Role of Histone Methylation in Extinction of Fear Memory

THESIS

SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
LUCKNOW
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN
APPLIED ANIMAL SCIENCES

Submitted By

Rohit Kanojia

(Enrolment No. 186/12)

Supervisor
PROF. KAMAL JAISWAL
Professor and Head
Department of Applied Animal Sciences
School of Biosciences and Biotechnology
Babasaheb Bhimrao Ambedkar University,
Vidya Vihar, Raebareli Road,
Lucknow-226025

Co-supervisor
PROF. D.R. MODI
Professor
Department of Biotechnology,
Dean, School of Biosciences and Biotechnology
Babasaheb Bhimrao Ambedkar University,
Vidya Vihar, Raebareli Road,
Lucknow-226025

DEPARTMENT OF APPLIED ANIMAL SCIENCES
(SCHOOL OF BIOSCIENCES AND BIOTECHNOLOGY)
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A Central University, NAAC 'A' Grade)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226025
UTTAR PRADESH INDIA

2020

DEDICATED

TO

MY PARENTS,

MY TEACHERS & MY LOVING

FRIENDS

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

बाबासाहेब भीमराव अम्बेडकर विश्वविद्यालय
(केन्द्रीय विश्वविद्यालय)
विद्या विहार, रायबरेली रोड, लखनऊ– 226025
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A Central University)
Vidya Vihar, Rae Bareli Road, Lucknow-226025

CERTIFICATE

This is to certify that the thesis titled **"Role of Histone Methylation in Extinction of Fear Memory"** submitted by **Mr. Rohit Kanojia** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other University.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations-1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

10/01/2020

Supervisor
PROF. KAMAL JAISWAL

Professor and Head
Department of Applied Animal Sciences
School of Biosciences and Biotechnology
Babasaheb Bhimrao Ambedkar University,
Vidya Vihar, Raebareli Road,
Lucknow-226025

Co-supervisor
PROF. D.R. MODI

Professor
Department of Biotechnology,
Dean, School of Biosciences and Biotechnology
Babasaheb Bhimrao Ambedkar University,
Vidya Vihar, Raebareli Road,
Lucknow-226025

Date : 10 Jan 2020

CANDIDATE'S DECLARATION

I, **Mr. Rohit Kanojia (Enrolment no. 186/12)**, certify that the work embodied in this thesis entitled **"Role of Histone Methylation in Extinction of Fear Memory"** is my own bonafide work carried out by me under the supervision of Prof. Kamal Jaiswal at Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, (A Central University), Vidya Vihar, Rai Bareilly Road, Lucknow-226025, INDIA. The matter authenticated in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged, given credit and referred to the research workers wherever their works have been cited in the text and the body of the thesis.

Date: 10 Jan 2020

Place: Lucknow

Rohit

Name: Rohit Kanojia

ACKNOWLEDGEMENTS

First of all, I bow in reverence to almighty God "the creator of the universe", the most benevolent and merciful, for providing enough patience, zeal and strength that enabled me to complete my Doctor of Philosophy successfully.

The present study was carried out at Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, (A Central University), Vidya Vihar, Rai Bareilly Road, Lucknow-226025, Uttar pradesh. I wish to express my deepest gratitude to my supervisor, ***Prof. Kamal Jaiswal, Head, Department of Applied Animal Sciences,*** *Babasaheb Bhimrao Ambedkar University, (A Central University), Vidya Vihar, Rai Bareilly Road, Lucknow-226025, Uttar pradesh.*

My sincere thanks are also due to my ***co-supervisor Prof. Dinesh Raj Modi,*** *Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, (A Central University), Vidya Vihar, Rai Bareilly Road, Lucknow-226025, Uttar pradesh. for his ideology and support with this study. I specially acknowledge him for inspiring ideas in areas of life sciences.*

I am extremely thankful to ***Dr. Suman Mishra and Dr. U.V Kiran for*** *his constant support and motivation in completing my research work. I also thank* ***Dr. V. Elongvan, Dr. Abha Mishra, Dr. Venkatesh, Dr. Sandhya,*** *and staff Member* ***Deepak sir, Arun sir, Kavita mam, Shashank and Deepti mam*** *of the department for their timely help, cooperation and care.*

I would also like to acknowledge ***Prof. Anand Prakash, Dr. Madhukar Saxena, Dr. Sarfraj Ahmad, Mr. Sanjay Singh, Ms. Sukanya*** *and all research scholars for their timely motivation. My special thanks to those subjects who were directly or indirectly involved in donating their blood for the study.*

My labmates and friends especially ***Amit mishra, Sanjay, Sarfaraz, Sukanya, Atul, Abishek, Raj kumar khalgo, Sneha mam, and Ms. Rashmi*** *helped me by putting up with me and my temper.*

In addition, I am indebted to my beloved and benevolent brother ***Rahul Kanojia, Roshan Kanojia****, and* ***my Sister Neha Kanojia*** *for their kind cooperation and constant support*

Last but not the least, I am short of words to express my heartfelt gratitude to my father ***Mr. Moolchand*** *and Mother Mrs.* ***Daya Kanojia.*** *I am indebted to their love, noble guidance and sincere efforts that inspired me to academics and do something meaningful in life. I owe them a lot more than a mere word of thanks.*

Date: 10 Jan 2020

Place: Lucknow

Rohit

Name: Rohit Kanojia

बाबासाहेब भीमराव अम्बेडकर विश्वविद्यालय

(केन्द्रीय विश्वविद्यालय)

विद्या विहार, रायबरेली रोड, लखनऊ–226 025

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A Central University)

Vidya Vihar, Raebareli Road, Lucknow-226 025

Letter No.-213.../COE/BBAU/2018 Dated: 20/07/2018

Ph.D. Course Work Certificate

This is to certify that **Rohit Kanojia**, Enrollment No. 186/12 Ph.D. Research Scholar, Department of Applied Animal Sciences of the University has successfully completed his Ph.D. Course work in the examination held during December, 2015.

Controller of Examinations

CONTENT

ABBREVIATIONS

AMPA : amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

BA : Basal nucleus of amygdala

BDNF : Brain Derived Neurotrophic Factor

BLA : Basolateral amygdala

CA : Cornu Ammonis

CBP : CREB Binding Protein

CBT : Combined behavioral therapy

CeA : Central nucleus of amygdala

CeL : Lateral central nucleus of amygdala

CeM : Medial central nucleus of amygdala

Cond : Condition/conditioned

CR : Conditioned Response

CREB : Cyclic-AMP Response Element Binding protein

CS : Conditioned Stimulus

Ctx : Context

DG : Dentate gyrus

DH : Dorsal hippocampus

EPM : Elevated plus maze

Ext : Extinction

HAT : Histone Acetyl Transfearse

HDAC : Histone Deacetylase

IHC : Immunohistochemistry

IL :	Infralimbic cortex of PFC
ITC :	Intercalated cell mass
LA :	Lateral nucleus of amygdala
LTM :	Long Term Memory
LTP :	Long Term Potentiation
MAPK:	Mitogen Activated Protein Kinase
NMDA :	N-methyl D-aspartate
PBS :	Phosphate Buffered Saline
PL :	Prelimbic cortex
PFC :	Prefrontal cortex
PKC :	Protein kinase C
PTSD :	Post Traumatic Stress Disorders
RT-PCR :	Real Time Polymerase Chain Reaction
SAHA :	Suberoylanilide Hydroxamic Acids
STM :	Short Term Memory
TSA :	Trichostatin A
US :	Unconditioned Stimulus
VH :	Ventral hippocampus
VPA :	Valproic acid

LIST OF FIGURES

ABSTRACT

Fear is psychological and psycho physiological response to a perceived external threat that is consciously recognized as a danger. Failure to extinguish traumatic memories can lead to the development of fear associated with anxiety. This is a major challenge amongst researchers and scientists to come up with newer paradigms of exposure therapies for the effective treatment of fear related anxiety disorders. Fear conditioning in rats is a valuable model for study of cellular/molecular mechanism related to learning as well as memory. Intercalated Cell Mass (ITCs) within the amygdala are key regulatory elements controlling emotional responses by controlling amygdala circuits. Central Amygdala (CeA) is the main output relay for the fear response and has innervations of fear traces from Basolateral Amygdala (BLA) as well as from ITCs. Histone methylation is one of the important mechanisms amongst other histone modifications for controlling behavioral outcome through the regulation of gene. In the present report the expression of histone H3/K9 methylation during fear learning and extinction in rats may change the behavioral outcomes as well as the role of histone modifications especially histone methylation was studied in correlation to ARC, CREB, p-CREB to find out whether fear extinction is a inhibitory learning, erasure of memory or both. We found that the expression of p-CREB was higher in extinction group as compared to their respective control groups in the LA region. However, the changes were insignificant between immediate and delayed extinction group. In BA, the expression of p-CREB was significantly higher in delayed extinction group ($p \leq 0.000$) While the CREB expression was higher in extinction groups as compared to their respective control groups but no significant changes were observed between immediate and delayed extinction groups in LA region. Similarly the ARC, expression was higher in extinction groups as compared to their respective control groups but no significant changes were observed between immediate and delayed extinction groups in LA region. Moreover, the level of histone methylation in amygdale was suppressed. The present research highlights the analysis of molecular events in parts of brain an has a great impact on the methods of physiological and psychological researches specially in the field of behavioral science. We further hypothesized that the mechanism, how timing of fear extinction for a specific fear affect the histone methylation and their effect in retracement. Fear memory acquisition followed by extension training was given to rats for 10 minutes which deficits in the retention of extinction memory when compared to the other which went for 24 hours of extinction after fear acquisition. The first one is immediate extinction (IE) and the second is delayed extinction (DE). We analyzed that the activity of

infralimbic prefrontal cortex (IL) to prelimbic cortex (PL) was decreased significantly in ($p \leq 0.000$) IE when compared to DE and confounded with the activity and expression of c-fos in mPFC. As a confirmation we further analyzed the methylation of histone H3/K9 and levels of CREB binding protein (CBP) which is a histone methyaltransferase (HAT) and found that this was associated with the activation of neuron and is significantly decreased in IL of IE as compared to the DE ($p \leq 0.000$) . We finally conclude that the deficits in IE is mainly due to the sustained activation of IL because of it is associated with the changes involved in histone methylation.

Introduction

Fear is psychological and psycho physiological response to a perceived external threat that is consciously recognized as a danger. Fear response includes increased alertness; concentration on the source of fear; attack and fight or flight behaviors and evidence of sympathetic nerve stimulation such as cardiovascular excitation, ,superficial vasoconstriction and dilation of the pupils. Failure to extinguish traumatic memories can lead to the development of fear associated anxiety disorders viz. Post traumatic stress disorder (PTSD) (Van Elzakker et al., 2014). These kind of people are mainly cured by exposure therapy based on extinction learning and its retention (Craske et al., 2008; Rothbaum et al., 2003; Bouton et al., 2001) and the most importantly reported by Pavlovian based fear conditioning in rats (Maren, 2005; Pare, 2004; LeDoux, 2000). In healthy subjects, the main brain regions i.e. prefrontal cortex (PFC), hippocampus and amygdala are critical for processing fearful and other kind of emotional stimuli and fear learning to extinguish fear in situation that are no longer threatening. This is a major challenge amongst researchers and scientists to come up with newer paradigms of exposure therapies for the effective treatment of fear related anxiety disorders (Muigg et al., 2008; Wessa and Flor, 2007; Myers and Davis, 2002). Any disturbance in it will develop fear related disorders such as post- traumatic Stress disorder (PTSD). The treatment paradigms for these fear related disorders are at par and the wounded have to lead a pathetic life. However, erasure of fear memory is a phenomenon during which there is reversal of molecular events taking place during fear acquisition and extinction. Previous reports suggest that the timing of extinction after fear learning had a varied effect on the strength of extinction (Golkar et al., 2012; Huff et al., 2009). Interestingly, it was found that extinction training performed immediately after the fear learning resulted in either “erasure” (Norrholm et al., 2008) or fear reduction (Chang and Maren, 2009). Signaling cascades in brain structures are reportedly important in fear acquisition and extinction through inhibitory learning. Although histone modification occurs throughout the sequence, the unstructured N-termini of histones (called histone tails) are most extensively modified. These modifications include acetylation, methylation, ubiquitylation, phosphorylation and sumoylation. Thus, -CH3 is found to induce repression of transcription and hence shows to influence a rat in memory consolidation as well as extinction. Fear

conditioning in rats is a valuable model for studying of cellular/molecular mechanism related to learning as well as memory. Intercalated Cell Mass (ITCs) within the amygdala is key regulatory element controlling emotional responses by controlling amygdala circuits. Central Amygdala (CeA) is the main output relay for the fear response and has innervations of fear traces from Basolateral Amygdala (BLA) as well as from ITCs. Extinction of fear involves extinction trace innervations into CeA from ITCs directly and from prefrontal cortex (PFC) via ITCs. Histone methylation is one of the important mechanisms amongst other histone modifications for controlling behavioral outcome through the regulation of gene. Amygdala micro-circuitry is integrated with different interconnected nuclei having different neuronal types and connections. Central Amygdala (CeA) is the main output circuitry for the fear response which has innervations of fear traces from basolateral amygdala (BLA) as well as from Intercalated cell mass (ITCs). As CeM (medial nucleus of the central amygdala) is the main output nucleus for the fear responses it is controlled by CeL (lateral nucleus of the central Amygdala) as well as by ITCv (ventral intercalated neurons) in different states of fear conditioning and extinction. During fear extinction new connections are formed in brain in amygdala, prefrontal cortex, hippocampus and brain stem (Herry et al., 2010). Deciphering the signaling pathways and circuitries leading to erasure of fear memory will be of immense importance as the treatment paradigms based on inhibitory learning are not adequate and fear memory gets reactivated even after several rounds of extinction training. Keeping in view the large number of people suffering from these conditions it becomes imperative to come up with newer drug targets which may help in overcoming these conditions. In the Present research, the impact of expression of histone H3/H4 methylation during fear learning and extinction on the behavioral outcomes. As hypothesized, the role of histone modifications especially histone methylation of H4/K9 was studied in correlation to activity regulated cytoskeleton associated protein (ARC), cAMP response element binding protein (CREB), phospho-cAMP response element binding protein (p-CREB) and its target gene and also explored whether fear extinction is a inhibitory learning erasure of memory or both.

Post traumatic stress disorder (PTSD), a major fear related anxiety disorders, is developed mainly due to the failure to extinguish traumatic memories in many persons (Van Elzakker et al., 2014). These persons are mainly under the treatment

therapy of exposure usually based on extinction learning followed by retention (Craske et al., 2008). Pavlovian translational model is the bench mark for researchers working on fear related anxiety disorders (Maren, 2005; Pare et al., 2004). The model well describes the effects of conditioned stimulus (CS) and unconditioned stimulus (US) (Myers and Davis, 2007; Bouton et al., 2006; Pavlov, 1927). Now this is been taken up as a challenge by many researchers around the globe and worked to design new therapies for the effective treatment for such disorders (Muigg et al., 2008; Wessa and Flor, 2007; Rosen and Schulkin, 1998). Published reports suggests that the fear learning followed by extinction timing had a varied effect on extinction strength (Golkar et al., 2012; Huff et al., 2009; Maren and Chang, 2006; Myers et al., 2006; Norrholm et al., 2008). It was reported that in fear learning followed by extinction training results either "erasure" (Norrholm et al., 2008) or may reduce the fear (Chang and Maren, 2009). Moreover, other reports published controversial results on fear extinction and suggest that the IE was not as effective as in the case of DE in inhibiting the return of fear, known as ''immediate extinction deficit (IED)" (Maren, 2014; Stafford et al., 2013; Long and Fanselow, 2012; Archbold et al., 2010; Kim et al., 2010; Wood et al., 2005). Apart from this, Chang and Maren (2009) found that the reduction in fear observed after IE is for short time which may be via. short term habituation and not a long term extinction.

From published reports, it was suggested that the altered neural activity basically, in the region of amygdala and IL subregion of mPFC plays a major role in the regulation of fear (Greenberg et al., 2013) as well as memory extinction (Sotres-Bayon et al., 2006; Quirk et al., 2000). This was supported by other studies where lesions of mPFC results the impairment to recall the memory extinction (Milad and Quirk, 2002). During fear and extinction learning, the infralimbic prefrontal cortex (IL) and prelimbic prefrontal cortex (PL) subregions of the mPFC play an important role (Quirk and Mueller, 2008) and the activity of IL positively correlates to recall the memory extinction (Milad and Quirk, 2002) as well as the PL activity to the expression of fear response (Burgos-Robles et al., 2009; Likhtik et al., 2005). Histone acetyl transferases (HATs), like CREB-binding protein (CBP/p300), are involved in the acetylation of histone at Lysine residues which is significantly associated with the consolidation of memory following fear and extinction learning (Alarcon et al., 2004; Levenson et al., 2004; Sintoni et al., 2013; Stefanko et al., 2009). Increased histone

methylation (H4) in neurons of IL-PFC is a well documented fact in the storage of fear extinction memories (Ferreira et al., 2015). Histone methylation (H3) in CA1 (field CA1 of the hippocampus) is important for contextual fear learning (Miller et al., 2008; Lubin and Sweatt, 2007). To hypothesize this, we have focused to find the effect of neuronal activity in IL with association of retention of extinction memory and changed neuronal activity in the IL following IE and may lead to the deficits in retention of memory extinction.

The limited neurological understanding especially the molecular mechanism involved in fear extinction has been attributed to the need for improved animal models for the treatment of anxiety disorders. It was hypothesized that how timing of fear extinction for a specific fear affect the histone methylation and their effect in retracement. Fear memory acquisition followed by extension training was given to rats for 10 minutes which deficits in the retention of extinction memory when compared to the other which went for 24 hours of extinction after fear acquisition. The first one is immediate extinction (IE) and the second is delayed extinction (DE).

We analyzed that the activity of infralimbic prefrontal cortex (IL) to prelimbic cortex (PL) was decreased in IE when compared to DE and confounded with the activity and expression of c-fos in mPFC. As a confirmation we further analyzed the acetylation of histone H3/H4 and levels of CREB binding protein (CBP) which is a histone acetyltransferase (HAT) and found that this was associated with the activation of neuron and is significantly decreased in IL of IE as compared to the DE.

The limited neurological understanding especially the molecular mechanism involved in fear extinction has been attributed to the need for improved animal models for the treatment of anxiety disorders. We presently hypothesized that the mechanism, how timing of fear extinction for a specific fear affect the histone methylation and their effect in retracement. Fear memory acquisition followed by extension training was given to rats for 10 minutes which deficits in the retention of extinction memory when compared to the other which went for 24 hours of extinction after fear acquisition. The first one is immediate extinction (IE) and the second is delayed extinction (DE). We analyzed that the activity of infralimbic prefrontal cortex (IL) to prelimbic cortex (PL) was decreased in IE when compared to DE and confounded with the activity and

expression of c-fos in mPFC. As a confirmation we further analyzed the acetylation of histone H3/H4 and levels of CREB binding protein (CBP) which is a histone acetyltransferase (HAT) and found that this was associated with the activation of neuron and is significantly decreased in IL of IE as compared to the DE. We finally conclude that the deficit in IE is mainly due to the sustained activation of IL because it is associated with the changes involved in histone methylation.

REVIEW OF LITERATURE

Review of Literature

The treatment paradigms for these fear related disorders are at par and the wounded have to lead a pathetic life. However, erasure of fear memory is a phenomenon during which there is reversal of molecular events taking place during fear acquisition and extinction. Previous reports suggest that the timing of extinction after fear learning had a varied effect on the strength of extinction (Golkar et al., 2012; Huff et al., 2009). Interestingly, it was found that extinction training performed immediately after the fear learning resulted in either “erasure” (Norrholm et al., 2008) or fear reduction (Chang and Maren, 2009). Signaling cascades in brain structures are reportedly important in fear acquisition and extinction through inhibitory learning. Although histone modification occurs throughout the sequence, the unstructured N-termini of histones (called histone tails) are most extensively modified. These modifications include acetylation, methylation, ubiquitylation, phosphorylation and sumoylation. Thus, -CH3 is found to induce repression of transcription and hence shows to influence a rat in memory consolidation as well as extinction. Fear conditioning in rats is a valuable model for studying cellular/molecular mechanism related to learning as well as memory.

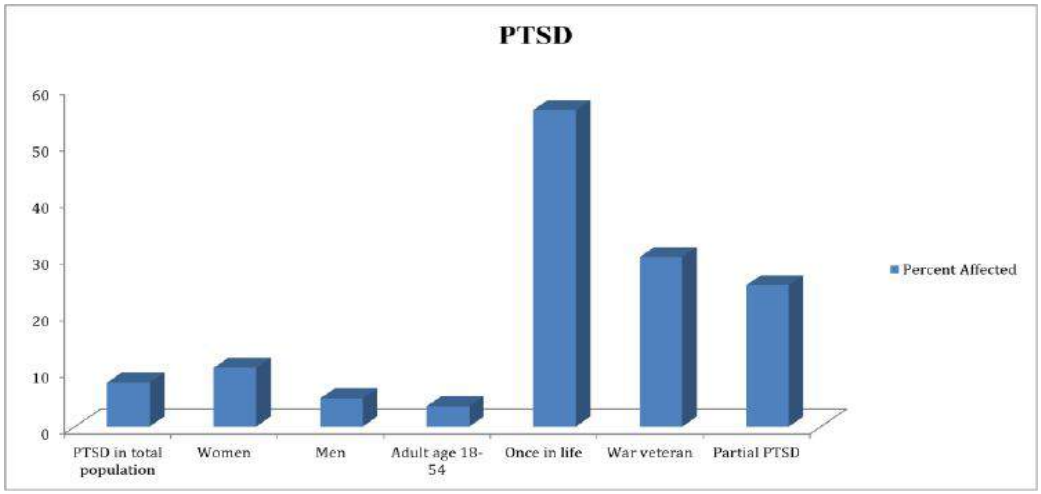


Figure 2.1. Occurrence of PTSD cases in the human population (Iribarren et al., 2005).

Intercalated Cell Mass (ITCs) within the amygdala is key regulatory element controlling emotional responses by controlling amygdala circuits. Central Amygdala (CeA) is the main output relay for the fear response and has innervations of fear traces from Basolateral Amygdala (BLA) as well as from ITCs. Extinction of fear involves extinction trace innervations into CeA from ITCs directly and from prefrontal cortex (PFC) via ITCs. Histone methylation is one of the important mechanisms amongst other histone modifications for controlling behavioral outcome through the regulation of gene. Amygdala micro-circuitry is integrated with different interconnected nuclei having different neuronal types and connections. Central Amygdala (CeA) is the main output circuitry for the fear response which has innervations of fear traces from basolateral amygdala (BLA) as well as from Intercalated cell mass (ITCs). As CeM (medial nucleus of the central amygdala) is the main output nucleus for the fear responses it is controlled by CeL (lateral nucleus of the central Amygdala) as well as by ITCv (ventral intercalated neurons) in different states of fear conditioning and extinction. During extinction new connections are formed in brain in amygdala, prefrontal cortex, hippocampus and brain stem (Herry et al., 2010). Deciphering the signaling pathways and circuitries leading to erasure of fear memory will be of immense importance as the treatment paradigms based on inhibitory learning are not adequate and fear memory gets reactivated even after several rounds of extinction training. Keeping in view the large number of people suffering from these conditions it becomes imperative to come up with newer drug targets which may help in overcoming these conditions.

