10.1007/s00018-011-0805-4

Persad, S., Attwell, S., Gray, V., Delcommenne, M., Troussard, A., Sanghera, J., & Dedhar, S. (2000). Inhibition of integrin-linked kinase (ILK) suppresses activation of protein kinase B/Akt and induces cell cycle arrest and apoptosis of PTEN-mutant prostate cancer cells. Proc Natl Acad Sci U S A, 97(7), 3207-3212. doi: 10.1073/pnas.060579697

Peyrat, J. P., Revillion, F., & Bonneterre, J. (1998). Plasma insulin-like growth factor in primary breast cancer patients treated with adjuvant chemotherapy. Br J Cancer, 77(10), 1669-1671.

Rao, V. R., & Finkbeiner, S. (2007). NMDA and AMPA receptors: old channels, new tricks. Trends Neurosci, 30(6), 284-291. doi: 10.1016/j.tins.2007.03.012

Reisinger, C., Yelamanchili, S. V., Hinz, B., Mitter, D., Becher, A., Bigalke, H., & Ahnert-Hilger, G. (2004). The synaptophysin/synaptobrevin complex dissociates independently of neuroexocytosis. Journal of Neurochemistry, 90(1), 1-8. doi: 10.1111/j.1471-4159.2004.02472.x

Rezvani, A. H. (2006). Involvement of the NMDA System in Learning and Memory. In E. D. Levin & J. J. Buccafusco (Eds.), Animal Models of Cognitive Impairment. Boca Raton (FL).

Rodier, F., Coppe, J. P., Patil, C. K., Hoeijmakers, W. A., Munoz, D. P., Raza, S. R., . . . Campisi, J. (2009). Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol, 11(8), 973-979. doi: 10.1038/ncb1909

Schagen, S. B., van Dam, F. S., Muller, M. J., Boogerd, W., Lindeboom, J., & Bruning, P. F. (1999). Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. Cancer, 85(3), 640-650.

Seruga, B., Zhang, H. B., Bernstein, L. J., & Tannock, I. F. (2008). Cytokines and their relationship to the symptoms and outcome of cancer. Nature Reviews Cancer, 8(11), 887-899. doi: Doi 10.1038/Nrc2507

Shonesy, B. C., Thiruchelvam, K., Parameshwaran, K., Rahman, E. A., Karuppagounder, S. S., Huggins, K. W., . . . Suppiramaniam, V. (2012). Central insulin resistance and synaptic dysfunction in intracerebroventricular-streptozotocin injected rodents. Neurobiol Aging, 33(2), 430 e435-418. doi: 10.1016/j.neurobiolaging.2010.12.002

Schagen, S. B., van Dam, F. S., Muller, M. J., Boogerd, W., Lindeboom, J., & Bruning, P. F. (1999). Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. Cancer, 85(3), 640-650.

Snyder, G. L., Allen, P. B., Fienberg, A. A., Valle, C. G., Huganir, R. L., Nairn, A. C., & Greengard, P. (2000). Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. J Neurosci, 20(12), 4480-4488.

Subramaniam, S., Subramaniam, S., & Shyamala Devi, C. S. (1994). Erythrocyte antioxidant enzyme activity in CMF treated breast cancer patients. Cancer Biochem Biophys, 14(3), 177-182.

Sultani, M., Stringer, A. M., Bowen, J. M., & Gibson, R. J. (2012). Anti-inflammatory cytokines: important immunoregulatory factors contributing to chemotherapy-induced gastrointestinal mucositis. Chemother Res Pract, 2012, 490804. doi: 10.1155/2012/490804

Suppiramaniam, V., Vaithianathan, T., & Parameshwaran, K. (2006). Electrophysiological analysis of interactions between carbohydrates and transmitter receptors reconstituted in lipid bilayers. Functional Glycomics, 417, 80-+. doi: Doi 10.1016/S0076-6879(06)17007-X

Sze, C. I., Troncoso, J. C., Kawas, C., Mouton, P., Price, D. L., & Martin, L. J. (1997). Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. J Neuropathol Exp Neurol, 56(8), 933-944.

Thompson, P. M., Hayashi, K. M., de Zubicaray, G., Janke, A. L., Rose, S. E., Semple, J., . . . Toga, A. W. (2003). Dynamics of gray matter loss in Alzheimer's disease. J Neurosci, 23(3), 994-1005.

Tomita, S., Adesnik, H., Sekiguchi, M., Zhang, W., Wada, K., Howe, J. R., . . . Bredt, D. S. (2005). Stargazin modulates AMPA receptor gating and trafficking by distinct domains. Nature, 435(7045), 1052-1058. doi: 10.1038/nature03624

Tsien, J. Z., Huerta, P. T., & Tonegawa, S. (1996). The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell, 87(7), 1327-1338.

Usuki, K., Okazaki, R., Iki, S., Muramatsu, M., Yamaguchi, Y., Totsuka, Y., & Urabe, A. (1998). Serum leptin levels during cancer chemotherapy. Ann Hematol, 77(4), 191-192.

Wang, J. Q., Arora, A., Yang, L., Parelkar, N. K., Zhang, G., Liu, X., . . . Mao, L. (2005). Phosphorylation of AMPA receptors: mechanisms and synaptic plasticity. Mol Neurobiol, 32(3), 237-249. doi: 10.1385/MN:32:3:237

Wilson, C. J., Finch, C. E., & Cohen, H. J. (2002). Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. J Am Geriatr Soc, 50(12), 2041-2056.

Woo, N. H., Teng, H. K., Siao, C. J., Chiaruttini, C., Pang, P. T., Milner, T. A., . . . Lu, B. (2005). Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat Neurosci, 8(8), 1069-1077. doi: 10.1038/nn1510

Xu, B., Gottschalk, W., Chow, A., Wilson, R. I., Schnell, E., Zang, K., . . . Reichardt, L. F. (2000). The role of brain-derived neurotrophic factor receptors in the mature hippocampus: modulation of long-term potentiation through a presynaptic mechanism involving TrkB. J Neurosci, 20(18), 6888-6897.

Yarlagadda, A., Alfson, E., & Clayton, A. H. (2009). The blood brain barrier and the role of cytokines in neuropsychiatry. Psychiatry (Edgmont), 6(11), 18-22.

Yirmiya, R., & Goshen, I. (2011). Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun, 25(2), 181-213. doi: 10.1016/j.bbi.2010.10.015

Zhang, S., Liu, X., Bawa-Khalfe, T., Lu, L. S., Lyu, Y. L., Liu, L. F., & Yeh, E. T. (2012). Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nat Med, 18(11), 1639-1642. doi: 10.1038/nm.2919