Memories are highly dynamic in nature and may be of good/ bad depending on the type of consequences. Dreadful events form persistent memory and if they remain for a longer duration of time or occurs in repeated events then they may get converted into fear memory and its associated disorders. This traumatic condition is now considered as a Post-traumatic disorder (PTSD). The prevalence of this is around ~10.0% in global population of world and it belongs to the 4th most common psychiatric disorder in America (Breslau et al., 1991; Kawaguchi, 1995; Schlenger et al., 2002). The best mechanism to be considered for the progression of PTSD includes the formation of associative learning occurred from the environmental cues at the onset of these traumatic events. To analyze this memory based alterations in the laboratory, the Pavlovian fear conditioning model has been proved to be the best

model paradigm.

A survival factor mainly fear memory, the incidence of a fearful event is determined by the animals and their response is generated in turn to get off from such condition. While, the extinction learning includes the therapeutic part where extinction of conditioned fear has direct parallels with cognitive-behavioral treatments like exposure therapy for anxiety disorders reported in humans (Bouton, 1988; Rothbaum and Schwartz, 2002; Mineka and Oehlberg, 2008). In the case of extinction, a previously conditioned stimulus (CS) is repeatedly presented in the absence of an unconditioned stimulus (US) (Pavlov, 1927) which results in suppression of learned fear behavior, often measured as freezing. Although the extinction learning is usually opposed by the effect of conditioned learning, this effect is caused by creating inhibitory learning in place of conditioning (Bouton, 1993; Maren, 2001; Bouton et al. 2006; Ji and Maren 2008) as fear return following the passage of time/context.
Now we can state that this extinguished fear shows association with the specific context. The return of fear memory after extinction is a substantial problem for maintaining long-lasting suppression of fear through exposure therapies (Rodriguez et al., 1999; Hermans et al., 2006; Effting and Kindt, 2007; Quirk and Mueller, 2008). These differences in response to extinction show the effect of environment on extinction which are mainly due to various molecular levels controlled by many mechanisms including changes at epigenetic level (Bredy and Barad, 2008).
Many reports and our present report analysis consolidate that it was identified that the region- specific molecular changes lead to such phenomenon.

The change at the molecular level i.e. chromatin modification plays a pivotal role in consolidation and extinction of fear. Many supporting studies have shown that regulation through histone acetylation/methylation proved to be critical for synaptic plasticity and the formation of memory (Guan et al., 2009; Levenson et al., 2004). However, it was also reported that consolidation and extinction of fear memory are mainly facilitated by the HDAC inhibition (Guan et al., 2009). These reports conclude that the global HDAC inhibitor can act as a target for improvement in learning and also to the memory deficits of neurodegenerative disorders in animals (Levenson et al., 2004; Fischer et al., 2007; Lattal et al., 2007; Vecsey et al., 2007; Barrett and Wood, 2008; Morris et al., 2013; Monsey et al., 2011). This was documented that

HDACs promote a transcriptionally inactive chromatin state usually by removing -acetyl groups from histone tail lysine residues. Mice treated with an HDAC inhibitor mainly affected long-term memory formation while exhibited less effect on short term memory formation (Haberland et al. 2009; Stefanko et al., 2009).

Recent reports and findings have focused on the functional roles of different HDACs in various kinds of brain functions (Guan et al., 2009; Montgomery et al., 2009; Kim et al., 2012). Most of the reports using HDAC inhibitor have revealed mainly the effect of HDAC inhibition on the cognitive function using various behavioral paradigms (e.g., Morris Water maze, fear conditioning), without demarcating whether the effect was for all HDAC classes or their subtypes (Levenson et al., 2004; Fischer et al., 2007; Lattal et al., 2007; Vecsey et al., 2007; Barrett and Wood, 2008; Bredy and Barad, 2008; Stefanko et al., 2009; Morris et al., 2013; Monsey et al., 2011). In different subsets of HDACs, HDAC1 was found to be required for extinction learning mainly via the regulation of histone acetylation (Bahari-Javan et al., 2012). While, working memory and extinction learning are mainly due to the loss of HDAC2 (Michael et al., 2013). However, HDAC3 negatively regulates the formation of long term memory (Sultan et al., 2011), HDAC4 positively regulates the learning and memory (Kim et al., 2012). Recent studies on HDAC6 found that it functions in the elimination of protein aggregates in oxidative stress as well as in mitochondrial transport and it plays as a negative regulator for associative and spatial memory formation (Simoes-Pires et al., 2013). Therefore, it becomes quite interesting to study the role of HDAC in memory formation and extinction so as to reveal the more specified pathway for treatment of disorders like PTSD and alter the treatment regimens.

Learning is an active process which involves various brain regions to function together for acquisition, processing, and storage. Animal organisms receive different kind of information from the environment and surroundings and process this information in such a way that it can be recalled at any stage of life. While fear is a form of emotional response which is generated in a traumatic or stressful condition and such response differs in the impact it creates on individual organism (LeDoux, 2014). In brief, it can be regarded as a condition where a conscious state develops due to the occurrence of threat condition or its imagination (Costanzi et al., 2011;

LeDoux, 2012; 2014).

The different stages of fear memory mainly include acquisition, storage and retrieval of information for stabilizing the fear memory. In animals, a high level of emotional response after fear learning causes very stable and powerful as well as long-lasting memories which are difficult or impossible to erase (Cahill and McGaugh, 1998; McGaugh, 2004).

In almost all the studies based on memory, the Pavlovian model is used. It involves the relation of a conditioned stimulus (CS) such as tone or context with an unconditioned stimulus (US) such as an electrical foot-shock. This results in the formation of stable memory consolidation for fear memory. Following the fear memory consolidation, memory can be enhanced by the second phase for stabilization of the memory known as retrieval which mainly involves re-exposure to the CS through reconsolidation process. Prolonged re-exposure to the CS may leads to the extinction of fear memory and this is a new form of inhibitory learning against the fear memory, where all animals learn not to fear in response to the CS [Figure 2.2 (A)]. While fear extinction is training in which the long lasting effect of fear memory can be controlled/suppressed by behavioral training. This was widely used as an exposure based therapy to rule out the effect of fear memory (Milad and Quirk, 2012). The exposure therapy in which the extinction training involves the exposure of the individual to a CS which results in suppression of the effect mainly caused by fear memory mainly related to the traumatic episodes. Sometimes after the passage of a long time of the training, fear returns which is due to the result in persistent fear for a long time. This effect can be regulated by the repeated presentation of extinction training with some intervals of time which mainly results in strong persistent suppression of fear memory.

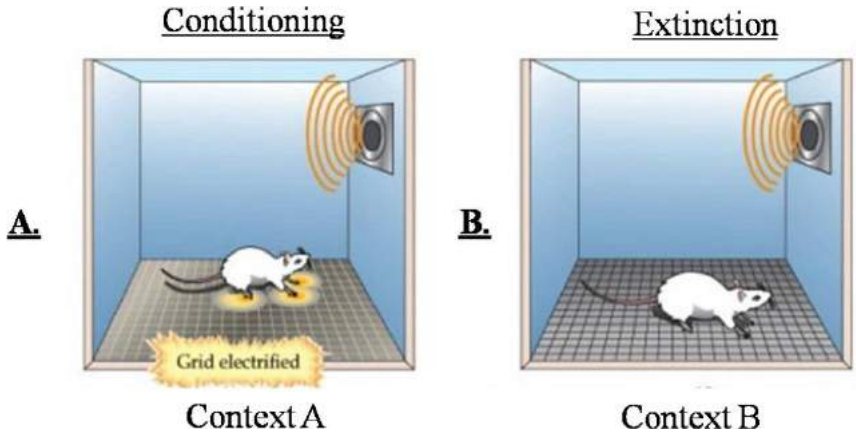


Figure 2.2. The training in conditioning (A) and extinction (B) (Siddiqui et al., 2017)

During extinction of the learning memory in laboratory condition, the animals are usually exposed to the repeated presentation of CS like tone in a context that is different from the conditioning context. The animals thus learn to dissociate the associative memory formed between CS and US by the formation of new memory which allows animals to learn memory that the CS is not associated with the US [Figure 2.2 (B)]. The experimental use of extinction training enhances the requirement for comprehending the mechanism mainly involved in fear extinction at molecular levels with the studies that are involved in the pharmacological intervention (Myers and Davis, 2002; Mueller et al., 2008, 2009; Holmes and Quirk, 2010; Davis, 2011).

Memory formation is a continuous process that involves different cortical regions of the brain. Tripartite realms involved are (Maren et al, 2013; Quirk and Mueller, 2008) amygdala, PFC and hippocampus (the limbic system) which act and function in the coordinated way for fear memory consolidation and extinction (Johansen et al, 2011). These brain parts function for the different phases of fear memory consolidation and extinction and are interrelated via synaptic connections (Izquierdo et al, 2016) (Figure 2.3).

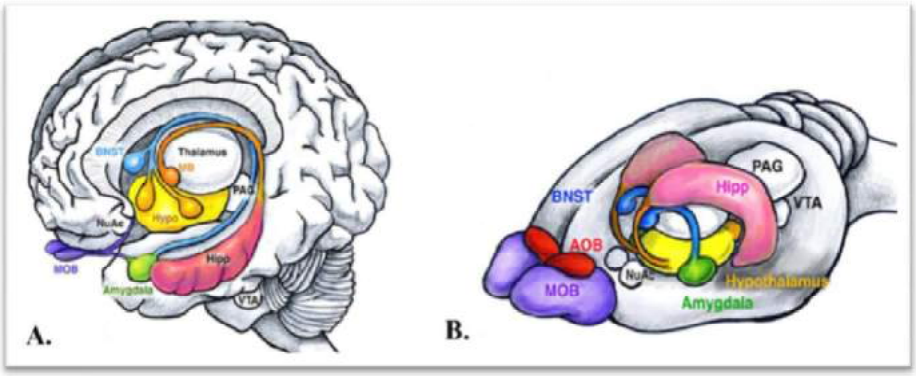


Figure 2.3. Lymbic system of human (A) and rat (B) involved in fear memory (Sokolowski et al., 2012). CA1: Cornu Ammonis 1; DG: Dentate Gyrus; CA3: Cornu Ammonis 3; BNST, bed nucleus of stria terminalis; Hipp, hippocampus; PAG, periaqueductal gray; NuAc, nucleus accumbens; VTA, ventral tegmental area; MOB, main olfactory bulbs; AOB, accessory olfactory bulbs.

The amygdala (almond-shaped structure) located deep on both the sides of the temporal part of the brain. It is composed of around 13 different subregions or nuclei which are formed by the cluster of neurons. The BLA (basolateral amygdala) is the largest subregion reported and this is formed by the lateral nucleus (LA), basolateral nucleus (BA) and accessory basal subregion (McDonald, 1998; Turner and Herkenham, 1991; Krettek and Price, 1978; Petrovich and Swanson, 1997; Veening et al., 1984). The BLA gets sensory inputs from thalamus, hippocampus and cortex which pertains and receives sensory information from the surrounding (Davis and Whalen, 2001). The LA is the center of BLA which functions as the entry point for the sensory information of the auditory, visual, olfactory and taste (LeDoux, 2007). Sensory information of the CS and US congregate in the LA mainly during the fear learning (Wilensky et al., 2006). The activated LA subregion activates the CeA (Central amygdala) which may serves as the output center of the amygdala for the expression of fear responce (Davis and Whalen, 2001). The CeA, in turn, innervates brainstem for the expression of fear responses which mainly results in specialized behavior and physiological response (LeDoux, 2007). The CeA is composed of CeL (centrolateral nucleus) and CeM (Centromedial amygdala) and these are involved in the all regulation/expression of the fear and emotional kind of memories. The three clusters (lITC- lateral intercalated cell masses, dITC- dorsal intercalated cell masses

and vITC- ventral intercalated cell masses) of GABAergic neurons are also known as intercalated cell masses (ITCs) which regulate the activity of BLA and CeA via inhibitory connections. The other two subregions of it the cortical nuclei and the medial nucleus mainly receive information through olfactory centers. Amygdala also shows its projections from the hypothalamus and brainstem. The hypothalamus functions in the regulation of emotional kind of responses through the regulation of endocrine secretion from the pituitary gland (Figure 2.4).

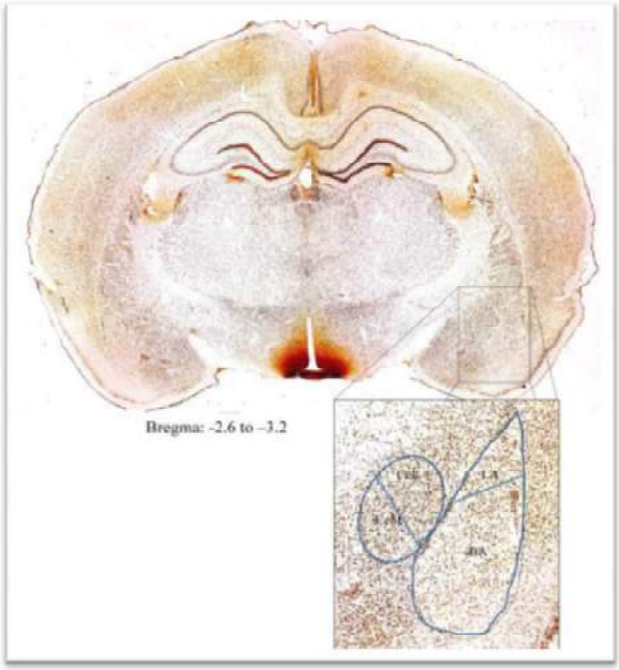


Figure 2.4. Region of whole Amygdala under inverted microscope from the section of brain (Myskiw et al., 2010). LA: Lateral Amygdala; BA: Basal Amygdala; CeL: Central Lateral; CeM: Central Medial.

Prefrontal cortex (PFC) or medial prefrontal cortex (mPFC) is located in the frontal lobe of the mammalian brain. In rodents, the PFC includes three parts the medially located mPFC, a ventrally located orbital prefrontal cortex (oPFC) and a lateral or sulcal prefrontal cortex (l-PFC) (Divac and Mogenson, 1985; Groenewegen, 1988). The mPFC is involved in the regulation of fear memory consolidation and extinction. It is further divided into four specialized subregions, prelimbic area, infralimbic area, medial precentral area (PrCm) or area Fr2 and anterior cingulate area (Krettek and Price, 1978). The PFC has been involved in the implementation of psychological functions that involves the capability to differentiate conflicting thoughts and memories like good, bad, better, best, same, different, etc.

The PFC receives innervations for a number of sensory stimulus from the cortices and it also receives connections with the mediodorsal thalamic nucleus (Miller et al., 2017). The lPFC got connections with temporal as well as parietal regions correlated with the cognitive memory functions (Siddiqui et al., 2008). The orbitofrontal and anterior cingulate cortical regions receive signals from the amygdala and medial temporal structures mainly involved with the emotional responses and their memory functions (Bonelli and Cummings, 2007). The dorsal subregion is innervated with the brain region associated with the cognition as well as attention however, the ventral subregion is associated with the emotional thoughts (Fanselow and Dong, 2010) (Figure 2.5).

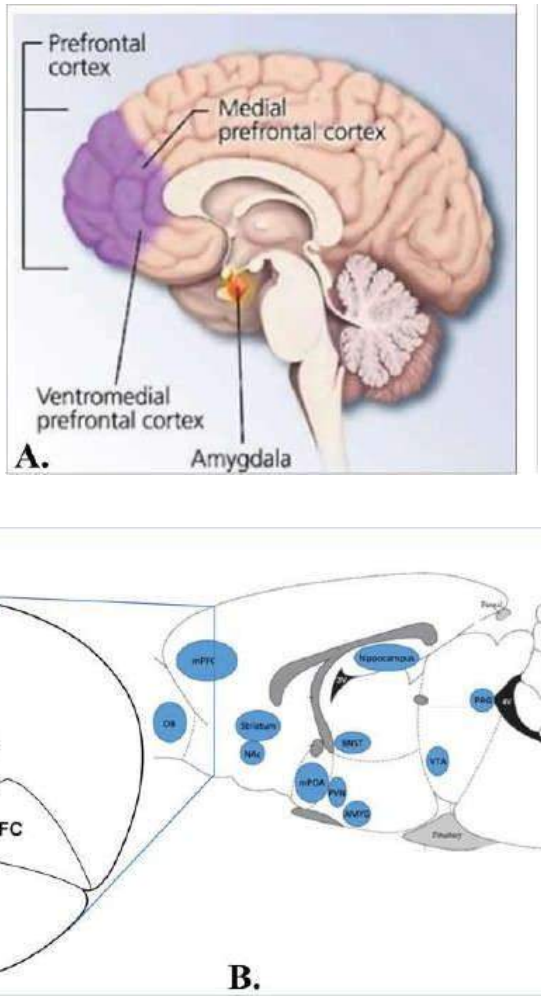


Figure 2.5. The anatomical structure (lateral view) of the Prefrontal cortex of (A) Human and (B) Rat (Zhou et al., 2009). ACd: dorsal anterior cingulated cortex; PL: prelimbic cortex; IL: infralimbic cortex; OFC: orbitofrontal cortex.

The hippocampus, small area of the vertebrate's brain is a major component of the limbic system mostly related with the contextual memory and spatial navigation (Orsini et al, 2012). It is an arched shape in structure located in medial temporal lobe in primates. The main function of it is strengthening of short-term memory to long-term memory and involved in spatial memory processes. Its primary function is to store the spatial information of the surrounding environment. The dorsal hippocampus (DH) processes spatial, verbal and conceptual information. Ventral hippocampus (VH) functions to promote fear while the intermediate hippocampus have function of both dorsal and ventral hippocampus (Figure 2.6).

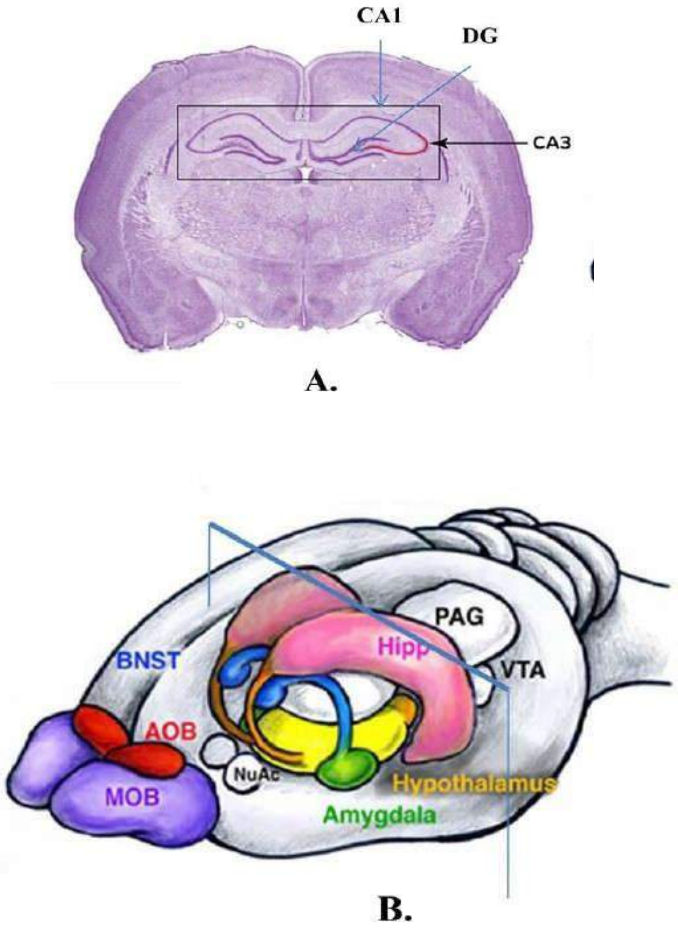


Figure 2.6. The Hippocampus (A) Immunohistochemistry sections and (B) structure of rat hippocampus (Ranjan et al., 2015). CA1: Cornu Ammonis 1; DG: Dentate Gyrus; CA3: Cornu Ammonis 3; BNST, bed nucleus of stria terminalis; Hipp, hippocampus; PAG, periaqueductal gray; NuAc, nucleus accumbens; VTA, ventral tegmental area; MOB, main olfactory bulbs; AOB, accessory olfactory bulbs.

Many groups of researchers have worked out to find the role of hippocampus in the formation of new memories through previously experienced events in their life which help in the finding of new events, places and stimuli from their previous experiences. Furthermore, the hippocampus is considered to be involved in the learning of environmental surrounding factors which are associated with the fearful learning. It has been reported that hippocampus functions in contextual fear learning, as lesion to the hippocampus creates amnesia in the organisms of contextual fear conditioning. However, lesions of the hippocampus following a time interval don't impair contextual fear conditioning (Sanders et al., 2003; Young et al., 1994).

The amygdala, PFC and hippocampus are at the center of the fear circuit and form a network of system known as limbic system (Maren, 2001; Fanselow and LeDoux, 1999). Practically memories do not emerge by activity of individual's brain, but formed from the interactions of a number of other associated subregions compositely forming the neural circuit. The neural circuit is associated and stands for the input, output and the processing of this information may results due to the effect of external environmental conditions.

Amygdala is involved in acquisition and expression of fear which was confirmed by the lesion studies resulting in disruption of both the acquisition and expression of fear conditioning in rodents (Maren et al., 1996; LeDoux et al., 1990). It is proved and evidenced by the studies that BLA subregion is the site for CS-US association during fear memory consolidation (Goosens and Maren, 2001). BLA then convey the signal of information to CeA which is acting as a gateway for the expression of resulting fear response (Maren, 2008; Zimmerman and Maren, 2010, 2011). All different subregions of the amygdala mainly function differently in the regulation of fear memory during conditioning as well as in extinction. During conditioning, the LA subregion receives sensory information for both the CS and US association from the cortical region and this activates BA and dITC of amygdala (Kim et al., 2006). The BA region activates CeM which is the output center point for the fear memory while dITC which is the GABAergic neuron center as already discussed and sends inhibitory connections to the vITC and inhibits its activity. The vITC has inhibitory GABAergic projections to the CeM which is restricted in conditioning. LA also sends the glutamatergic connection to the CeL and activates PKC zeta-on neurons which inhibit PKC zeta-off neurons involved in inhibition of the CeM activity (Pare and

Duvarci, 2012). During extinction, the LA subregion receives CS input signals but the lITC with GABAergic inputs to LA inhibit their activity. However, the extinction neurons in BA are mainly activated by the contextual inputs to BA (Pare and Duvarci, 2012), extinction neurons inhibit fear neurons (Lee et al., 2013). The activated extinction neurons project to vITC which finally activate through GABAergic connection and inhibit the activity of CeM (Pare and Duvarci, 2012). The vITC during extinction don't receive inputs from dITC and finally inhibit CeM activity directly via its inhibitory connection to enhance extinction (Pare and Duvarci, 2012).

The mPFC contain two subdivisions Prelimbic cortex (PL) and Infralimbic cortex (IL) in rodents and their human homologs, which exhibited specialized roles for the activity of fear circuit. The dorsally located PL is considered to regulate fear expression, while the ventrally located IL mediates the suppression of fear (Quirk and Beer, 2006; Sotres-Bayon and Quirk, 2010; Milad and Quirk, 2012; Riga et al., 2014). The PL has innervations which are bi-directionally from the BA where it activates the fear neurons and modulates the activity of BA (Lee et al., 2013). The IL furthermore innervates extinction of neurons in BA and vITC neurons during extinction of learning to enhance extinction (Lee et al., 2013). The hippocampus receives contextual information and conveys it to the BLA for further processing (Maren, 2001; Orsini and Maren, 2012; LeDoux, 2014).

Furthermore, the direct monosynaptic connections of the hippocampus to BLA also exist via mPFC and entorhinal cortex (Saunders et al., 1988). Tronson et al. (2009) found that CA1 activity was increased in conditioning and extinction as evident by the c-fos and pERK expression (Tronson et al., 2009), whereas the optogenetic inhibition of CA1 resulted in a long lasting inhibition of fear retention (Goshen et al., 2011). Hippocampus is divided into three major regions as CA1, CA3 and DG and these brain parts are associated with the fear learning. The CA1 is the output of the hippocampus from the information which projects to subiculum and entorhinal cortex (Cenquizca and Swanson, 2007). CA1 and CA3 have been found to be essential for acquisition and retention of fear memory in contextual learning (Jin and Maren, 2015). BL the most important subregion of BLA was found to innervate the hippocampal CA1 and CA3 regions (Pikkarainen et al., 1999). The hippocampal innervations to mPFC which modulate the activity of it and involved in the regulation of the activity of the amygdala during both conditioning and extinction of fear memory (Godsil et al., 2013). The hippocampal innervation to IL is mainly involved

in regulation of the extinction of fear memory (Herry et al., 2008; Tronson et al., 2009; Myskiw et al., 2010; Gilmartin et al., 2014) (Figure 2.7).

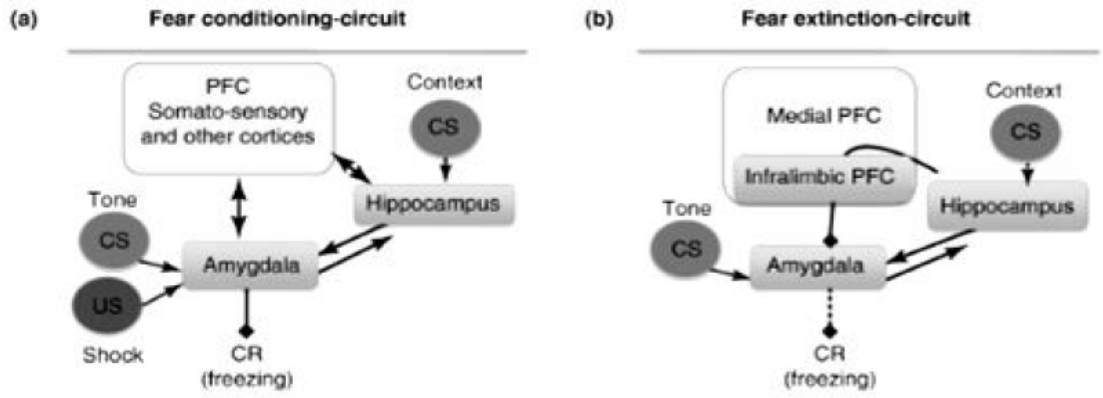


Figure 2.7. Diagrammatic representation of (a) Fear conditioning and (b) Fear extinction circuitry involving amygdala, PFC, and Hippocampus (Herry et al., 2008).

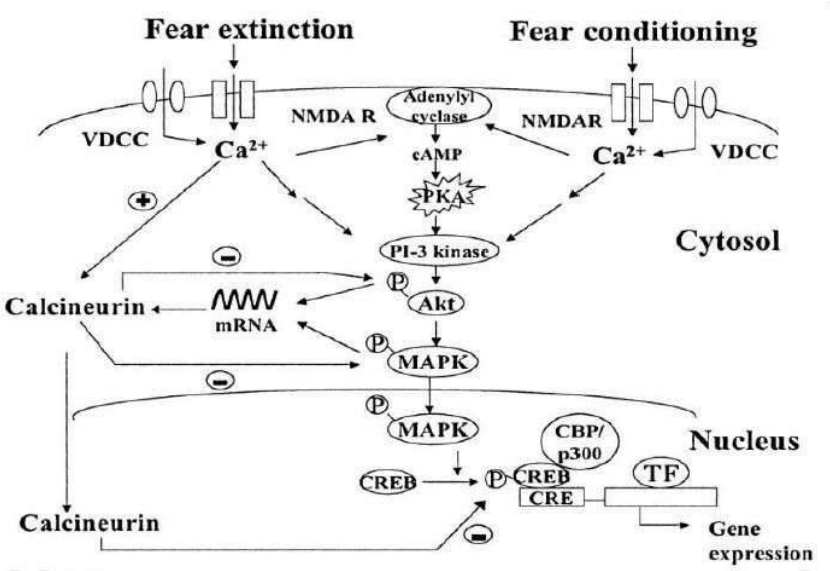
The consolidation and extinction of fear is a dynamic process which requires the synthesis of a number of proteins such as transcription factor, neurotransmitters and cytoskeleton proteins (Alberini, 2009; McGaugh, 2000). Recent research showed that immediate early genes c-fos and JunB are required for the consolidation and extinction in contextual fear memory and show different responses during conditioning and extinction learning (Huff et al., 2006; Strekalova et al., 2003).

The synthesis and function of these proteins are under the control of inter and intracellular signaling cascades in the amygdala, PFC, and hippocampus. The activated signaling in fear memory consolidation and extinction initiates neural plasticity in these subregions which involves NMDA receptor activity in postsynaptic neurons (Johansen et al., 2011; Zimmerman and Maren, 2010). The NMDA receptor activity in synapses conveys calcium ion signaling which is important for synaptic plasticity as well as for the formation of memory (Johansen et al., 2011). Activated NMDA receptor results in elevation of intracellular calcium level which further increases the auto-phosphorylation of CaMKII (Ca2+/Calmodulin (Cam)-dependent protein kinase II) (Silva, 2003; Rodrigues et al., 1999). Moreover, the CaMKII activates the phosphorylation of AMPA receptor (GluA1 subunit) which results in enhanced synaptic plasticity in LA in fear conditioning and extinction (Malinow and Malenka, 2002; Yeh et al., 2005). The CaMKII then activates PKA (Protein kinase A)

which then further activates MAPK/ERK pathways. The activated MAPK/ERK then activates the phosphorylation of CREB (cAMP response element binding), a factor required for transcription of plasticity-related genes (Orsini and Maren, 2012). Although the role of CREB in conditioning and extinction is well known but some results showing CREB phosphorylation (Hall et al., 2001; Mamiya et al., 2009) while others showing its inhibition in consolidation and extinction of fear memory (Lin et al., 2003). The CREB activity was not found to be associated in the hippocampus for contextual fear extinction (Tronson et al., 2009), however, the involvement of protein synthesis has been extensively studied in the hippocampus during extinction. The studies using intra hippocampal administration of the protein synthesis inhibitor anisomycin has been found to disrupt learning in inhibitory avoidance paradigm (Cammarota et al., 2005; Vianna et al., 2001). The inhibitory neuro transmission in LA subregion has been found to be associated with the conditioning and regulate the activity of neurons (Pare´ et al., 2003; Ehrlich et al., 2009). Neural plasticity during fear learning is also regulated by the metabotropic glutamate receptor activity (Nakanishi, 1994). The formation of LTM for conditioning and extinction requires synaptic strengthening and activates secondary messenger proteins for further signaling (Kandel, 2001; Alberini, 2009; Herry et al., 2006).

The activated transcription factors thus activate RNA which translates into proteins associated with the memory formation (Hernandez and Abel, 2008; McGaugh, 2000; Davis and Squire, 1984). It has been observed that inhibition of protein synthesis by the infusion of protein synthesis inhibitor anisomycin in LA disrupts the LTM formation (Schafe and LeDoux, 2000; Duvarci et al., 2008). The broad spectrum RNA inhibitors also have been found to disrupt LTM stabilization through inhibition of RNA transcription (Duvarci et al., 2008, Hoeffer et al., 2011).

Overall, the transcription and translation processes are required for fear memory consolidation and extinction (Jarome et al., 2011). Although transcription events happen in cyton but the translation takes place both in soma and dendrites that results in synaptic strengthening (Helmstetter et al., 2008). The Arc/Arg3.1 protein (Activity-regulated cytoskeletal-associated protein) is one of the transcript products at synapses which are found to be up regulated during conditioning in LA (Guzowski et al., 2000; Ploski et al., 2008). Recently the researchers have also shown the association of



glucocorticoids in fear memory consolidation and extinction (Rodrigues et al., 2009)

Figure 2.8. Model of the molecular mechanism involved in fear memory consolidation and extinction in the amygdala (Lin et al., 2003).

The epigenetic mechanism involves a change in the state of chromatin and its expression without posing a change in underlying DNA sequence. The mechanism includes histone acetylation, methylation, phosphorylation, sumoylation and DNA methylation which alter the expression profile (Ruthenburg et al., 2007; Peixoto and Abel, 2013). By the last few decades, epigenetic associations of fear memory and other cognitive processes have been well established by a number of studies in rodents and primates. Of which histone acetylation is the most studied epigenetic mechanism associated with the fear memory consolidation and extinction. Histone acetylation at specific lysine residue on N-terminal histone tail promotes transcription of genes (Ruthenburg et al., 2007; Grayson et al., 2010). Interestingly, the formation of memory has been found to be associated with an increase in histone acetylation in the different brain regions of the rodents (Alarcon et al., 2004; Guan et al., 2002; Levenson et al., 2004; Vecsey et al., 2007). The histone acetylation at various H4 and H3 residues promotes accessibility of the DNA for various transcription factors resulting in expression of the gene (Clayton et al., 2006). Both the cued and contextual fear memory are regulated by increased histone H3 and H4 acetylation which could further be strengthened by the use of HDACi (e.g. TSA, SAHA) (Peixoto

and Abel, 2013).

The acetylation of histone is regulated by the activity of histone acetyl transferases (HATs) and histone deacetylases (HDACs). Moreover, the mutations or deregulation of different KATs or HDACs results in various neurological and neurodegenerative dysfunction. The histone acetylation is found to be associated with the active gene transcription, whereas reduced or no histone acetylation represses gene transcription (Fischer et al., 2010). Researchers have shown that the enhanced histone acetylation is an essential molecular mechanism involved with fear memory consolidation and extinction (Guan et al., 2009: Bredy et al., 2007).

The role of histone acetylation was also confirmed by the studies showing enhanced histone H3K14 acetylation in CA1 following contextual fear conditioning (Levenson et al., 2004). Though histone acetylation is associated with the memory but different types of learning and learning paradigm shows different association with the epigenetic changes (Levenson et al., 2004). This increased histone acetylation during memory formation regulates the expression of genes such as cfos, CREB, BDNF, Erg1, involved in different phases of memory (Lubin et al., 2008; Bredy et al., 2007). Recent evidence has shown that extinction learning is associated with the altered HAT activity. Following extinction, there is enhanced expression of the HAT (p300/CBP-associated factor, PCAF) in the rodent IL, and intra-IL infusion of the PCAF activator (SPV106) facilitates extinction and inhibits the renewal of fear (Wei et al., 2012). The evidence has shown that the histone acetylation is associated with the consolidation and extinction of fear memory (Whittle and Singewald, 2014), and increased histone H4 acetylation at the promoter region have been studied at bdnf exon IV following extinction learning (Bredy et al., 2007). Furthermore, the acetylation of histone 3 (H3) in CA1 is found to be associated with the consolidation of contextual fear memory which was enhanced by the use of histone deacetylases (HDAC) inhibitors when given prior to the training experiment (Wood et al., 2005; Vecsey et al., 2007; Levenson et al., 2004) .

Histone deacetylases (HDACs) are the class of enzymes that catalyze the removal of acetyl groups from the lysine residues of histone and non-histone proteins and suppress the activity of the gene. There are 18 different HDAC subtypes, and based on sequence similarities have been classified into four classes from class I to class IV.

The class I, II and IV are known as classical HDACs as their activity is inhibited by the TSA (Trichostatin A) and shows zinc-dependent activity, while Class III HDACs, known as sirtuins are not affected by the trichostatin A and are NAD+- dependent proteins. Class I HDACs include HDAC1, HDAC2, HDAC3 and HDAC8 isoforms. Of these HDACs, HDAC1 and HDAC2 are known for their extensive involvement in memory like functions (Seto and Yoshida, 2014).

The Class I HDACs are generally localized into the nucleus except for HDAC3, which shuttle between nucleus and cytoplasm, and HDAC8 which is present both in cytoplasm and nucleus. Class II HDACs include HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10 which are able to shuttle from nucleus to cytoplasm to target nonhistone proteins (Seto and Yoshida, 2014). Class III HDACs includes sirtuins (SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, and SIRT7) which are localized in cytosol and nucleus both. There is little information about the Class IV HDAC, HDAC11 which is localized in nucleus and cytoplasm both. HDAC11 is considered to regulate the stability of the protein CDT1 which is involved in DNA replication (Seto and Yoshida, 2014).

Recent findings in research have shown that Class I HDACs, and particularly HDAC1 and HDAC2, are actively involved in CNS development and synaptic transmission (Montgomery et al., 2009). Studies using HDAC1 and HDAC2 deletions in embryonic stages showed abnormal development in the cortical and Hippocampal region (Montgomery et al., 2009). However, the deletion of HDAC2 results in improvement of learning and memory processes (Guan et al., 2009) while its overexpression results in impaired fear memory. The knockout studies for HDAC2 again show enhanced extinction learning in fear conditioning paradigm (Morris et al., 2013). Likewise, the class I HDAC, HDAC3 is also known to be a negative regulator of the fear memory, and its deletion improved memory in an object recognition task (McQuown et al., 2011). As compared to the class I HDACs less information is available about the functions of Class II HDACs. As compared to other HDACs of class I HDAC, the deletion of HDAC4 in the forebrain of mice results in impaired learning in the Morris water maze experiment (Kim et al., 2012).
The immense involvement of HDAC inhibitors in the promotion of fear memory consolidation and extinction has raised a number of suggestions to decipher the role

of HDACs in the treatment of various cognitive dysfunctions (Figure 2.9). Thus it has become a novel target to study the epigenetic involved in memory formation and extinction.

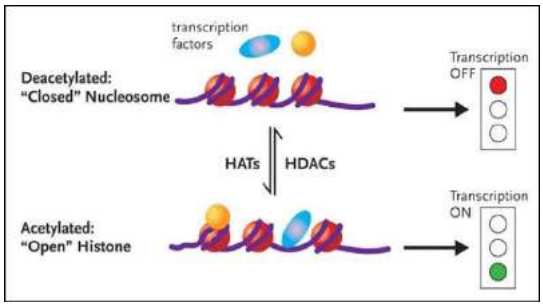


Figure 2.9. The mechanism of HDACs in the regulation of gene expression (Kim et al., 2012).

After analyzing the role of HDACs and its inhibitors in memory formation and extinction the scientists turned their focus towards them as a newer therapeutic approach. The valproic acid (VPA) a neuroprotective drug and potent HDAC inhibitors have been used in mice with Alzheimer's disease (Qing et al., 2008). The Vorinostat (suberoylanilide hydroxamic acid or SAHA) promotes fear extinction through enhanced histone acetylation in the promoter region of NMDA receptor and in the P4 promoter region of BDNF exon IV which is involved in synaptic plasticity and long-term memory (Bredy et al., 2007; Fujita et al., 2012). The widely used HDAC inhibitors selectively affect class I HDACs which affects the stabilization of learning and memory, establishing HDAC class I as a potential target to control fear learning and cognitive functions (Haggarty and Tsai, 2011).

Later it was shown that the impairment of HDAC2 promotes the synaptic plasticity and related genes involved in LTP during fear consolidation and extinction (Guan et al., 2009; Morris et al., 2013). These results suggest that HDAC2 can be a potential target molecule in the regulation of fear memory and target this HDAC might be helpful for PTSD conditions through CBT. Later, the role of HDAC1 on fear extinction promotion has been studied through HDAC1 over expression studies in mice (Bahari-Javan et al., 2012). Another study has shown that HDAC1 siRNA introduction or HDAC inhibitor introduction promotes histone acetylation at BDNF exon IV promoter (Yasuda et al., 2009). Similar to HDAC2, HDAC3 is known for its association with inhibition of long-term memory in fear learning as its ablation

enhanced the memory for spatial learning and CPP (conditioned place preference) task (McQuown et al., 2011; Malvaez et al., 2010). Furthermore, HDAC8 functions as an inhibitor for the extinction of fear memory through inhibition CREB pathway suggesting CREB as a potential target for enhancing extinction learning (Kida et al., 2002; Gao et al., 2009).

The use of HDAC inhibitors in cognitive enhancement is extensively studied; the use of systematic or in local introduction promotes the conditioning and extinction of fear memory (Morris et al., 2013; Maddox and Shafe, 2011). Similarly, intra hippocampal HDAC administration enhanced the extinction learning for a number of conditioning paradigm (Lattal and Wood, 2013; Lattal et al., 2007; Bredy and Barad, 2008). The types of HDAC isoform as in case of HDAC1 and HDAC2 subtypes affect the extinction memory differently as the gene silencing of HDAC2 but not HDAC1 promotes extinction learning (Grayson et al., 2010; Morris et al., 2013). Thus the studies converge with the use of individual HDAC subtype inhibitors in the enhancement of extinction learning and cognitive functions. The global HDAC inhibitor such as valproic acid is advantageous over other HDACs as it is widely used as a mood stabilizer with no side effect (Peterson and Naunton, 2005). The Valproic acid in brain targets mainly the class I HDACs and also target GABAergic signaling (Mimaki et al., 1984).

The drawback of exposure therapy in the treatment of anxiety disorder is the limitations caused by the therapy as the psychological conditions of anxiety often relapse with the passage of time and the change in context. The extinction memory caused by the exposure therapy is generally labile which raise a question for the development of new effective method. The use of pharmacotherapy together with exposure therapy is a current method for treatment of anxiety disorders. Of which the use of HDAC inhibitors is commonly used cognitive enhancers to promote extinction learning. Although the HDAC inhibitors are the promising target for the therapy but the use of HDAC subtype selective target are still in demand as all HDACs are not an inhibitor of extinction learning (e.g. HDAC1).

AIMS & OBJECTIVES

Aims and Objectives:-

1. To investigate whether extinction of fear memory is just inhibitory learning or it also has some erasure component.
2. To find the molecular mechanism involved in fear memory taking place during erasure / inhibitory as compared to the inhibitory learning.
3. To find the association of molecular events of inhibitory learning with differential histone methylation in the erasure or inhibitory of fear memory.

MATERIALS AND METHODS

Materials and Methods

Animals

The experiments were conducted on rat as subject for characterizing the neuronal activity in both the Basal Amygdala (BA) and Lateral Amygdala (LA), following immediate and delayed extinction. For the study male Sprague-Dawley rats (250- 300 gm) were used. Rats were housed individually with access to food and water adlibitum. Rats were handled for 15 days up to 2-5 minutes each day on a 12-h light/dark cycle, 23°C temperature. All experiments were carried out under strict compliance with committee for the purpose of control and supervision of experiments on animals as per the CPCSEA guidelines (853/AC/04/CPCSEA), Govt. of India, New Delhi. All subjects were on light/dark cycle, ambient temperature and proper food and water resources and they have individual cases. 20-22 rats' animals were taken as sample size in each group and all experiments were performed in triplets.

Subject: Male Sprague-Dawley rats weighing (250-300 gm) were used as subjects for the study. Male Sprague-Dawley rats (3 weeks old) were procured from Indian Institute of Toxicological Research (IITR) Lucknow. They were housed one per cage under standard conditions at a temperature of 23°C ± 1°C and 50 % humidity with a 12-hour light/dark cycle. Food and water was made available ad libitium to them in due meliu of guidelines established by the Institutional Animal Care and Use Committee of BBA University, Lucknow (IACUC).

Behavior Apparatus

Two plexiglass identical observation chambers kept in sound proof cabinets were used for all training such as fear and extinction. The chambers were made as per standard protocols and sophistication. A speaker was mounted outside the chamber to capture the acoustic CS. The chambers were fully ventilated and have a fan. First chamber is used as conditioning (Context A) and other is for extinction (Context B).

Conditioning apparatus: Behavioral fear testing experiments were conducted in two chambers 'A' and 'B' in various contexts. In current study, the experiment was carried out in separate departmental experimental laboratory of biotechnology at BBAU. Context 'A' served as conditioning chamber while context 'B' served as

extinction chamber. Both contexts had two transparent walls and stainless steel grid floors, however, the grid floor in context B was covered with flat white acrylic inserts to minimize context generalization. Context A was wiped down before testing with 10% ethanol and context B with 10% methanol. Personalized video monitoring cameras were mounted in the ceiling of each chamber which are connected with a quad processor to an advanced recorder and monitor for videos and scoring of freezing. Grid floors were connected to a scrambled shock source. Auditory stimuli were delivered via a speaker in the chamber wall. Delivery of stimuli was controlled with a personal computer and was maintained at 80 dB throughout behavioral testing.

Fear Conditioning: For acclimatizing the subjects, they were handled for 5-10 min everyday for a week prior to the experiment. After a week, the rats were brought in a separate white cage and were placed directly into the conditioning. Exposure to the room environment was avoided and the subjects were left in the chamber for stabilizing in the conditioning context, before initiating the fear training session the readings of base line freezing were recorded. Further, the subjects were exposed to five trials of Conditioning Stimulus (CS) – Unconditional Stimulus (US) and the readings of freezing were recorded in the mode when no body movements are shown expect breathing and heart-beat. Each trial lasted for 60 seconds with an Inter-trial interval (ITI) of 1 minute at every 10 seconds and then the rats were subjected to the CS. The CS was provided for 10 seconds with the 80 dB intensity tone of coterminous with US (shock) of 0.70 mA (for 1 second). The inter-trial interval between shock and the next tone was 60 seconds and the freezing behavior was calculated by both the observer and the software (V.J. instruments, India). One minute after the completion of the behavioral session, rats were returned to their home cage. Protocol for the study is summarized in Figure 4.1.

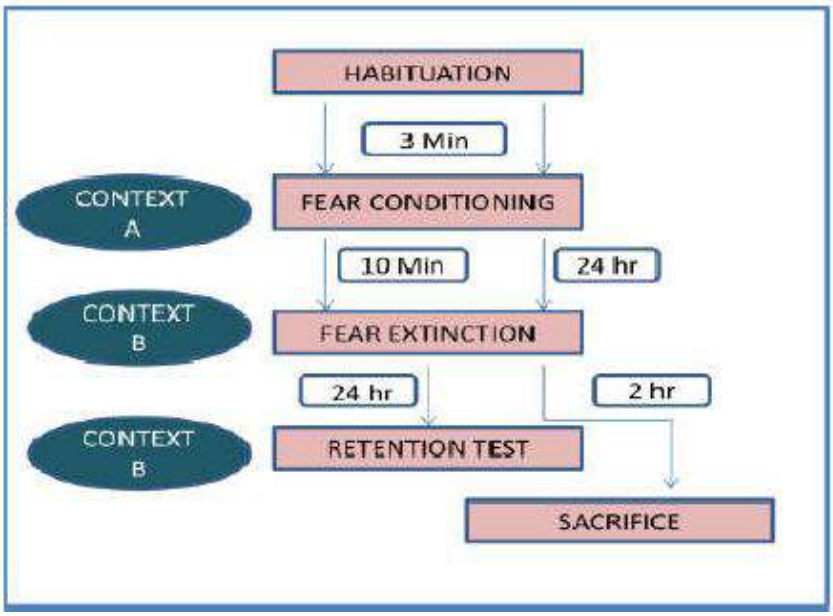


Figure 4.1. Outline for Immediate and Delayed Extinction.

Conditioning

The conditioning was for 7 days for at least 5 minutes each day. Firstly the rats were directly placed into the first chamber which is dark to expose the fear learning and to adapt themselves for the environment in context A for at least 3 minutes and the Freezing baseline was recorded. This was then followed by five consecutive sessions and documented the freezing when no movement is recorded. The 10 second exposure of CS (tone) having the intensity of 80 dB coterminous with US (shock) of 1 second having intensity of 0.70 mA. The difference between these two trials was 1 minute and the data was recorded both by software and manually.

Fear Extinction: The extinction training was performed after animal behavioral conditioning (fear learning) in a separate room chamber for animal behavior. It was performed at two different stages with duration of 10 minutes and 24 hours. After fear learning the subjects were placed in a fear extinction chamber for stabilizing the fear extinction context and recorded base line of the freezing. The subjects were placed in the context for 3 min during the extinction process and both the tone and shock were administered on the subjects. The freezing percentages of the subjects were recorded without application of both the conditions after the baseline sessions. 30 CS (tone 80 dB for 10 seconds) was administered to the rats with an ITI of 10 seconds. Behavior

was measured in the absence of US. In the whole extinction process thirty trials were analyzed and were reported as five trial blocks. Average of every six consecutive trials (six trial = 1 T block, Thus 30 trial = 55 block) was taken. The study included four groups i.e immediate extinction, immediate no extinction, delayed extinction or delayed no extinction to their corresponding two control groups in the absence of tone CS or shock US. Minutes of acclimatization period and extinction training was performed in a context B (V.J. Instruments). Animals (N=10 per cage) were sacrificed for Immunohistochemistry (IHC) analysis, two hours after extinction training. An additional group of the same aged subjects, who has not received any experimental manipulation, were used as naive control in all experimental procedures. Conditioning, extinction as well as control group underwent retention test for 24 h. Moreover, the training involved the presentation of the CS (5 tones, total duration 10s and 80 dB) Inter trial interval: 10 s in context B in the absence of foot shock. The animals which were used for IHC did not experience retention test.

Extinction

Context B was used for extinction training at two point scale i.e. 10 mins and 24 hours after fear learning. Before hand the baseline freezing was recorded for all the study groups followed by exposure to the 3 minutes extinction context by keeping the period free from tone or shock. 30 CS tone of 80 dB for 10 sec with interval of 10 sec in monitoring the freezing behavior and the data were recorded. Total 5 blocks having 30 trials i.e. 6 repetitive trials were taken for analysis with two controls one is immediate no extinction and delayed no extinction to find the correlation.

Retention test

Ten animals of the total twenty animals were taken as sample for the retension test having 24 hours of extinction training and the other ten were sacrificed for immunohistochemistry of brain after 2 hr of extinction. In this test, the subjects were taken in 5 consecutive trials of 80 dB for 10 sec as extinction context to record the freezing score to analyze the retention of extinction learning.

Control groups: For removing the confounding effect of behavior, additional group of animals were included as tone only (CS alone), context only (expose for the same duration to context) and shock only (Shock treated without tone). The context only

group (n=10) were subjected to experience the context A and context B in absence of any tone or foot-shock for conditioning and extinction group, respectively. Context only group was allowed to expose the conditioning and extinction context for the same duration as conditioned and extinction group. In tone only group, the rats were confronted with 80 dB for 10 sec, ITI, 60 sec in conditioning chamber (n=10). The shock only group received 5 trials of shock (0.7 mA), ITI 60 sec in the absence of any tone and freezing was measured for the overall duration.

Study of Brain Sub-Regions

Brains of sacrificed subjects were taken and the antibodies against each portion were used and the data were recorded as mean of positive neurons from each subject.

Details of Brain Sub-Regions under Study: Two brain regions (amygdale and prefrontal cortex) involve in fear memory consolidation and extinction were taken into the study. Amygdala consist of LA and BA (Mc Donald, 1998; Turner and Herkerham, 1991; Krettek and Price 1978) which regulate the expression of fear in rodents, respectively, (Giustino and Maren, 2015) especially in IL and PL regions of PFC.

Behavioral training: Naive subjects were not trained at all and remained in their home cages until sacrifice. Subjects were trained for fear conditioning and extinction inside a conditioning chamber (V. J. Instrument). Before experiments, subjects were allowed to acclimatize in the chamber for 3 minutes. For conditioning of fear, subjects were exposed to five trials of an acoustic signal (80 dB, 10 sec) of tone co-terminated with a foot-shock of intensity of 1 mA for 1 sec. The ITI were used of 10 sec after each trial. Subjects were then placed into their home cages. After 24 hours, subjects were re-introduced to the chamber for extinction experiment by providing 30 trials of a tone (80 dB, 10 sec) in the chamber with context B in the absence of shock and 10 sec ITI. Subjects were then again placed into their home cages. Freezing behavior was recorded and analyzed via a video camera connected to video tracking software (V. J. Instrument).

Behavioral test: 24 hours after conditioning, fear retention test was performed by the presentation of the CS (5 tones, 10 sec, 80 dB, inter-trial interval: 10 sec) in context B

for all groups from both the aims. The subjects of the test group were not used for molecular studies while the groups which were used for IHC, did not undergo retention test.

Figure 4.2. Performing behavioral experiment.

Tissue Sectioning

Subjects were anesthetized in both the trial blocks and perfused transcardially using normal saline followed by chilled 4.0 % paraformaldehyde (PFA). The resulted brains were then stored in 4.0 % PFA for at least 24 hours. Serial sucrose solution of 10, 20 and 30 % were used in next day to settle the brains followed by isopentane freezing at -300 C for 30 minutes. The samples were then stored in -80 0C deep freezer for immunohistochemistry.

Perfusion: Perfusion was done as following :

- The rats were anaesthetized with 50 mg/Kg body weight using Sodium pentobarbital (Nembutal) and to expose the heart, a cut is made from sternum.
- Needles was inserted from left ventricle into the right aorta and clip it to keep in place, then right auricle was cut and cold normal saline was passed immediately.
- After passing approximately 100 ml normal saline, approximately 400 ml ice cold 4% paraformaldehyde was also passed to fix the animal.
- The brains were then taken out carefully and kept in 4% paraformaldehyde for 24 hours for post fixation.

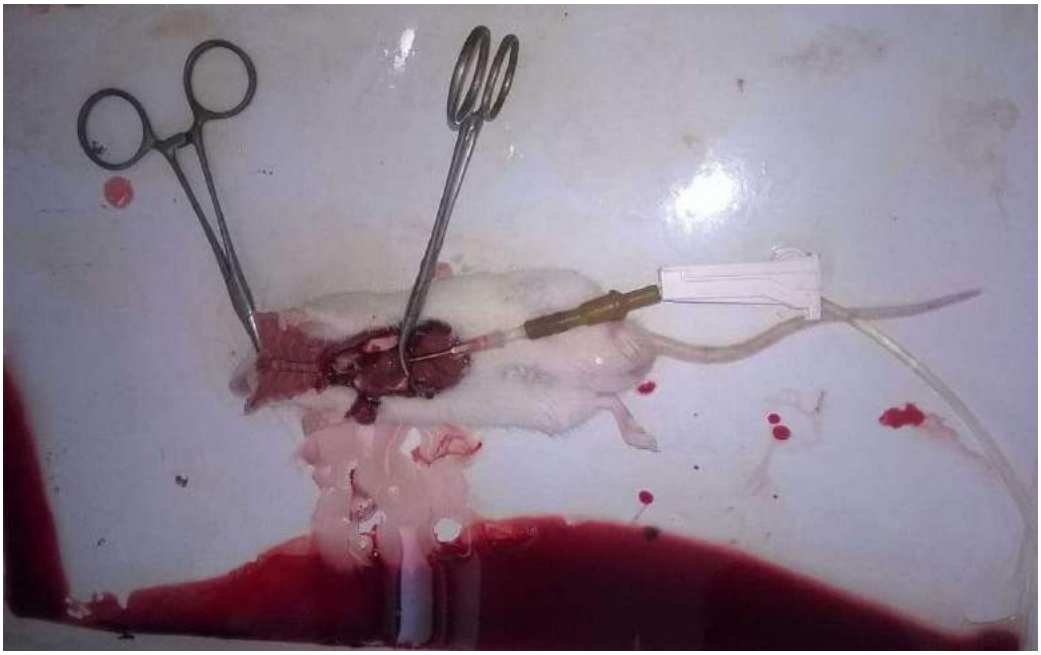


Figure 4.3. Performing perfusion.

Cryosectioning: Brains were transferred from paraformaldehyde to 10% sucrose, kept overnight (tissue had to sink fully to the bottom) and transferred to 20% sucrose (for the brain tissue to sink fully, it may take more than 24 hours) then transferred to 30% sucrose (for the brain tissue to sink fully, it took more than 24 hours) followed by freezing of brains (Figure 4.4 and 4.5).

Figure 4.4. Isolation of brain after perfusion for sectioning.

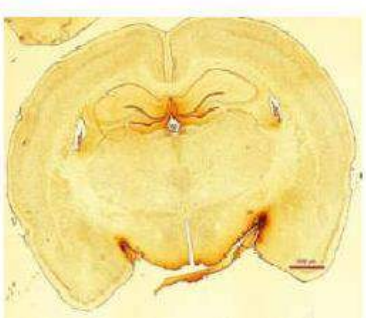


Figure 4.5. Represents the Bregma of amygdala used in the study.

Immunohistochemistry

- Place the slides in a 56-60 °C oven for 15 min.
- Shake off excess liquid and rehydrate slides in two changes of fresh absolute ethanol for 3 min. each. Shake off excess liquid and place slides in fresh 90% ethanol for 3 min. followed by fresh 80% ethanol for 3 min.
- Rinse the slides in gently running tap water for 30 seconds.
- Place in PBS wash bath for further rehydration (30 min. at room temperature).
- Apply 0.1% trypsin in PBS or 0.1% protease in PBS for 2-30 min. at 37 °C. Rinse in PBS for 10 min. and wash the slides with deionized H_2O. Make sure slides are fully covered with solution. This process can be repeated 2-3 times.
- Add enough drops of 3% hydrogen peroxide to cover the whole section.
- Incubate 5 min. at room temperature and rinse with PBS for 2 min.
- Allow the slides to drain, shake off excess fluid with a brisk motion and carefully wipe each slide around the sections.
- Dilute the primary antibody or negative control reagent to its optimal dilution in diluent. The diluent alone may be used as a negative control. A positive control slide (a tissue known to contain the antigen under study) should also be run.
- Apply 100 µl primary antibody solution to the appropriate slides, covering the tissue sections. Tilt each slide in two different directions, so the liquid is spread evenly over the slide.

- Incubate for at least 60 min. at 37 °C in humidified chamber. Rinse gently with PBS from a wash bottle for 5 min.
- Dilute the biotinylated secondary antibody in diluent to its optimal concentration. Apply 100 µl to each slide, covering the tissue sections.
- Tilt each slide in two different directions. Incubate in a humidity chamber for at least 30 min. at room temperature. Rinse gently with PBS from a wash bottle for 5 min.
- Apply 100 µl to all slides; cover the section. Tilt each slide in two different directions for at least 20 min. at room temperature.
- Rinse gently with PBS from a wash bottle for 5 min.
- Allow the slide to drain. Shake off excess fluid with a brisk motion and carefully wipe the slide as before.
- Dilute the conjugated secondary antibody in the diluent to its optimal dilution.
- Apply 100 µl to all slides, covering the tissue sections. Tilt each slide in two different directions.
- Incubate 30 min. at room temperature or at 37 °C in humidified chamber.
- Rinse gently with PBS from a wash bottle for 5 min.
- Apply enough drops of freshly prepared substrate mixture to cover the tissue section.
- Incubate 5-10 min. or until desired color reaction is observed when monitored with the microscope. Terminate the reaction before background staining appears in the negative controls by rinsing gently with distilled water from a wash bottle.
- Mount the sections using aqueous mounting medium such as glycerol gelatin. Coverslip may be sealed with clear nail polish.

Rats from each group were anesthetized using pentobarbital (50 mg/kg) and animals were perfused intracardially with paraformaldehyde. Brains were desected followed by sucrose treatment and freezing in isopentane. 3'-Diaminobenzidine (DAB), immunostaining was performed for cAMP response element-binding protein (CREB), Pre- cAMP response element-binding protein (p-CREB). Accurate nucleus of the hypothalamus (ARC) and Dimethyl H3K9me2 coronal brain sections (20 µm) from amygdala region were prepared using cryostat. Brain sections were blocked with normal goat serum (NGS) followed by incubation for overnight (8-12 hrs) at room temperature (25^0C ± 2^0C) with- CREB (1:500) (# Cat. No. 35-0900; Invitrogen, USA), p-CREB (1:500) (# Cat. No. 44-297G; Invitrogen, USA), ARC (1:500) (# Cat. No. C-6827; Invitrogen, USA) and Di- methyl (1:500) antibody (# Cat. No. 49-1008; Invitrogen, USA). Sections were washed and incubated with biotinylated goat anti mouse IgG for 2 hours. DAB staining was performed.

Cryostat sectioning of 20 µm thickness containing mPFC coronal brain sections were collected. These sections were blocked with 1% NHS (NHS Vecta-stain Elite ABC kit, Vector Laboratories, Burlingame, CA, USA) and 0.25 % tween 20 and followed by overnight incubation with c-fos primary antibody, methyl H3K9 (# Cat. No. 49-1007; Invitrogen, USA). After this the sections were incubated with the biotinylated secondary antibody for 2 hours followed by ABC complex for 2 hours at 25 ^{0}C. Finally, DAB substrate was added and the immunostained sections were then fixed mounted on clean slides. Reading of PL and IL sub regions were recorded. All readings were recorded in triplicate. Untreated normal rat brains were used as control to correlate the results.

Statistical Analysis

Behavioral data has been shown as mean ± standard error and the data was analyzed using three-way repeated measures ANOVA. Post-hoc corrections were made by Tukey's post hoc analysis. Two-way ANOVA was used to analyze other data using online software. For each session, the freezing data was transformed to the percent value. Retention test data and IHC data were analyzed using two-way ANOVA. All the statistical calculations were performed by the ezANOVA software. Pearson correlation was performed between % freezing observed during retention test versus expression of CREB, p-CREB, ARC and histone methylation in the BA and LA Sub region.

Image analysis

The sections were analyzed for the positive neurons of different Immunostaining under the up-right microscope from Nikon using NIS Element NS-BR image acquisition software, Nikon. All the images were obtained at 4x and 40x and immune positive neurons were counted manually as well as with the help of NS-BR image acquisition software.

The sections were analyzed for the positive neurons of different Immunostaining under the up-right microscope from Nikon using NIS Element NS-BR image acquisition software, Nikon. All the images were obtained at 4x and 40x and immune positive neurons were counted manually as well as with the help of NS-BR image acquisition software.

RESULTS

Results

Behavioral training for fear Conditioning and Extinction

Behavior: In the present study, the effect of timing of extinction training relative to fear learning on retention of extinction memory was explored. The rats were subjected to extinction training either 10 min (Immediate extinction) or 24 hours after fear learning (delayed extinction). Before the onset of conditioning, during acclimatization period of 3 min, baseline freezing was recorded indicating that all the rats possess similar low level of freezing response with no significant difference across the groups. During fear learning, the freezing response increased with each successive trial across the groups and the result was confirmed by three-way repeated measure of conditioning. Data revealed a significant main effect of trials [$F(3,116) = 70.12$, $p \leq 0.0001$] and the interaction of trial [$F(3,116) = 24.10$, $p \leq 0.05$] in the immediate and delayed extinction group during fear learning [Figure 5.1(A)]. Tukey's posthoc test confirmed significant differences between the trials (between 5 trials) in conditioning (all $p \leq 0.001$). Prior to the commencement of extinction learning, the baseline freezing was low across all the groups with no significant difference. During extinction learning, both the immediate and delayed extinction groups showed attenuation in the freezing response with each consecutive trial. However, immediate extinction group exhibited a comparatively higher level of freezing as compared to the delayed extinction group during the initial trials. The freezing during the last trial was similar in both the groups ($p \leq 0.05$). The immediate no extinction and delayed no extinction groups maintained low freezing throughout the session. The result was confirmed by three-way repeated measure which showed significant main effect of extinction condition and trial [$F(3,116) = 7.144$, $p \leq 0.0001$] followed by post-hoc analysis which confirmed that extinction groups froze more significantly as compared to the extinction groups ($p \leq 0.001$) and there was a significant effect of trials on freezing response ($p \leq 0.0.01$). 24 hours after successful extinction learning, retention test was performed to gauge the retention of fear [Figure 5.1 (B)].

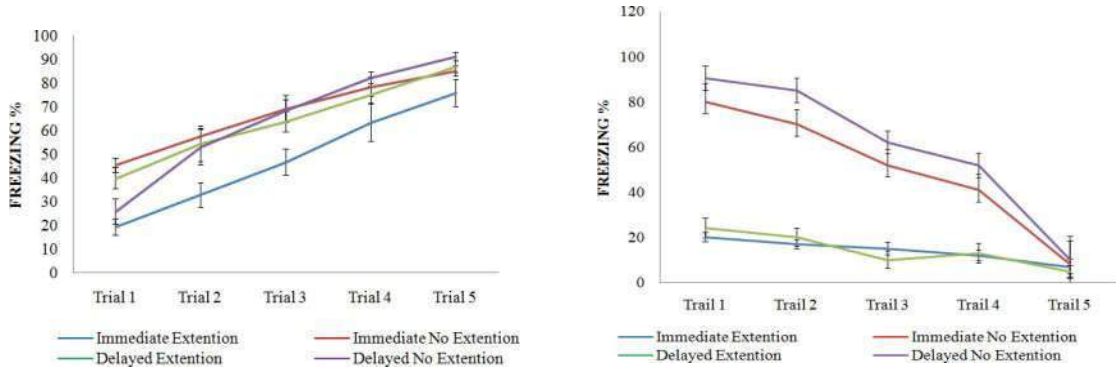


Figure 5.1. (A) In freezing response of pre-conditioning baseline was very low in all groups of fear learning memory. During experiment of fear learning memory of all the groups is found to be significant. All each consecutive trials, each trial showed freezing also with no significant difference between each groups ($p>0.05$).

(B) The pre –extinction base line of freezing response of all the groups followed by extinction learning memory. During experiment extinction learning memory, immediate extinction and delayed extinction both groups showed a significant decrease in freezing behavior with each consecutive trial in experiment. However, the percentage freezing during the initial trials of experiment was lower in delayed extinction group as compared to immediate extinction group. Both the control group exhibited low percent freezing response throughout the trial.

Retention test showed that delayed extinction group exhibited the least level of freezing as compared to the immediate extinction and no extinction group. Two- way ANOVA of retention test showed highly significant association w.r.t. time [$F(3,116) = 8.20$, $p \leq 0.005$] and condition X time interaction [$F(3,116) = 17.37$, $p \leq 0.0005$] but no significant main effect of condition [$F(3, 116) = 12.36$, $p \leq 0.00005$] was observed on freezing response. Tukey's post-hoc analysis confirmed that delayed extinction had a low level of freezing response as compared to the immediate extinction group ($p \leq 0.0005$) and delayed no extinction group ($p \leq 0.0005$) (Figure 5.2).

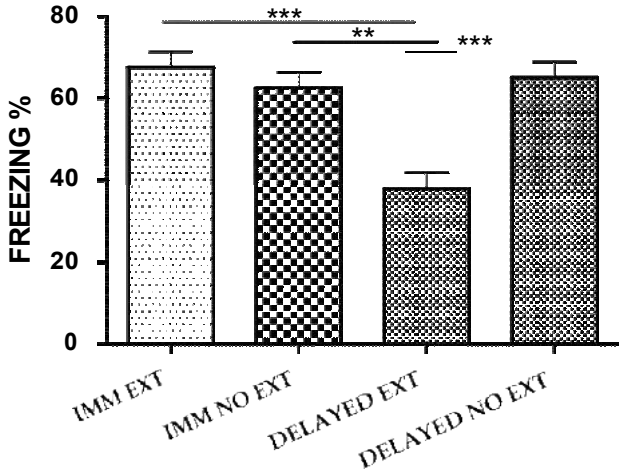


Figure 5.2. Animal behavior retention test experiment, performed after one day 24 hours of the extinction and no extinction training test. During experiment retention test, group delayed extinction exhibited a significantly low level of freezing than the immediate extinction group and control group. [N=60-100, n=40 animal SD (Sprague Dawley) rat in each group (n = 20 for immunohistochemistry and n = 20 for retention test).

Overall the immediate extinction group had deficits in the retention of fear extinction memory whereas delayed extinction group had stable long-term retention of fear extinction. The di-methyl (H3K9me2) expression has been extensively used as a neuronal activity marker in various studies and its expression has been correlated to region-specific activity in different brain regions. Our next aim was to examine the region-specific neuronal activity in the BA and LA sub regions of the amygdala following immediate and delayed extinction. To accomplish this objective, Coronal brain sections containing the Amygdala were immunostained for di-methyl (H3K9me2) from each group.

p-CREB expression following immediate and delayed extinction in BLA

region: The expression of p-CREB was found to be higher in extinction group as compared to their respective control groups in the LA region. However, the changes were insignificant between immediate and delayed extinction group. Two-way ANOVA analysis for p-CREB expression in LA region revealed a significant main effect of extinction condition (extinction vs. no extinction) [$F(3, 26) = 184$, $p \leq 0.0001$], Figure 5.3, However in BA, the expression of p-CREB was significantly

higher ($p \leq 0.0001$) in delayed extinction group as compared with the immediate extinction group and delayed no extinction group. The expression of p-CREB was further confirmed by two ways ANOVA that revealed a significant main effect of extinction condition (extinction vs. no extinction) [$F(3, 26) = 184$, $P \leq 0.0001$] extinction time (immediate extinction vs. delayed extinction) [$F(3, 26) = 146$, $P \leq 0.0001$] as well as extinction condition and extinction time interaction [$F(3, 26) = 14.4$, $P \leq 0.0001$]. In other words, the two sub regions of Amygdala responded differentially to the two extinction paradigms. This differential expression of p-CREB exemplifying the activity in the BA and LA in the immediate and delayed extinction group may be responsible for the deficit in the retention of extinction memory as observed after immediate extinction [Figure 5.3 (A) and (B)].

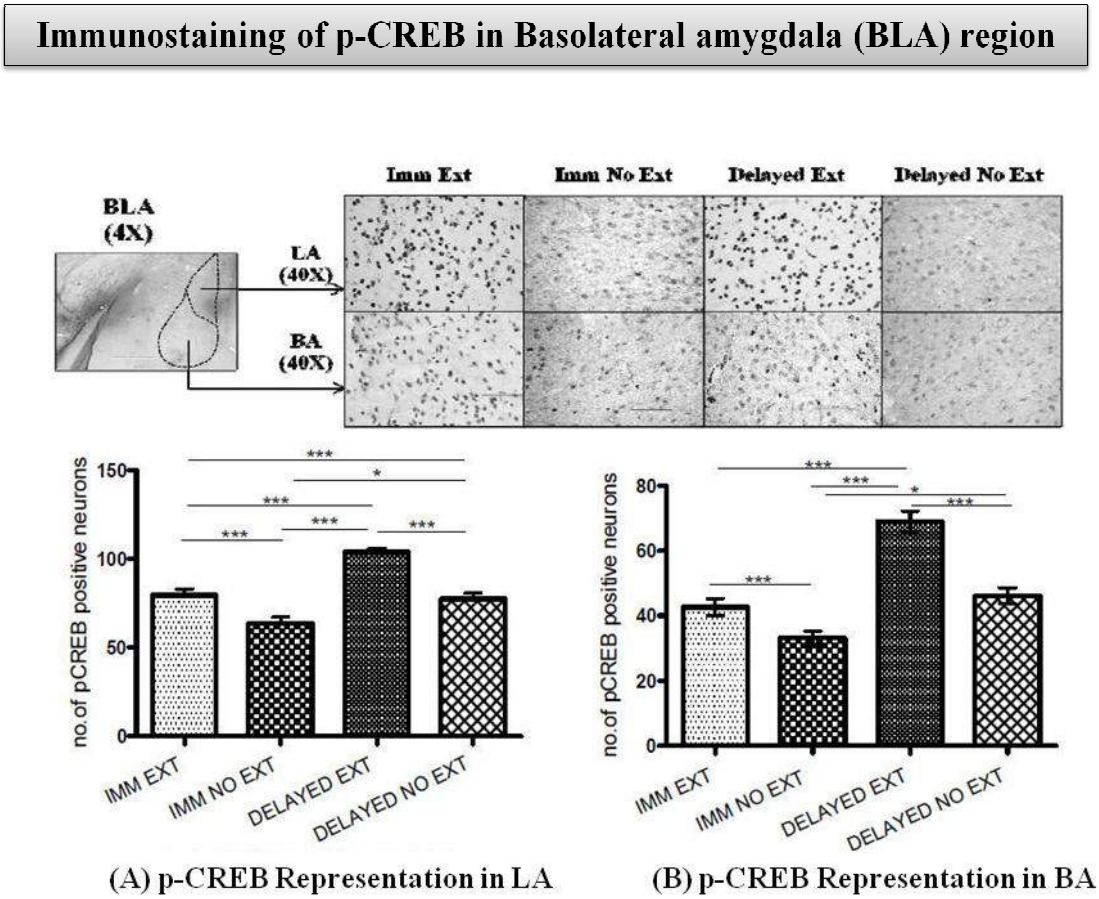


Figure 5.3. p-CREB expression in amygdala following immediate and delayed extinction learning: The p-CREB expression was elevated in the BA and LA of both immediate and delayed extinction group when compared to the immediate no extinction and delayed no extinction control groups. However, the p-CREB expression was significantly higher ($p \leq 0.000$) in LA of the delayed extinction group

as compared to immediate extinction group and to the immediate and delayed no extinction controls ($p \leq 0.000$).

CREB expression following immediate and delayed extinction in BLA region:

The CREB expression was higher in extinction groups as compared to their respective control groups but no significant changes were observed between immediate and delayed extinction groups in LA region (Figure 5.4). Two-way ANOVA analysis for CREB expression in LA region revealed significant main effect of extinction condition (extinction vs. no extinction) [$F (3, 26) = 71.2$, $p \leq 0.0001$] but no effect of extinction time (immediate vs. delayed [$F (3, 26) = 445$, $p \leq 0.0001$] and condition x time interaction [$F (3, 26) = 5.75$, $p \leq 0.0001$] [Figure 5.4(A)]. However in BA, a significantly increased expression of CREB was observed in delayed extinction group as compared with the immediate extinction group and delayed no extinction group. This information was further supported by two way ANOVA analysis that exhibited significant main effect of extinction condition [$F (3, 26) = 94.1$, $p \leq 0.0001$], extinction time [$F (3, 26) = 9.50$, $p \leq 0.0001$] [(Figure 5.4 (B)]. Expression of CREB seems to be associated with neuronal activity in the BA and LA following immediate and delayed extinction. We next wanted to see whether the increased CREB levels in these regions culminated in methylation of H3 and H4 suggesting methylation of Histone at various lysine residues are to be associated with enhanced expression of genes required for synaptic activity and memory consolidation.

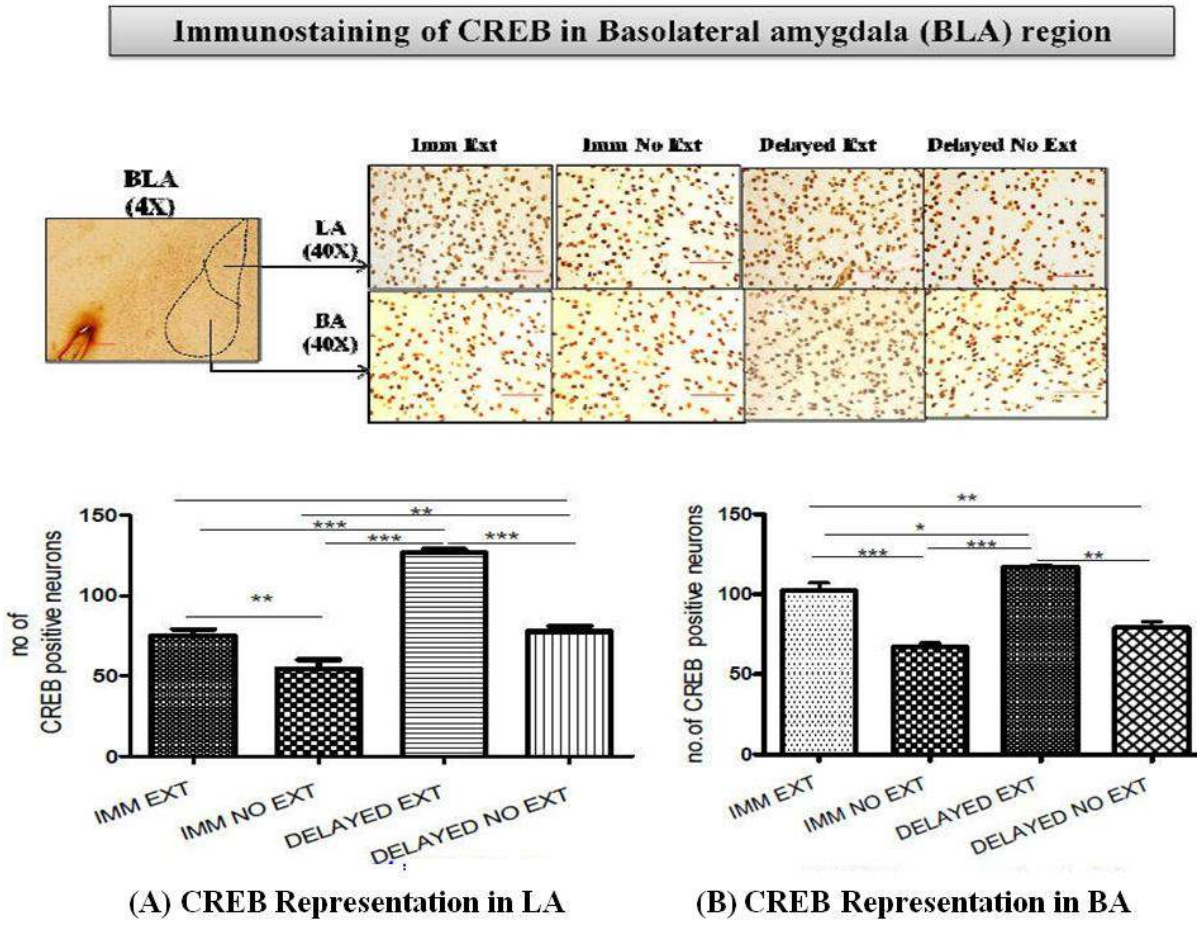
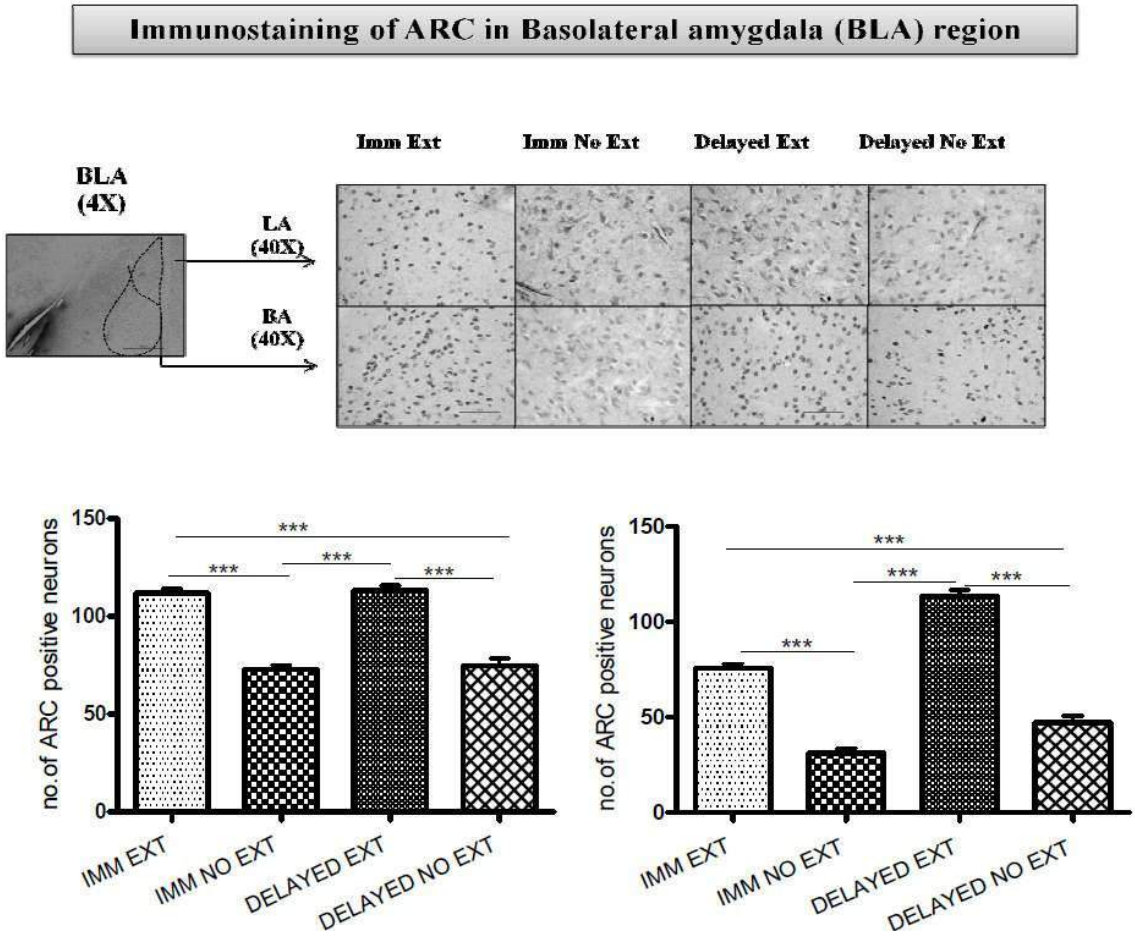


Figure 5.4. CREB expression in amygdala following immediate and delayed extinction fear learning. (A) The CREB expression was elevated in the BA and LA of both immediate and delayed extinction group when compared to the immediate no extinction and delayed no extinction control groups ($p \leq 0.000$). (B) CREB expression was significantly higher in BA of the delayed extinction group as compared to immediate extinction group and to the immediate and delayed no extinction controls ($p \leq 0.000$).

ARC expression following immediate and delayed extinction in BLA region:

Similar to the ARC, expression was higher in extinction groups as compared to their respective control groups but no significant changes were observed between immediate and delayed extinction groups in LA region. Two-way ANOVA analysis for CREB expression in LA region revealed significant main effect of extinction condition (extinction vs. no extinction [$F(3, 26) = 162.2$, $P \leq 0.0001$] [Figure 5.5(A)] but no effect of extinction time (immediate vs. delayed) and condition x time interaction. However in BA, a significantly increased expression of CREB was

observed in delayed extinction group as compared with the immediate extinction group and delayed no extinction group. This information was further supported by two way ANOVA analysis that exhibited significant main effect of extinction condition [F (3, 26) =195.2, P ≤ 0.0001] [Figure 5.5(B)]. Expression of ARC seems to be associated with neuronal activity in the BA and LA following immediate and delayed extinction. This will be further clarified when the increased ARC levels in these regions culminated in methylation of H3 and H4 and the reason being the methylation of Histone at various lysine residues associated with enhanced expression of genes required for synaptic activity and memory consolidation in rats.



(A) ARC Representation in LA (B) ARC Representation in BA

Figure 5.5. ARC expression in amygdala following immediate and delayed extinction fear learning: (A) The number of ARC positive neurons was elevated in the BA and LA of both immediate and delayed extinction group when compared to the immediate no extinction and delayed no extinction control groups ($p \leq 0.000$).

(B) The number of ARC positive neurons was significantly higher in LA of the

immediate extinction group as compared to immediate extinction group in BA and to the immediate and delayed no extinction controls ($p \leq 0.000$).

Histone methylation following immediate and delayed extinction in BLA region: To correlate the neuronal activation to histone methylation the histone methylation in the LA and BA following immediate and delayed extinction were checked. We gauged the levels of methyl H3 at lysine residue 9 (K9) in LA and BA following the extinction paradigms. Expression of methyl H3K9 in LA region showed that there was no significant difference between immediate extinction and delayed extinction group, however Immediate and delayed extinction group has a lower level of methyl H3K9 positive neurons with respect to immediate no extinction and delayed no extinction group. Two-way ANOVA analysis confirmed that there was significant main effect of extinction condition (extinction vs. no Extinction) [$F (3, 26) = 73.3$, $P \leq 0.0001$], in comparison to the effect of extinction time (immediate vs. delayed) [$F (3, 26) = 8.46$, $p \leq 0.0001$] [Figure 5. 6 (A)]. On the other hand in LA region, the expression of H3K9 was significantly lower in delayed extinction group as compared to the delayed no extinction group and immediate extinction group. Two-way ANOVA analysis of BA [$F (3, 26) = 96.6$, $P \leq 0.0001$] revealed significant main effect of extinction condition (extinction vs. no Extinction) [$F (3, 26) = 96.6$, $p \leq 0.0001$] and extinction time (immediate vs. delayed) [$F (3, 26) = 5.35$, $p \leq 0.0001$] (Figure 6b) [Figure 5. 6 (B)]. These changes in the H3 methylation were linked to the neuronal activity i.e., the ARC, CREB, p-CREB in a region-specific manner. Similar to the H3K9 me2, the immediate and delayed extinction group exhibited lower level of H3K9 expression, when compared to their respective control groups in BA region, but no significant difference between immediate and delayed extinction group was observed. Two-way ANOVA analysis confirmed that there was significant main effect of extinction condition (extinction vs. no Extinction) [$F (3, 26) = 96.6$, $p \leq 0.0001$]. But no significant difference was observed for extinction time (immediate vs. delayed extinction) [$F (3, 26) = 5.35$, $p \leq 0.0001$] (Figure 5.6).

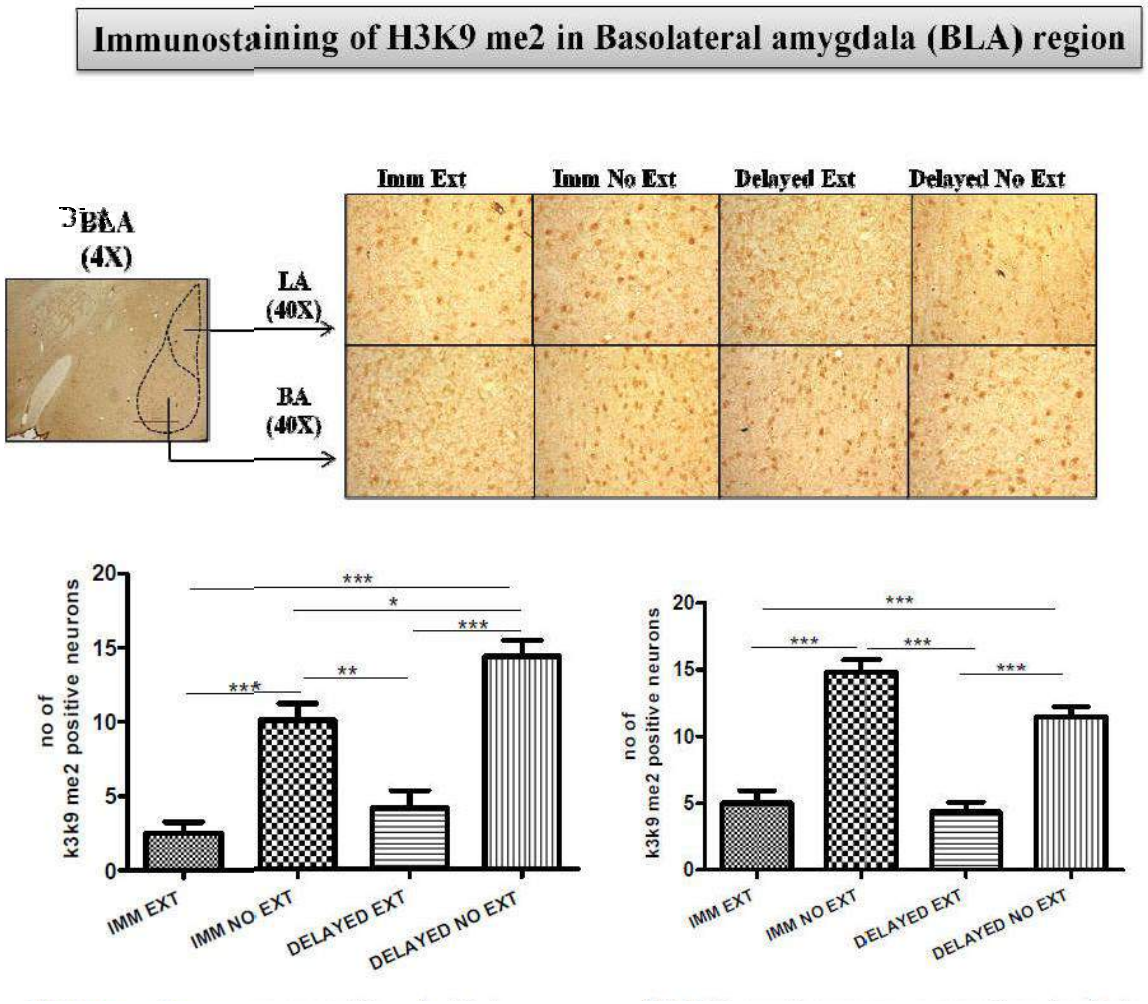


Figure 5.6. Methyl H3K9me2 expression in amygdala following immediate and delayed extinction learning: Methylation of histone H3 at the 9th residue of lysine illustrated decrease expression in both immediate and delayed extinction group in both the (A) LA and (B) BA when compared to their respective control groups. Delayed extinction group exhibited significant lower no. of Methyl H3K9 positive nuclei in BA and LA than the control group.

p-CREB expression following immediate and delayed extinction in PFC region : The expression of p-CREB was found to be higher in extinction group as compared to their control in the PL region however; the changes were non-significant between IE and DE group. Two-way ANOVA analysis for p-CREB expression in PL region revealed a significant main effect of extinction condition (extinction vs. no

Extinction) [F (3, 26) =163.3, p ≤ 0.0001], However in IL, the expression of p-CREB was significantly higher in DE group as compared with the IE group and delayed no extinction and this was confirmed by two way ANOVA analysis [F (3, 26) =139.0, p ≤ 0.0001]. Extinction time (IE vs. DE) [F (3, 26) = 5.820, p ≤ 0.0001] as well as extinction condition and extinction time interaction [F (3, 26) = 176.0, p ≤ 0.0001]. We may say that the two sub regions of Amygdala responded differentially to respective two extinction conditions. This differential expression of p-CREB exemplifying the activity in the PL and LA in the IE and DE group which may be responsible for the deficit in the retention of extinction memory as observed after IE (Figure 5.7).

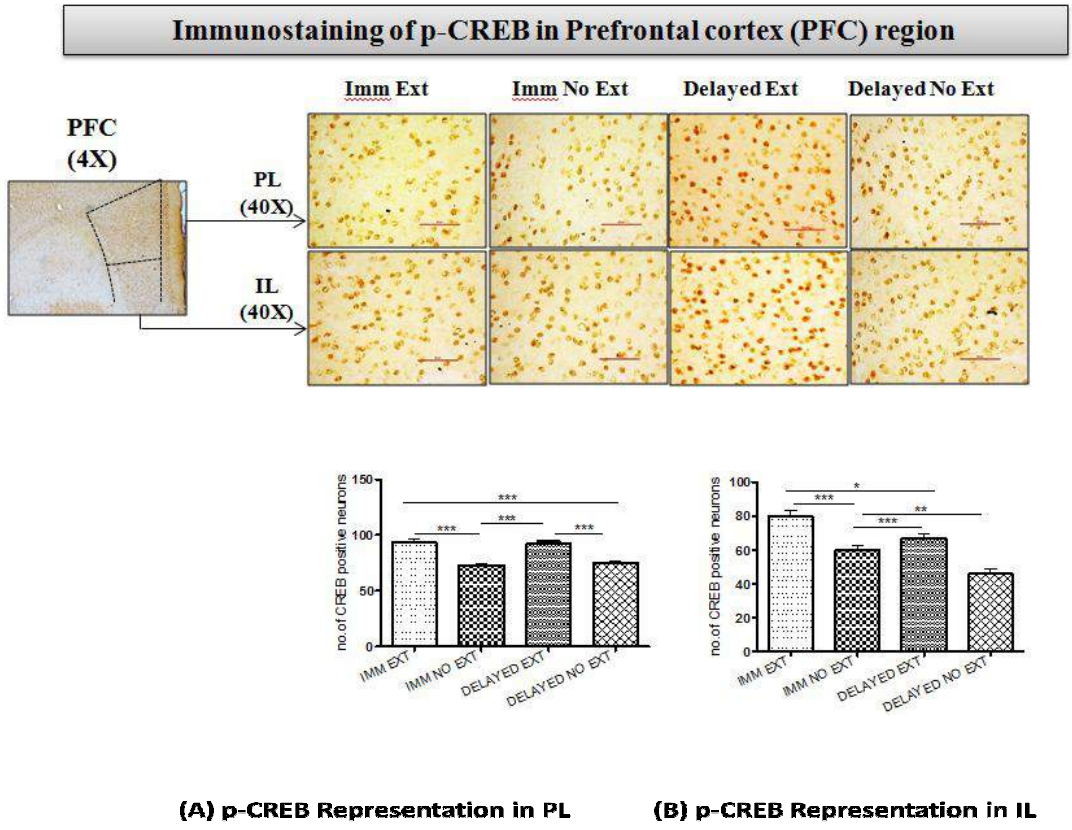


Figure 5.7. The expression of p-CREB in PFC following IE and DE learning: The expression of p-CREB was increased in the (A) PL and (B) IL of both IE and DE when compared to their respective controls. While, the expression of p-CREB was significantly increased in PL of DE as compared IE.

CREB expression following immediate and delayed extinction in PFC region:

The CREB expression is quite similar to p-CREB and higher in extinction groups when compared to their control. However, no significant changes were observed between IE and DE groups in PL region. Two-way ANOVA analysis for CREB expression in PL region showed significant association [$F(3, 26) = 83.19$, $P \leq 0.0001$] but no effect of extinction time in IL of IE vs. DE [$F(3, 26) = 191.3$, $P \leq 0.0001$].

Moreover in IL, significant increment in CREB expression was observed in DE. The data further supported by using two way ANOVA analysis that explains the same significant association [$F(3, 26) = 5.565$, $P \leq 0.0001$] extinction time [$F(3, 26) = 9.50$, $P \leq 0.0001$]. Expression of CREB also seems to be highly associated with neuronal activity in the IL and PL following IE and DE. We further analyzed that whether the increased CREB levels in these subregions culminated in methylation of H3 and H4 which are being studied and suggest that the methylation of Histone at various lysine residues to be associated with increased gene expression required for synaptic activity and memory consolidation (Figure 5. 8).

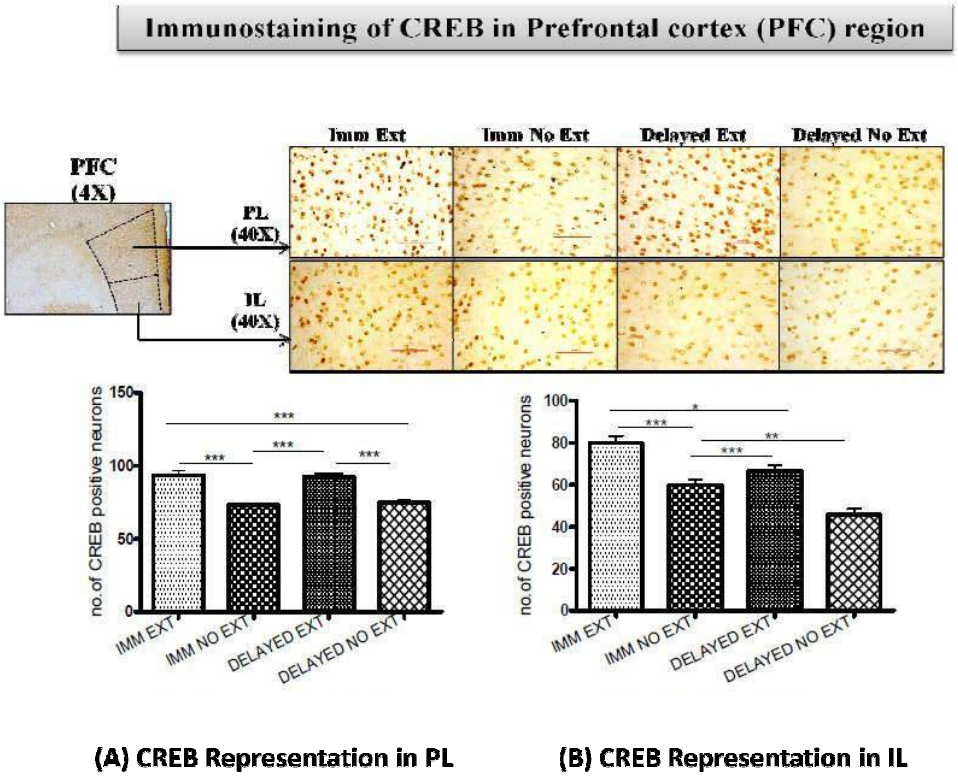
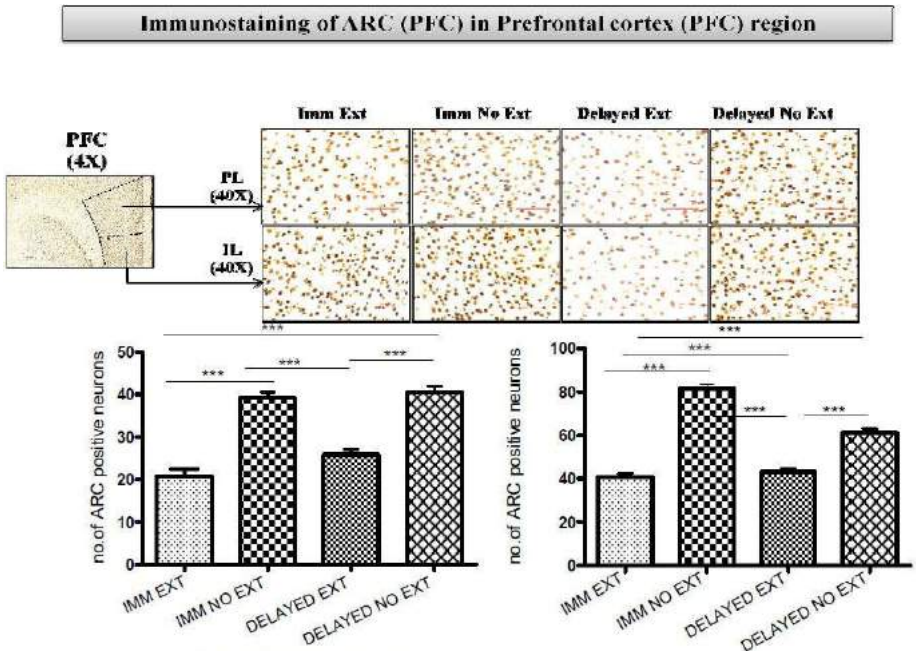


Figure 5.8. The expression of CREB in PFC following IE and DE learning: The expression of CREB was increased in the (A) PL and (B) IL of both IE and DE when

compared to their respective controls. While, the expression of CREB was significantly increased in PL of DE as compared IE.

ARC expression following immediate and delayed extinction in PFC region :

The same were observed in this i.e. the expression was higher in extinction groups however no significant association were observed between IE and DE in PL region. The same two-way ANOVA analysis for CREB expression in PL region revealed significant association with extinction condition [$F(3, 26) = 131.7$, $p \leq 0.0001$] but no effect of extinction time (IE vs. DE) and condition with time interaction. However in IL, there was a significant increment in gene expression of CREB when observed in DE as compared with IE and delayed no extinction group as supported by two way ANOVA analysis [$F(3, 26) = 310.6$, $p \leq 0.0001$]. Expression of ARC seems to be highly associated with neuronal activity in the PL and IL following IE and DE. We further want to clear whether the increased ARC levels in these regions were related to the methylation of H3 and H4, suggesting methylation of Histone at various lysine residues which may be associated with increased gene expression (Figure 5.9).

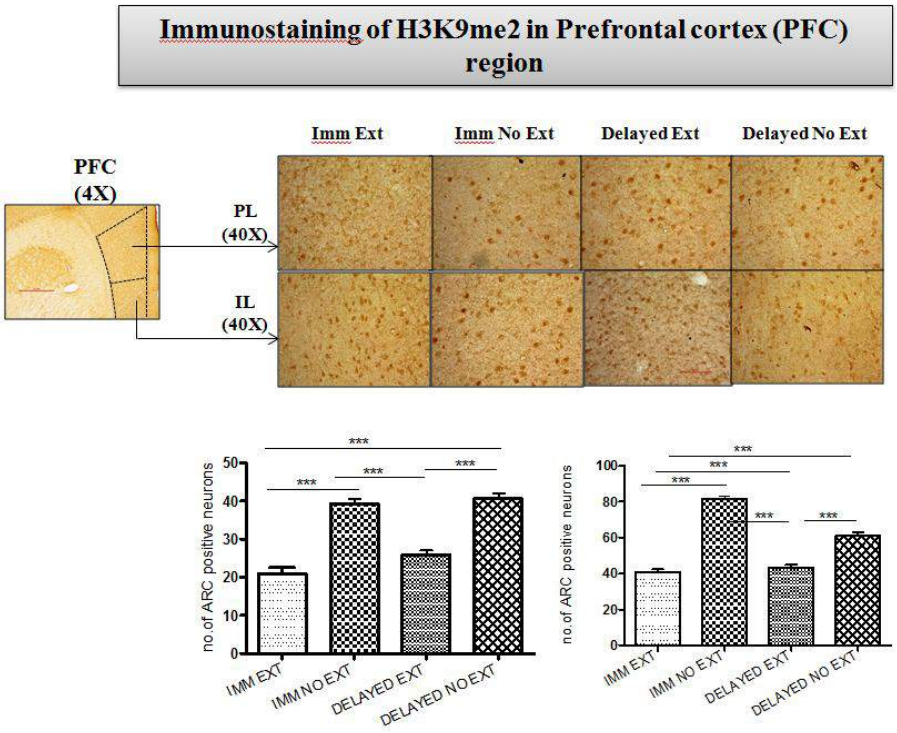


(A) ARC Representation in PL **(B) ARC Representation in IL**

Figure 5.9. The expression of ARC in PFC following IE and DE learning: The number of ARC + ve neurons was higher in (A) PL and (B) IL of both IE and DE. While, the numbers of ARC + ve neurons was significantly increased in IL of the IE as compared IE in PL.

Histone methylation following immediate and delayed extinction in PFC region:

Further, histone metylation in the PL and IL following IE and DE. We gauged the levels of methyl H3 at lysine residue 9 (K9) and methyl H4 at lysine residue 5 (K5) in PL and IL following two extinctions. Expression of methyl H3K9 in LA region showed that there was no such significant association between IE and DE however, IE and DE, has a decreased level of methyl H3K9 positive neurons as compared to immediate no extinction and delayed no extinction group. Two-way ANOVA analysis confirm the result [$F(3, 26) = 148.4$, $p \leq 0.0001$] and also the effect of extinction time (IE vs. DE) [$F(3, 26) = 16.48$, $p \leq 0.0001$]. While in PL region, the expression of H3K9 was significantly decreased in DE using two-way ANOVA analysis of IL [$F(3, 26) = 172.6$, $p < 0.0001$] and showed significant association [$F(3, 26) = 96.6$, $p \leq 0.0001$] and extinction time (IE vs. DE) [$F(3, 26)$ $p \leq 0.0001$]. These changes in the H3 methylation were definitely linked to the neuronal Activity i.e., the ARC, CREB, p-CREB in a region-specific manner; similar to the H3K9me2, the IE and DE exhibited decreased level of H3K9 expression. While we compared to their respective control groups in IL region but no such significant association was observed between IE and DE. The result was further confirmed by two-way ANOVA analysis. However, no significant association was also observed for Extinction time (IE vs. DE) [$F(3, 26) = 45.8$, $p \leq 0.0001$] (Figure 5.10).



(A) H3K9me2 Representation in PL **(B) H3K9me2 Representation in IL**

Figure 5.10. The expression of methyl H3K9me2 in PFC following IE and DE learning: Histone H3 methylation at 9th residue of lysine was found to be decreased in both IE and DE in both (A) PL and (B) IL. DE exhibited highly significant decrease in number of Methyl H3K9 +ve nuclei in PL and IL as compared to their respective controls.

DISCUSSION

In the present study, the effect of immediate and delayed extinction on retention of extinction memory along with neuronal activation and histone methylation in amygdala was examined. A deficit in the retention of extinction memory was observed in the immediate extinction group when compared to that in the delayed extinction group. Many previous studies have also reported such deficit in retention of extinction memory after immediate extinction (Chang and Maren, 2011; Thompson et al., 2010; Kim et al., 2010). However, there are conflicting reports about the outcome of immediate extinction. Most of the studies have reported deficit in the extinction memory except one which reports "erasure" of fear memory (Maren et al., 2013). Fear circuitry in amygdala poses its effect of fear expression via its interconnected nuclei. An increase in activity of these nuclei leads to different behavior outcomes governed by the stimuli received. Fear learning and extinction induces synapse formation by new neuronal connections, activating different sets of genes within the amygdala which in turn regulate the activity of that part. Acetylation of histone leads to activation of a gene by allowing the access of transcription factors which is the case here also in consolidation and extinction.

This form of learning is characterized by a decrease in a fear response when the contingent relationship between a conditioned stimulus (e.g. a sound) and an unconditioned stimulus (e.g. an electric shock) is compromised. This situation is most commonly implemented when the CS is repeatedly presented in the absence of the shock (Myers and Davis, 2002). The resilience of traumatic memories to extinction represents a serious obstacle for treating disorders characterized by abnormal fear and anxiety. Therefore understanding the basic mechanisms underlying fear extinction will lead to improvements in the treatment of this anxiety disorders (Myers and Davis, 2007). Studies have pointed out environmental effect on memory formation via the regulation of neuronal histone acetylation (Levenson et al., 2004). It has been demonstrated that CREB-binding protein (CBP), a potent HAT and transcriptional co activator is critical for long- lasting forms of synaptic plasticity (the activity-dependent changes in the strength of neuronal connections) and long-term memory (Levenson and Sweatt, 2006).

Firstly it was discovered in the 1960's, histone methylation is a common histone mark that occurs by the addition of a methyl group (-CH3) onto a lysine or arginine amino

acid residue (Murray, 1964; Greer and Shi, 2012). Studies of animals with genetic manipulations of the G9a (Gupta-Agarwal et al., 2012) and Mll2/Kmt2b genes (Kerimoglu et al., 2013) have firmly defined a role for histone methylation in both learning and memory.

A role for HDAC3 has also been identifed for addictions, where inhibition of this enzyme enhanced behavior performance in fear memory formation and the extinction of drug- seeking behavior (Malvaez et al., 2013). Effect of epigenetic modifications in addiction is not unidirectional or simple. Histone methylation can activate or repress gene transcription depending on the specific lysine residue modified and as a result can have both negative and positive effects on drug- associated behaviors.

Methylation of H3K9 in the NAc inhibits behavioral responses to cocaine and morphine (Maze et al., 2010), but methylation of H3K4 in the NAc enhances methamphetamine-induced conditioned place preference (Aguilar- Valles et al., 2014). However, histone methylation is necessary for proper long term consolidation of contextual fear memories. However, histone modifications like histone acetylation/phosphorylation facilitate the unraveling of DNA around the histone proteins resulting in the recruitment of the transcriptional machinery which is responsible for mediating gene expression of cell. Apart from this other histone modifications like histone methylation have controversial effects on gene transcription depending on the type of amino acid residue modification and the number of methyl groups present.

As per previous reports, histone methylation was implicated in the regulation of chromatin structure present in nervous system (Tsankova et al., 2006; Huang et al., 2007). Moreover, till date no such reports of histone methylation regulation in the nervous system functioning is available to represent the process of long-term memory storage.

Histone methylation as a potential upregulator of gene transcription in memory consolidation and may be involved in bidirectionally modify gene expression which is depending on the modification of histone residue as well as number of methyl groups present on a given residue. For example, histone tri-methylation is linked to gene repression at H3K9 (H3K9me3), but associated to gene activation at H3K4 (H3K4me3).

Histone modifications are catalyzed by various enzymes and the modifications are reversible. As per the previous reports, it was found that the enzymes are histone acetyltransferases (HAT) and histone deacetylase complexes (HDACs). HATs transfer an acetyl group to lysine residues, whereas HDACs remove acetyl groups. Histone post-translational modifications are key epigenetic processes controlling the regulation of gene transcription. In recent scenario, it has become apparent that chromatin modifications contribute to cognition through the modulation of gene expression required for the expression and consolidation of memories.

Most of the studies on epigenetics in cognition have focused on DNA methylation and histone H3 (de-) acetylation and have been summarized in excellent recent reviews (Miller et al., 2010). While DNA methylation is generally considered to inhibit gene transcription and histone acetylation is considered to be activating, histone methylation can achieve both gene activation and repression.

Histone methylation initially received less attention, because it was long assumed to be rigid and therefore of little interest to dynamic processes involved in cognition, such as learning and memory. It has been suggested that histone methylation could also be dynamically regulated (Gräff & Mansuy, 2008; Agarwal et al., 2012). In recent years, numerous studies have provided evidence that changes in histone lysine methylation, leading to gene expression activation or repression, are also required for the formation and consolidation of long-term memory (LTM) in particular brain regions.

However, epigenetics is an umbrella term used for all the changes that affect gene expression without changing the underlying gene sequence. It includes modifications of histones mostly at lysine residues by addition of acetyl group, methyl group etc. or modification of DNA by addition of mainly methyl residues. Many studies have directly correlated epigenetic changes to memory formation, storage and behavioral outcomes (Jarome and Lubin, 2014) as well as epigenetic mechanism for the consolidation of long-term memory was also reported (Levenson et al., 2004; Guan et al., 2002) and which is an important consideration when studying epigenetic mechanisms that are involved in cancer formation (Gong et al., 2016) Like histone modifications, DNA methylation may constitute an epigenetic code. These complex gene transcription programs initiated during cellular differentiation and division appear to be epigenetically regulated. Epigenetic changes can simply be viewed as

one of the final steps (or perhaps the final step) in a long cascade of events that leads to learning-related gene transcription.

These epigenetic mechanisms are involved in both biochemical and behavioral responses to drugs of abuse. The first of these studies employed a chromatin immunoprecipitation approach to identify histone modifications at individual gene-targets in the nucleus accumbens following cocaine treatment. (Murray, 1964; Lubin et al., 2011; Greer and Shi, 2012).

The unwinding story of these two sub-regions viz. LA and BA and hippocampus which regulate the consolidation and retention of fear and extinction in memory (Penke et al., 2016). It was found that the expression of p-CREB was found to be higher in extinction group as compared to their respective control groups in the LA region however, the changes were insignificant between immediate and delayed extinction group. However in BA, the expression of p- CREB was significantly higher in delayed extinction group as compared with the immediate extinction group and delayed no extinction group.

This predicts that the two sub regions of Amygdala responded differentially to the two extinction paradigms. This differential expression of p-CREB exemplifying the activity in the BA and LA in the immediate and delayed extinction group may be responsible for the deficit in the retention of extinction memory. While the CREB expression was higher in extinction groups as compared to their respective control groups no significant changes were observed between immediate and delayed extinction groups in LA region.

However, in BA, there was a significantly increased expression of CREB in delayed extinction group as compared with the immediate extinction group and delayed no extinction group and it was expressed that CREB seems to be associated with neuronal activity in the BA and LA, following immediate and delayed extinction. Furthermore, the increased CREB levels in these regions culminated in methylation of H3 and H4, the reason being methylation of Histone at various lysine residues to be associated with enhanced expression of genes required for synaptic activity and memory consolidation (Parkel et al., 2013; Petrovich et al., 1997; Akbarian and Huang, 2009; Tzeng et al., 2007; Veening et al., 1984).

Similar to the ARC, expression was higher in extinction groups as compared to their respective control groups but no significant changes were observed between immediate and delayed extinction groups in LA region.

However, the expression of ARC seems to be associated with neuronal activity in the BA and LA following immediate and delayed extinction. Expression of methyl H3K9 in LA region showed that there was no significant difference between immediate extinction and delayed extinction group however immediate and delayed extinction group has a lower level of methyl H3K9 positive neurons with respect to immediate no extinction and delayed no extinction group and these changes in the H3 methylation were linked to the neuronal activity i.e., the ARC, CREB, p-CREB in a region-specific manner similar to the H3K9 me2, The immediate and delayed extinction group exhibited lower level of H3K9 expression.

Our results on behavioral acclimatization of fear memory in relation with histone methylation in correlation with accurate nucleus of the hypothalamus (ARC), cAMP response element-binding protein (CREB), p-CREB as well as its target gene suggest that the activation of neurons in the amygdala region is compromised during fear memory-extinction in both the BA and LA regions. However, the level of histone methylation in amygdale is suppressed.

The present report depicts the effect of IE and DE extinction on retention of extinction memory as well as the neuronal activation and histone methylation in mPFC. A great loss in the retention of extinction memory was found in IE as compared to the DE. Many published studies support our observations (Chang and Maren, 2009; Chang and Maren, 2011; Thompson et al., 2010; Kim et al., 2010). However, some counteracts the data with IE (Myers et al., 2006). Our data is dependent on the decreased levels that were observed in any form of recovery in the early extinction. Moreover, this is quite hard to prove the statement of hypothesis. So, we finally conclude that the IE is less effective as compared to the DE, and this was supported by previous reports of Myers (2006). While, Chang and Maren (2009) reported that short term context independent suppression of fear after IE was high mainly due to habituation not extinction.

Apart from this in the present report we didn't use probe CS to test the extinction retention after extinction for 15 minutes and the deficit in retention of extinction as observed next day during the retention test was almost alike to published reports. Further studies are required for its confirmation.

To make the data effective we correlated the changes usually occurring in the two subregions of brain mainly PL and IL of mPFC, which along with the amygdala and

hippocampus. They are involved to regulate the consolidation and fear retention as well as memory extinction (Marek et al., 2013; Preston and Eichenbaum, 2013; Corcoran and Maren, 2001; Maren et al., 2013). DE and IE exhibited neuron activation in PL and IL subregion of mPFC as demarcated by the c-fos expression after extinction exposure. While, IL neuronal activity increment for DE was increased in relation to IE. Our results also support the previous reports (Thompson et al., 2010). However, some other reports showed different for fear behavior regulated by PL in relation IL (Sotres-Bayon et al., 2006; Milad et al., 2004; Courtin et al., 2014) through up/down regulated neuron activation in these regions (Sierra- Mercado et al., 2011; Sotres-Bayon and Quirk, 2010) as suggested in our present report.

The loss in retention of memory extinction was observed in the IE in our report and is similar to the previously published reports (Chang and Maren, 2009; Chang and Maren, 2011; Thompson et al., 2010; Kim et al., 2010). This is mainly due to the result of suppressed activation of IL neurons as well as electrical stimulation of mPFC which results in elimination of this loss (Kim et al., 2010). While expression of c-fos, we had looked at expression of CBP which was found to be increased in IL than that of IE which positively correlates with the expression of c-fos expression. As a known fact that CBP is associated with histone acetyltransferase and its increased activity results in the acetylation of histones H3/H4 which may modulate the expression of gene in the formation of memory (Peixoto and Abel, 2013). Our result suggested that there is decreased activity of neurons in IL of rats which fail to relate the fear as compared to extinguish fear normally. This is usually associated with the lysine residues which are present in the core of histone proteins (Roth et al., 2001). This report predicts that the neuronal activity in the IL and PL is associated with histone methylation. The methylation of H3 at residues K9 was significantly increased in IL and PL regions following IE and DE showed significant association. Our major findings suggest long-term extinction is minimal when extinction is conducted for a short time after fear learning in rats and this loss is usually by the decreased neuronal activation in IL of rats which are in IE. This loss in long term extinction is shown that it may be related to the level of histone methylation in the IL of the IE. The data from the present report may be useful in systematic planning and execution of psychological and pharmacological interventions.

The current study involves understanding the role of Histone methylation in fear memory consolidation and extinction. In the study, the histone methylation and

demethylation were found to be playing an important role in fear regulation and the mechanism is controlled differentially by different mechanisms. The basic mechanism of fear memory, functions by regulated control over histone methylation involved in different stages of fear memory as well as for spatial function in different brain regions (e.g. amygdala and PFC) (Morris et al, 2013). Basically, the fear memory has its path inside the brain through a complex connection known as fear circuitry which connects amygdale and PFC to produce a fear response (Giustino and Maren, 2015). Though the existence of fear circuitry is well known in animals (e.g. rodents and mammals) the molecular mechanism involved in fear memory is still an enigma (Blackiston et al, 2015). In this study, it has been observed that following fear memory consolidation animals response towards conditioning increased successively from first to the last trial which is similar with the previous studies (Datta and O'Malley, 2013; Siddiqui et al., 2017). However during extinction animals exhibited a reduction in fear response in each successive trial block of behavior as reported in earlier studies (Quirk, 2002; Siddiqui et al., 2017). Earlier studies have figured out that fear memory consolidation and its extinction involves different but overlapping neuronal circuitry known as fear and extinction circuit (Pare and Duvarci, 2012; Sah et al., 2003). The current study similarly signifies the role of different subregions of the amygdale and PFC following fear memory consolidation and extinction ($p \geq 0.000$). To understand the association of different brain activity the expression of IEG c-fos, which is a neuronal activity marker protein, was analyzed in the amygdale and PFC following fear memory consolidation and extinction ($p \geq 0.000$). In the amygdala, the activity of LA and BA showed positive association ($p \geq 0.000$) with the conditioning and extinction both, however, the activity was associated with the fear memory consolidation ($p \geq 0.000$) but not for the extinction learning.

Similarly, the histone methylation was associated with the expression of IEG c-fos during conditioning and extinction. Further more, the CBP expression shows similarity for the mRNA expression as exhibited by the c-fos mRNA expression in BLA ($p \leq 0.000$). These results are showing that the enhanced histone methylation is associated with the activation of the different subregions of the amygdala in conditioning and extinction. In light of the previous study that describes the presence of fear circuit within the amygdala, it may be hypothesized that these molecular changes observed in the present study are associated with the functionality of the

components of fear circuitry (Pare and Duvarsi, 2012; Kim et al., 2015). The presence of different types of neuronal population in LA and BA might be the reason for such differential molecular mechanism in conditioning and extinction. The studies have shown that the two types of neuronal populations namely dLAd (dorsal) and vLAd (ventral) exist in LA which response during conditioning and extinction respectively, (Repa et al., 2001). So it might be possible that this enhanced expression of c-fos and histone methylation is associated with these different neuronal populations in LA in conditioning and extinction.

Further more, it was reported that the variation of histone gene expression in such LA neurons might have an association with the conditioning and extinction, differentially. Also in LA the mGluR2/3 positive neuronal population respond only during fear extinction (Kim et al., 2015), which make another possibility that the histone expression is suppressed in such mGluR2/3 positive neurons might be associated with the extinction and these neuronal populations most probably confined to vLAd neuronal populations. Although the current work did not involve the study of such neuronal populations, the possible mechanism should be worked out for further clarification. Furthermore, in BA different neuronal populations known as fear and extinction neurons are present (Pare and Duvarci, 2012) that respond to conditioning and extinction respectively.

It is most probable that the increased histone methylation and activity of BA during conditioning and extinction is associated with the fear and extinction neurons, respectively. The PKC-zeta on and off neuronal population exist which respond to the conditioning and extinction respectively (Pare and Duvarci, 2012; Kim et al., 2015). The increased histone methylation in other neuronic regions may be associated with this PKC-zeta on and off neuronal activity during conditioning and extinction. The increased activity through enhanced histone methylation in PKC-zeta on neurons should be associated with the conditioning while enhanced activity through increased histone methylation in PKC-zeta off neurons might be associated with the extinction learning.

However, the different histone gene expression in neurotic regions of brain might be associated with the regulation of histone methylation in these neuronal populations

during conditioning and extinction (Siddiqui et al., 2017). The decreased gene expression of histone methylation during conditioning suggests that it is caused by the increased histone methylation activity. During extinction, the gene expression of histone was enhanced, which results in regulation of the fear memory expression. As most of the histone modifications are suppressed during conditioning but only one of them retains its activity for the extinction memory and might function to create a break on fear expression (Siddiqui et al., 2017). So it may be speculated that only one histone modification alone or in combination with other may regulate the activity of neurotic regulation mainly by the histone methylation. As histone methylation for H3 and H4 exhibited a positive correlation in LA and BA during conditioning with freezing behavior, the effect is mostly due to the activation of fear circuitry involving these brain regions (Siddiqui et al., 2017). During extinction, there is an increase in histone methylation in LA and BA which might be due to the enhanced activity of extinction circuitry while no activity in other region of the amygdala suggests for its suppression or control by the inhibitory neurons. Fear and extinction circuitry overlaps in terms of brain sub-region but the difference lies in the type of neurons (i. e. fear or extinction neurons, excitatory or inhibitory neurons) (Pare and Duvarci, 2012). Previous studies have focused the role of LA and BA in fear memory consolidation and extinction as lesion prior to consolidation and extinction in these region results in impaired expression of fear during consolidation and extinction respectively (Nader et al., 2001; Anglada-Figueroa and Quirk, 2005; Sierra- Mercado et al., 2011). Kim et al., (2015) in his research suggested that mGluR2/3 receptor is required for retention of extinction learning in LA (Kim et al., 2015). This might be the reason behind differential histone gene expression in LA following conditioning and extinction (Kim et al., 2015) as it is clear from the above discussion as well as with previous studies that LA contain different neuronal populations (Cells that respond during conditioning and cells that respond during extinction).

It has been concluded from the studies that LA receives input from IL and MGm to promote extinction by reversing the conditioning-induced potentiation at MGm-LA inputs (Park and Choi, 2010). The PL and IL of Prefrontal cortex act differentially during fear memory consolidation and extinction (Pelloux et al., 2013; Giustino and Maren, 2015). PL has a potent role for activating fear circuitry while the activity of IL is associated with the activation of fear extinction circuitry (Peters et al., 2009;

Knapska and Maren, 2009). The results showed increased activity and histone methylation in PL during fear memory consolidation while in IL during fear extinction. The Prefrontal cortex exhibited different patterns of histone gene expression during fear memory consolidation and extinction. In PL, the histone gene expression were negatively associated with the conditioning as exhibited by the conditioning group. As previous studies have focused for the presence of a glutamatergic population in PL (Patton et al., 2013; Marek et al., 2013), the decreased expression of two important histones might be involved in enhancement of histone methylation during conditioning which results in enhanced PL activity.

During extinction, the histone gene expression shows enhanced expression which should be associated with the suppression of PL and the most probable mechanism might be through GABAergic inhibitory connections through IL (Saffari et al., 2016; Jones et al., 2005). Overall the result suggests that for conditioning both the histone subtype should be suppressed which in turn increases histone acetylation, while for extinction only histone gene function is necessary for PL.

It may also be concluded from the others result that histone gene expression is a potent regulator of PL activity during extinction (Saffari et al., 2016; Jones et al., 2005). In IL, both the histones exhibited different expression following fear memory consolidation and extinction. histone gene expression decreased following conditioning while there was no change in other histone expression. As both the PL and IL sends inhibitory GABergic connections to each other (Saffari et al., 2016; Jones et al., 2005), it might be possible that the activation of these neuronal population in PL and IL is associated with the histone subtype activity.

In previous studies related to the other regions it was reported that CA1, CA3 and DG were activated following fear memory consolidation and extinction (Siddiqui et al., 2008). The result shows similarity with the previous studies, showing the role of CA1, CA3 and DG in consolidation (Lee and Kesner, 2004) and extinction (Bernier et al., 2017; Ji and Maren, 2008) using different lesion studies. Although the study showed the involvement of CA1, CA3 and DG in conditioning and extinction but no clear evidence is available showing the presence of different neuronal population in these subregions associated with the conditioning and extinction. Although some studies

have pointed that different neuronal population responds during conditioning and extinction in CA1, CA3 and DG (Vlachos et al., 2011). The activation mechanism was associated with the increased histone methylation and HAT activity (CBP/p300 activity) and increased histone methylation promotes the activity in conditioning and extinction. Although studies by Tronson et al (2009) confirmed the current result, where, an increased c-fos expression in conditioning and extinction was found to be associated with the contextual learning. Furthermore, Sanders et al (2003) and Maren et al (1997) showed in his experiment the role of this region in context and tone conditioning both, as pre and post-training lesion of the dorsal produced a modest tone conditioning deficits. So it might be possible that conditioning and extinction activate this region for the acquisition of CS part of learning through increased histone acetylation. Extinction similar to the conditioning is a new type of learning which require almost similar molecular and cellular mechanism (Myers et al., 2006) and results in activation of other subregion following conditioning and extinction. The information can be further increased by the experiments showing the activity of different sets of it (e.g. in CA1) in different contexts (Vlachos et al., 2011).

However, the CA3 and DG regions were also studied in different populations of neurons showing activity in different processes (Leutgeb et al., 2007; Knierim, 2002; Kentros et al., 2004). In subregion, both the HDACs showed different expression during conditioning and extinction. During conditioning the histone gene expression was suppressed in CA1 and CA3 while other histone expression was increased in DG. During extinction the one histone molecule gene expression is increased in CA1, CA3 and DG while the other one expression decreased in CA1, CA3 and DG subregion of it. Although limited information is available about the neuronal population in this subregion. It might be possible that some neurons respond to conditioning and others to extinction, and this differential activity of neuronal population is associated with the different activity of histone gene expression during conditioning and extinction.

The conditioning of fear is influenced by the valproic acid activity as per previous reports (Siddiqui et al., 2017). The result suggests that valproic acid given prior to the conditioning promotes conditioning of fear. Furthermore, the enhancement of conditioning learning was followed by the molecular changes associated with the formation of memory. When analyzed the activity in the amygdala, PFC and

hippocampus it was observed that valproic acid mediated histone inhibition enhanced the activity of subregions of the brain as well as histone methylation differentially, following conditioning and extinction. During conditioning, the activity of LA and BA were enhanced by the histone mediated inhibitor valproic acid which leads to the activation of the fear circuitry resulting in downstream activation of other neurotic region. This activity might be under the influence of increased histone methylation and showing similar activity within the LA and BA. The histone gene expression is exhibited a positive correlation with the histone methylation and activity of amygdala subregion, while it other molecule of histone subtype exhibited a negative correlation.

In brief the result suggests that the histone methylation promotes the activation of fear circuitry and this activity is supported by the enhanced histone gene expression (Siddiqui et al., 2017). The differential expression of both the histone gene expression in amygdala might be associated with the activation and suppression of different component of fear circuitry. Although valproic acid promotes histone methylation and fear learning, its target might be the histone modification in the one subtype but not by the other one as shown by the histone gene expression inhibition during conditioning. So it may be concluded that the major inhibitory mechanism is caused by the histone methylation in the amygdala, and its inhibition enhanced histone methylation in the amygdala during conditioning. Histone gene modifications may inhibit the different neurotic activity in PFC subregion in conditioning. Although the histone inhibitor is associated with the enhanced PL activity in conditioning, it did not affect the IL activity. In PL, these inhibitor showed its role to enhance the activity and histone methylation which results in enhanced conditioning response through strengthening PL activity.

In LA and BA the gene expression was increased while modification in the histone gene expression was suppressed by the histone molecule inhibitor (Guan et al., 2009). Other reported studies showed that the modification in histone gene expression is the main target of its inhibitor during extinction. The IL which is involved in extinction learning (Quirk and Mueller, 2008) exhibited enhanced activity in histone inhibitor-treated extinction group; however, there was no effect of it on PL activity in extinction group. These inhibitor enhanced the expression of histone methylation in IL while in PL the this inhibitor enhanced the activity in drug only group but not in

extinction groups. Thus, it may be concluded that enhanced histone gene expression in PL and decreased histone gene expression in IL is required for the extinction learning, and these inhibitor is doing this by acting on the histone molecule.

Studies have focused on the association of different histone modifications in other parts of the brain dependent contextual fear memory consolidation and extinction (Itzhak et al., 2013; Kwapis and Wood, 2014). Our result adds to that knowledge by showing that histone gene expression has a different spatial association with cued fear memory consolidation and its extinction. To our knowledge, this is the first report that directly assesses the relationship between histone methylation and its specific regulation of it through the interplay of histone gene expression. It has an important implication for the understanding and treatment of cognitive, psychiatric and neurodegenerative disorders related to impaired histone methylation.

CONCLUSION

The current study focused on the role of histone methylation and the association of histone gene expression with the methylation during fear memory consolidation and extinction. The amygdale and PFC showed differential activity for these two distinct processes of fear memory. Though the conditioning and extinction are different learning, same brain regions are shown to be involved in the association of both conditioning and extinction. Amygdala, which comprises of LA and BA showed differential activity during conditioning and extinction of fear. The LA and BA were active in conditioning while during extinction all the subregions. This might be the result of overlapping circuitries being engaged following fear and extinction learning. Likewise, the histone methylation was enhanced in LA and BA in conditioning while during extinction the LA and BA shows enhanced histone methylation. The result suggests that the histone methylation (H3K9) in these brain parts is associated with the activity of these brain regions during conditioning and extinction of fear memory. The increased histone methylation might be associated with the regulation of the activity of these brain regions for consolidation of fear and extinction memory. Both the histone demethylases exhibited an association with the activity of these brain regions as well as with the histone methylation for the fear memory consolidation and extinction. In LA and BA, the histone methylation modification which shows its association during conditioning as well as in extinction. A decreased histone gene expression was observed with increased histone methylation in LA and BA during conditioning and extinction. This suggests that the role of histone methylation in the regulation of histone methylation during conditioning. However during extinction, only the one of it showed association with the regulation of histone methylation as its expression increased together with the decreased expression of histone methylation. So, from the result, it may be speculated that histone gene expression is associated mainly with the regulation of the histone methylation in LA and BA in conditioning and extinction. In LA and BA the enhanced histone gene expression during conditioning and extinction might be associated with the suppression of those neurons that inhibit the activity of fear and extinction circuit during conditioning and extinction, respectively.

The PFC activity in conditioning and extinction suggests its association with the histone acetylation. The histone methylation as well as the activity of PL increased in conditioning while it increased in IL during extinction. The histone gene expression

decreased in PL and IL, following conditioning and extinction respectively. Histone gene expression decreased in PL and IL both following conditioning and its expression increased in PL following extinction. From this it may be speculated that increased histone methylation in PL following conditioning and in IL during extinction regulates the activity of PL and IL in conditioning and extinction respectively. Furthermore, it may also be concluded that in PL both the histone molecules which together regulate the expression of histone methylation in conditioning while it also regulates the histone methylation in PL following extinction. However, on the other hand the histone gene expression in IL might be associated alone with the regulation of histone methylation during extinction.

REFERENCES

REFERENCES

- Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. (2012). The effects of oxidative stress on female reproduction: a review. Reprod. Biol. Endocrinol. 10, 49.
- Aguilar-Valles A, Kim J, Jung S, Woodside B, Luheshi GN. (2014). Role of brain transmigrating neutrophils in depression-like behavior during systemic infection. Mol Psychiatry. 19, 599-606.
- Akbarian S and Huang HS. (2009). Epigenetic Regulation in Human Brain – Focus on Histone Lysine Methylation. Biol. Psychiatry. 65, 198 - 203.
- Alarcón, J. M., Malleret, G., Touzani, K., Vronskaya, S., Ishii, S., Kandel, E. R., & Barco, A. (2004). Chromatin Acetylation, Memory, and LTP Are Impaired in CBP+/− Mice. Neuron, 42(6), 947–959.
- Alberini, C. M. (2009). Transcription factors in long-term memory and synaptic plasticity. Physiological Reviews, 89(1), 121–45.
- Anglada-Figueroa, D., & Quirk, G. J. (2005). Behavioral/Systems/Cognitive Lesions of the Basal Amygdala Block Expression of Conditioned Fear But Not Extinction.
- Archbold, G.E.B., Bouton, M.E., Nader, K., 2010. Evidence for the persistence of contextual fear memories following immediate extinction. Eur. J. Neurosci. 31, 1303–1311. https://doi.org/10.1111/j.1460-9568.2010.07161.x
- Bahari-Javan, S., Maddalena, A., Kerimoglu, C., Wittnam, J., Held, T., Bahr, M.,… Sananbenesi, F. (2012). HDAC1 Regulates Fear Extinction in Mice. Journal of Neuroscience, 32(15), 5062–5073.
- Barrett, R. M., & Wood, M. A. (2008). Beyond transcription factors: The role of chromatin modifying enzymes in regulating transcription required for memory. Learning & Memory, 15(7), 460–467.
- Bernier, B. E., Lacagnina, A. F., Ayoub, A., Shue, F., Zemelman, B. V., Krasne, F. B., & Drew, M. R. (2017). Dentate Gyrus Contributes to Retrieval as well as Encoding: Evidence from Context Fear Conditioning, Recall, and Extinction. The Journal of Neuroscience, 37(26), 6359–6371.
- Blackiston, D. J., Shomrat, T., & Levin, M. (2015). The stability of memories during brain remodeling: A perspective. Communicative & Integrative

Biology, 8(5), e1073424.

- Bonelli, R. M., & Cummings, J. L. (2007). Frontal-subcortical circuitry and behavior. Dialogues in Clinical Neuroscience, 9(2), 141–51.
- Bouton ME, Mineka S, Barlow DH. (2001). A modern learning theory perspective on the etiology of panic disorder. Psychol. Rev. 108, 4 - 32.
- Bouton, M. E. (1988). Context and ambiguity in the extinction of emotional learning: Implications for exposure therapy. Behaviour Research and Therapy, 26(2), 137– 149.
- Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. Psychological Bulletin, 114(1), 80– 99.
- Bouton, M. E., García-Gutiérrez, A., Zilski, J., & Moody, E. W. (2006). Extinction in multiple contexts does not necessarily make extinction less vulnerable to relapse. Behaviour Research and Therapy, 44(7), 983–994.
- Bredy, T. W., & Barad, M. (2008). The histone deacetylase inhibitor valproic acid enhances acquisition, extinction , and reconsolidation of conditioned fear, 39–45.
- Bredy, T. W., Wu, H., Crego, C., Zellhoefer, J., Sun, Y. E. Y. E., & Barad, M. (2007). Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learning & Memory (Cold Spring Harbor, N.Y.), 14(4), 268–76.
- Breslau, N., Davis, G. C., Andreski, P., & Peterson, E. (1991). Traumatic events and posttraumatic stress disorder in an urban population of young adults. Archives of General Psychiatry, 48(3), 216–22.
- Burgos-Robles, A., Vidal-Gonzalez, I., Quirk, G.J., 2009. Sustained Conditioned Responses in Prelimbic Prefrontal Neurons Are Correlated with Fear Expression and Extinction Failure. J. Neurosci. 29, 8474–8482. https://doi.org/10.1523/JNEUROSCI.0378- 09.2009
- Cahill, L., & McGaugh, J. L. (1998). Mechanisms of emotional arousal and lasting declarative memory. Trends in Neurosciences, 21(7), 294–9.
- Cammarota, M., Bevilaqua, L. R. M., Barros, D. M., Vianna, M. R. M., Izquierdo, L. A., Medina, J. H., & Izquierdo, I. (2005). Retrieval and the Extinction of Memory. Cellular and Molecular Neurobiology, 25(3–4), 465–474.
- Cenquizca, L. A., & Swanson, L. W. (2007). Spatial organization of direct

hippocampal field CA1 axonal projections to the rest of the cerebral cortex. Brain Research Reviews, 56(1), 1–26.

- Chang C and Maren S. (2009). Early extinction after fear conditioning yields a context- independent and short-term suppression of conditional freezing in rats. Learn. Mem. 16, 62 - 68.
- Chang C and Maren S. (2011). Medial prefrontal cortex activation facilitates re- extinction of fear in rats. Learn. Mem. 18, 221 - 225.
- Clayton, A. L., Hazzalin, C. A., & Mahadevan, L. C. (2006). Enhanced Histone Acetylation and Transcription: A Dynamic Perspective. Molecular Cell, 23(3), 289–296.
- Corcoran, K.A., Maren, S., 2001. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. J. Neurosci. 21, 1720–6.
- Costanzi, M., Cannas, S., Saraulli, D., Rossi-Arnaud, C., & Cestari, V. (2011). Extinction after retrieval: effects on the associative and nonassociative components of remote contextual fear memory. Learning & Memory (Cold Spring Harbor, N.Y.), 18(8), 508–18.
- Craske MG, Kircanski K, Zelikowsky M, Mystkowski J, Chowdhury N, Baker A. (2008). Optimizing inhibitory learning during exposure therapy. Behav. Res. Ther. 46, 5 - 27.
- Craske, M.G., Kircanski, K., Zelikowsky, M., Mystkowski, J., Chowdhury, N., Baker, A., 2008. Optimizing inhibitory learning during exposure therapy. Behav. Res. Ther. 46, 5– 27. https://doi.org/10.1016/j.brat.2007.10.003
- Datta, S., & O'Malley, M. W. (2013). Fear extinction memory consolidation requires potentiation of pontine-wave activity during REM sleep. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 33(10), 4561– 9.
- Davis, H. P., & Squire, L. R. (1984). Protein synthesis and memory: a review. Psychological Bulletin, 96(3), 518–59.
- Davis, M. (2011). NMDA receptors and fear extinction: implications for cognitive behavioral therapy. Dialogues in Clinical Neuroscience, 13(4), 463–74.
- Davis, M., & Whalen, P. J. (2001). The amygdala: vigilance and emotion. Molecular Psychiatry, 6(1), 13–34.
- Duvarci, S., Nader, K., & Le Doux, J. E. (2008). De novo mRNA synthesis is

required for both consolidation and reconsolidation of fear memories in the amygdala. Learning & Memory (Cold Spring Harbor, N.Y.), 15(10), 747–55.

- Effting, M., & Kindt, M. (2007). Contextual control of human fear associations in a renewal paradigm. Behaviour Research and Therapy, 45(9), 2002–2018.
- Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C., & Lüthi, A. (2009). Amygdala Inhibitory Circuits and the Control of Fear Memory. Neuron, 62(6), 757–771.
- Fanselow, M. S., & Dong, H.-W. (2010). Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? Neuron, 65(1), 7– 19.
- Fanselow, M. S., & LeDoux, J. E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron, 23(2), 229–32.
- Ferreira, A.N., Yousuf, H., Dalton, S., Sheets, P.L., 2015. Highly differentiated cellular and circuit properties of infralimbic pyramidal neurons projecting to the periaqueductal gray and amygdala. Front. Cell. Neurosci. 9. https://doi.org/10.3389/fncel.2015.00161
- Fischer, A., Sananbenesi, F., Dobbin, M., & Tsai, L. (2007). Recovery of learning and memory is associated with chromatin remodelling, 3–5
- Fischer, A., Sananbenesi, F., Mungenast, A., & Tsai, L.-H. (2010). Targeting the correct HDAC(s) to treat cognitive disorders. Trends in Pharmacological Sciences, 31(12), 605–617.
- Fujita, Y., Morinobu, S., Takei, S., Fuchikami, M., Matsumoto, T., Yamamoto, S., & Yamawaki, S. (2012). Vorinostat, a histone deacetylase inhibitor, facilitates fear extinction and enhances expression of the hippocampal NR2B-containing NMDA receptor gene. Journal of Psychiatric Research, 46(5), 635–643.
- Gilmartin, M. R., Balderston, N. L., & Helmstetter, F. J. (2014). Prefrontal cortical regulation of fear learning. Trends in Neurosciences, 37(8), 455– 464.
- Giustino TF and Maren S. (2015). The role of the medial prefrontal cortex in the conditioning and extinction of fear. Front. Behav. Neurosci. 9, 298.
- Godsil, B. P., Kiss, J. P., Spedding, M., Er, T., & Jay, E. M. (2013). The hippocampal– prefrontal pathway: The weak link in psychiatric disorders? European Neuropsychopharmacology, 23, 1165–1181.

- Golkar A, Bellander M, Olsson A, Ohman A. (2012). Are fear memories erasable?– reconsolidation of learned fear with fear-relevant and fear-irrelevant stimuli. Front. Behav. Neurosci. 6, 1 - 80.
- Gong F, Chiu LY, Miller KM. (2016). Acetylation reader proteins: linking acetylation signaling to genome maintenance and cancer. PLoS Genet. 12, e1006272.
- Goosens, K. A., & Maren, S. (2001). Contextual and Auditory Fear Conditioning are Mediated by the Lateral, Basal, and Central Amygdaloid Nuclei in Rats. Learning & Memory, 8(3), 148–155.
- Goshen, I., Brodsky, M., Prakash, R., Wallace, J., Gradinaru, V., Ramakrishnan, C., & Deisseroth, K. (2011). Dynamics of Retrieval Strategies for Remote Memories. Cell, 147(3), 678–689.
- Graff J and Mansuy IM. (2008). Epigenetic codes in cognition and behaviour. Behav. Brain. Res. 192, 70 - 87.
- Grayson, D. R., Kundakovic, M., & Sharma, R. P. (2010). Is There a Future for Histone Deacetylase Inhibitors in the Pharmacotherapy of Psychiatric Disorders? Molecular Pharmacology, 77(2), 126–135.
- Greenberg, T., Carlson, J.M., Cha, J., Hajcak, G., Mujica-Parodi, L.R., 2013. Ventromedial prefrontal cortex reactivity is altered in generalized anxiety disorder during fear generalization. Depress. Anxiety 30, 242– 250. https://doi.org/10.1002/da.22016
- Greer EL and Shi Y. (2012). Histone methylation: a dynamic mark in health, disease and inheritance. Nat. Rev. Genet. 13, 343 - 357.
- Guan Z, Giustetto M, Lomvardas S, Kim JH, Miniaci MC, Schwartz JH, Thanos D, Kandel ER. (2002). Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. Cell. 111, 483 - 493.
- Guan, J.-S., Haggarty, S. J., Giacometti, E., Dannenberg, J.-H., Joseph, N., Gao, J., Tsai, L.-H. (2009). HDAC2 negatively regulates memory formation and synaptic plasticity. Nature, 459(7243), 55–60.
- Gupta-Agarwal S, Jarome TJ, Fernandez J, Lubin FD. (2014). NMDA receptor- and ERK-dependent histone methylation changes in the lateral amygdala bidirectionally regulate fear memory formation. Learn Mem. 21, 351 - 62.

- Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., McGaugh, J. L., Worley, P. F., & Barnes, C. A. (2000). Inhibition of activitydependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 20(11), 3993– 4001.
- Haberland, M., Montgomery, R. L., & Olson, E. N. (2009). The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nature Reviews Genetics, 10(1), 32–42.
- Haggarty, S. J., & Tsai, L.-H. (2011). Probing the role of HDACs and mechanisms of chromatin-mediated neuroplasticity. Neurobiology of Learning and Memory, 96(1), 41–52.
- Hall, J., Thomas, K. L., & Everitt, B. J. (2001). Fear memory retrieval induces CREB phosphorylation and Fos expression within the amygdala. The European Journal of Neuroscience, 13(7), 1453–8.
- Helmstetter, F. J., Parsons, R. G., & Gafford, G. M. (2008). Macromolecular synthesis, distributed synaptic plasticity, and fear conditioning. Neurobiology of Learning and Memory, 89(3), 324–37.
- Hermans, D., Craske, M. G., Mineka, S., & Lovibond, P. F. (2006). Extinction in Human Fear Conditioning. Biological Psychiatry, 60(4), 361–368.
- Hernandez, P. J., & Abel, T. (2008). The role of protein synthesis in memory consolidation: Progress amid decades of debate. Neurobiology of Learning and Memory, 89(3), 293–311.
- Herry C, Ferraguti F, Singewald N et. al. (2010). Neuronal circuits of fear extinction. Eur. J. Neurosci. 31, 599 - 612.
- Herry, C., Ciocchi, S., Senn, V., Demmou, L., Müller, C., & Lüthi, A. (2008). Switching on and off fear by distinct neuronal circuits. Nature, 454(7204), 600–606.
- Herry, C., Trifilieff, P., Micheau, J., Lüthi, A., & Mons, N. (2006). Extinction of auditory fear conditioning requires MAPK/ERK activation in the basolateral amygdala. European Journal of Neuroscience, 24(1), 261–269.
- Hoeffer, C. A., Cowansage, K. K., Arnold, E. C., Banko, J. L., Moerke, N. J., Rodriguez, R., Klann, E. (2011). Inhibition of the interactions between eukaryotic initiation factors 4E and 4G impairs long-term associative memory

consolidation but not reconsolidation. Proceedings of the National Academy of Sciences, 108(8), 3383– 3388.

- Holmes, A., & Quirk, G. J. (2010). Pharmacological facilitation of fear extinction and the search for adjunct treatments for anxiety disorders - the case of yohimbine. Trends in Pharmacological Sciences, 31(1), 2–7.
- Huang HS, Matevossian A, Whittle C, Kim SY, Schumacher A, Baker SP, Akbarian S. (2007). Prefrontal dysfunction in schizophrenia involves mixed-lineage leukemia 1- regulated histone methylation at GABAergic gene promoters. J. Neurosci. 27, 11254 - 11262.
- Huff NC, Hernandez JA, Blanding NQ, LaBar KS. (2009). Delayed Extinction Attenuates Conditioned Fear Renewal and Spontaneous Recovery in Humans. Behav. Neurosci. 123, 834 - 843.
- Huff, N. C., Frank, M., Wright-Hardesty, K., Sprunger, D., Matus-Amat, P., Higgins, E., & Rudy, J. W. (2006). Amygdala Regulation of ImmediateEarly Gene Expression in the Hippocampus Induced by Contextual Fear Conditioning. Journal of Neuroscience, 26(5), 1616–1623.
- Itzhak, Y., Liddie, S., & Anderson, K. L. (2013). Sodium butyrate-induced histone acetylation strengthens the expression of cocaine-associated contextual memory. Neurobiology of Learning and Memory, 102, 34–42.
- Izquierdo, I., Furini, C. R. G., & Myskiw, J. C. (2016). Fear Memory. Physiological Reviews, 96(2), 695–750.
- Jarome TJ and Lubin FD. (2014). Epigenetic mechanisms of memory formation and reconsolidation. Neurobiol. Learn. Mem. 115, 116 - 127.
- Jarome, T. J., Werner, C. T., Kwapis, J. L., & Helmstetter, F. J. (2011). Activity Dependent Protein Degradation Is Critical for the Formation and Stability of Fear Memory in the Amygdala. PLoS ONE, 6(9), e24349.
- Ji, J., & Maren, S. (2008). Differential roles for hippocampal areas CA1 and CA3 in the contextual encoding and retrieval of extinguished fear. Learning & Memory (Cold Spring Harbor, N.Y.), 15(4), 244–51.
- Jin, J., & Maren, S. (2015). Fear renewal preferentially activates ventral hippocampal neurons projecting to both amygdala and prefrontal cortex in rats. Scientific Reports, 5, 8388.
- Johansen, J. P., Cain, C. K., Ostroff, L. E., & LeDoux, J. E. (2011). Molecular Mechanisms of Fear Learning and Memory. Cell, 147(3), 509–524.

- Jones, B. F., Groenewegen, H. J., & Witter, M. P. (2005). Intrinsic connections of the cingulate cortex in the rat suggest the existence of multiple functionally segregated networks. Neuroscience, 133(1), 193–207.
- Kandel, E. R. (2001). The Molecular Biology of Memory Storage: A Dialogue Between Genes and Synapses. Science, 294(5544), 1030–1038.
- Kawaguchi, Y. (1995). Physiological subgroups of nonpyramidal cells with specific morphological characteristics in layer II/III of rat frontal cortex. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 15(4), 2638– 55.
- Kentros, C. G., Agnihotri, N. T., Streater, S., Hawkins, R. D., & Kandel, E. R. (2004). Increased attention to spatial context increases both place field stability and spatial memory. Neuron, 42(2), 283–95. Retrieved from
- Kerimoglu C, Agis-Balboa RC, Kranz A, Stilling R, Bahari-Javan S, Benito-Garagorri E, Halder R, Burkhardt S, Stewart AF, Fischer A. (2013). Histone-methyltransferase MLL2 (KMT2B) is required for memory formation in mice. J. Neurosci. 33, 3452 - 3464.
- Kida, S., Josselyn, S. A., de Ortiz, S. P., Kogan, J. H., Chevere, I., Masushige, S., & Silva, A. J. (2002). CREB required for the stability of new and reactivated fear memories. Nature Neuroscience, 5(4), 348–355.
- Kim SC, Jo YS, Kim IH, Kim H, Choi JS. (2010). Lack of Medial Prefrontal Cortex Activation Underlies the Immediate Extinction Deficit. J. Neurosci. 30, 832 - 837.
- Kim, J., An, B., Kim, J., Park, S., Park, S., Hong, I., Choi, S. (2015). mGluR2/3 in the Lateral Amygdala is Required for Fear Extinction: Cortical Input Synapses onto the Lateral Amygdala as a Target Site of the mGluR2/3 Action. Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology, 40(13), 2916–28.
- 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. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 32(32), 10879–86.
- Kim, S., & Kaang, B.-K. (2017). Epigenetic regulation and chromatin remodeling in learning and memory. Experimental & Molecular Medicine,

49(1), e281.

- Kim, Y. K., Seo, D.-W., Kang, D.-W., Lee, H. Y., Han, J.-W., & Kim, S.-N. (2006).
- Involvement of HDAC1 and the PI3K/PKC signaling pathways in NF-κB activation by the HDAC inhibitor apicidin. Biochemical and Biophysical Research Communications, 347(4), 1088–1093.
- Knapska, E., & Maren, S. (2009). Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. Learning & Memory (Cold Spring Harbor, N.Y.), 16(8), 486–493.
- Knierim, J. J. (2002). Dynamic interactions between local surface cues, distal landmarks, and intrinsic circuitry in hippocampal place cells. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 22(14), 6254–64.
- Krettek JE and Price JL. (1978). Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. J. Comp. Neurol. 178, 225 - 254.
- Kwapis, J. L., & Wood, M. A. (2014). Epigenetic mechanisms in fear conditioning: implications for treating post-traumatic stress disorder. Trends in Neurosciences, 37(12), 706–20.
- Lattal, K. M., & Wood, M. A. (2013). Epigenetics and persistent memory: implications for reconsolidation and silent extinction beyond the zero. Nature Neuroscience, 16(2), 124–129.
- Lattal, K. M., Barrett, R. M., & Wood, M. A. (2007). Systemic or intrahippocampal delivery of histone deacetylase inhibitors facilitates fear extinction. Behavioral Neuroscience, 121(5), 1125– 31.
- LeDoux JE. (2000). Emotion Circuits in the Brain. Annu. Rev. Neurosci. 23, 155 - 184. LeDoux, J. (2007). The amygdala. Current Biology, 17(20), R868–R874.
- LeDoux, J. E. (2012). Evolution of human emotion. In Progress in brain research (Vol. 195, pp. 431–442).
- LeDoux, J. E. (2014). Coming to terms with fear. Proceedings of the National Academy of Sciences, 111(8), 2871–2878.
- LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The

lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 10(4), 1062–9.

- Lee, I., & Kesner, R. P. (2004). Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextualfearconditioning. Hippocampus, 14(3), 301–310.
- Lee, S., Kim, S.-J., Kwon, O.-B., Lee, J. H., & Kim, J.-H. (2013). Inhibitory networks of the amygdala for emotional memory. Frontiers in Neural Circuits, 7, 129.
- Leutgeb, J. K., Leutgeb, S., Moser, M.-B., & Moser, E. I. (2007). Pattern Separation in the Dentate Gyrus and CA3 of the Hippocampus. Science, 315(5814), 961–966.
- Levenson JM and Sweatt JD. (2006). Epigenetic mechanisms: a common theme in vertebrate and invertebrate memory formation. Cell. Mol. Life. Sci. 63, 1009 - 1016.
- Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD. (2004). Regulation of histone acetylation during memory formation in the hippocampus. J. Biol. Chem. 279, 40545 - 40559.
- Likhtik, E., 2005. Prefrontal Control of the Amygdala. J. Neurosci. 25, 7429–7437. https://doi.org/10.1523/JNEUROSCI.2314-05.2005
- Lin, C.-H., Yeh, S.-H., Lu, H.-Y., & Gean, P.-W. (2003). The similarities and diversities of signal pathways leading to consolidation of conditioning and consolidation of extinction of fear memory. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 23(23), 8310–7.
- Long, V.A., Fanselow, M.S., 2012. Stress-enhanced fear learning in rats is resistant to the effects of immediate massed extinction. Stress 15, 627–636. https://doi.org/10.3109/10253890.2011.650251
- Lubin FD, Gupta S, Parrish RR, Grissom NM, Davis RL. (2011). Epigenetic mechanisms: critical contributors to long-term memory formation. Neuroscientist. 17, 616 - 632.
- Lubin, F. D., Roth, T. L., & Sweatt, J. D. (2008). Epigenetic Regulation of bdnf Gene Transcription in the Consolidation of Fear Memory. The Journal of Neuroscience, 28(42), 10576–10586.
- Lubin, F.D., Sweatt, J.D., 2007. The IkappaB kinase regulates chromatin

structure during reconsolidation of conditioned fear memories. Neuron 55, 942–57. https://doi.org/10.1016/j.neuron.2007.07.039

- Maddox, S. A., & Schafe, G. E. (2011). Epigenetic alterations in the lateral amygdala are required for reconsolidation of a Pavlovian fear memory. Learning & Memory, 18(9), 579–593.
- Malinow, R., & Malenka, R. C. (2002). AMPA Receptor Trafficking and Synaptic Plasticity. Annual Review of Neuroscience, 25(1), 103– 126.
- Malvaez M, McQuown SC, Rogge GA, Astarabadi M, Jacques V, Carreiro S, Rusche JR, Wood MA. (2013). HDAC3-selective inhibitor enhances extinction of cocaine- seeking behavior in a persistent manner. Proc. Natl. Acad. Sci. USA. 110, 2647 - 2652.
- Malvaez, M., Sanchis-Segura, C., Vo, D., Lattal, K. M., & Wood, M. A. (2010). Modulation of Chromatin Modification Facilitates Extinction of Cocaine-Induced Conditioned Place Preference. Biological Psychiatry, 67(1), 36–43.
- Mamiya, N., Fukushima, H., Suzuki, A., Matsuyama, Z., Homma, S., Frankland, P. W., & Kida, S. (2009). Brain Region-Specific Gene Expression Activation Required for Reconsolidation and Extinction of Contextual Fear Memory. Journal of Neuroscience, 29(2), 402–413.
- Marek, R., Strobel, C., Bredy, T. W., & Sah, P. (2013). The amygdala and medial prefrontal cortex: partners in the fear circuit. The Journal of Physiology, 591(10), 2381–91.
- Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. Annual Review of Neuroscience, 24(1), 897–931.
- Maren, S. (2008). Pavlovian fear conditioning as a behavioral assay for hippocampus and amygdala function: cautions and caveats. European Journal of Neuroscience, 28(8), 1661–1666.
- Maren, S., 2005. Building and burying fear memories in the brain. Neuroscientist. https://doi.org/10.1177/1073858404269232
- Maren, S., 2014. Nature and causes of the immediate extinction deficit: A brief review. Neurobiol. Learn. Mem. https://doi.org/ 10.1016/j.nlm. 2013.10.012
- Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behavioural

Brain Research, 88(2), 261–74.

- Maren, S., Aharonov, G., Stote, D. L., & Fanselow, M. S. (1996). N-methyl-D- aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. Behavioral Neuroscience, 110(6), 1365–74.
- Maren, S., Chang, C. -h., 2006. Recent fear is resistant to extinction. Proc. Natl. Acad. Sci. 103, 18020–18025. https://doi.org/10.1073/pnas.0608398103.
- Maren, S., Phan, K. L., & Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. Nature Reviews Neuroscience, 14(6), 417–428.
- Maze I, Covington HE 3rd, Dietz DM, LaPlant Q, Renthal W, Russo SJ, Mechanic M, Mouzon E, Neve RL, Haggarty SJ, Ren Y, Sampath SC, Hurd YL, Greengard P, Tarakhovsky A, Schaefer A, Nestler EJ. (2010). Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. Science. 327, 213 - 216.
- McDonald AJ. (1998). Cortical pathways to the mammalian amygdala. Prog. Neurobiol. 55, 257 - 332.
- McGaugh, J. L. (2000). Memory--a century of consolidation. Science (New York, N.Y.), 287(5451), 248–51.
- McGaugh, J. L. (2004). The Amygdala Modulates The Consolidation Of Memories Of Emotionally Arousing Experiences. Annual Review of Neuroscience, 27(1), 1–28.
- McQuown, S. C., Barrett, R. M., Matheos, D. P., Post, R. J., Rogge, G. A., Alenghat, T., Wood, M. A. (2011). HDAC3 is a critical negative regulator of long-term memory formation. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 31(2), 764–74.
- Milad, M. R., & Quirk, G. J. (2012). Fear Extinction as a Model for Translational Neuroscience: Ten Years of Progress. Annual Review of Psychology, 63(1), 129– 151.
- Milad, M.R., Vidal-Gonzalez, I., Quirk, G.J., 2004. Electrical Stimulation of Medial Prefrontal Cortex Reduces Conditioned Fear in a Temporally Specific Manner. Behav. Neurosci. 118, 389–394. https://doi.org/10.1037/0735-7044.118.2.389
- Milad, M.R.R., Quirk, G.J.J., 2002. Neurons in medial prefrontal cortex signal

memory for fear extinction. Nature 420, 70–74. https://doi.org/10.1038/nature01144.1.

- Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant. Cell. Environ. 33, 453 - 467.
- Miller, C.A., Campbell, S.L., Sweatt, J.D., 2008. DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. Neurobiol. Learn. Mem. 89, 599–603. https://doi.org/10.1016/j.nlm.2007.07.016
- Miller, R. L. A., Francoeur, M. J., Gibson, B. M., & Mair, R. G. (2017). Mediodorsal Thalamic Neurons Mirror the Activity of Medial Prefrontal Neurons Responding to Movement and Reinforcement during a Dynamic DNMTP Task. eNeuro, 4(5).
- Mimaki, T., Yabuuchi, H., Laird, H., & Yamamura, H. I. (1984). Effects of seizures and antiepileptic drugs on benzodiazepine receptors in rat brain. Pediatric Pharmacology (New York, N.Y.), 4(4), 205–11.
- Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. Acta Psychologica, 127(3), 567– 580.
- Monsey, M. S., Ota, K. T., Akingbade, I. F., Hong, E. S., & Schafe, G. E. (2011). Epigenetic Alterations Are Critical for Fear Memory Consolidation and Synaptic Plasticity in the Lateral Amygdala. PLoS ONE, 6(5), e19958.
- Montgomery, R. L., Hsieh, J., Barbosa, A. C., Richardson, J. A., & Olson, E. N. (2009). Histone deacetylases 1 and 2 control the progression of neural precursors to neurons during brain development. Proceedings of the National Academy of Sciences, 106(19), 7876–7881.
- Morris, M. J., Mahgoub, M., Na, E. S., Pranav, H., & Monteggia, L. M. (2013). Loss of Histone Deacetylase 2 Improves Working Memory and Accelerates Extinction Learning. Journal of Neuroscience, 33(15), 6401–6411.
- Morrison, A. K. (2009). Cognitive behavior therapy for people with schizophrenia. Psychiatry (Edgmont (Pa. : Township)), 6(12), 32–9.
- Mueller, D., Olivera-Figueroa, L. A., Pine, D. S., & Quirk, G. J. (2009). The effects of yohimbine and amphetamine on fear expression and extinction in rats. Psychopharmacology, 204(4), 599–606.

- Mueller, D., Porter, J. T., & Quirk, G. J. (2008). Noradrenergic Signaling in Infralimbic Cortex Increases Cell Excitability and Strengthens Memory for Fear Extinction. Journal of Neuroscience, 28(2), 369– 375.
- Muigg P, Hetzenauer A, Hauer G, Hauschild M, Gaburro S, Frank E, Landgraf R, Singewald N. (2008). Impaired extinction of learned fear in rats selectively bred for high anxiety - Evidence of altered neuronal processing in prefrontal-amygdala pathways. Eur. J. Neurosci. 28, 2299 - 2309.
- Murray K. (1964). The occurrence of epsilon-N-methyl lysine in histones. Biochemistry. 3, 10 - 15.
- Myers, K. M., & Davis, M. (2002). Behavioral and Neural Analysis of Extinction, 36, 567–584.
- Myers, K.M., 2006. Different mechanisms of fear extinction dependent on length of time since fear acquisition. Learn. Mem. 13, 216–223. https://doi.org/10.1101/lm.119806
- Myers, K.M., Davis, M., 2007. Mechanisms of fear extinction. Mol. Psychiatry. https://doi.org/10.1038/sj.mp.4001939
- Myskiw, J. C., Fiorenza, N. G., Izquierdo, L. A., & Izquierdo, I. (2010). Molecular mechanisms in hippocampus and basolateral amygdala but not in parietal or cingulate cortex are involved in extinction of one-trial avoidance learning. Neurobiology of Learning and Memory, 94(2), 285–291.
- Nader, K., Majidishad, P., Amorapanth, P., & LeDoux, J. E. (2001). Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. Learning & Memory (Cold Spring Harbor, N.Y.), 8(3), 156–63.
- Nakanishi, S. (1994). Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. Neuron, 13(5), 1031–7.
- Norrholm SD, Vervliet B, Jovanovic T, Boshoven W, Myers KM, Davis M, Rothbaum B, Duncan EJ. (2008). Timing of Extinction Relative to Acquisition: A Parametric Analysis of Fear Extinction in Humans. Behav. Neurosci. 122, 1016 - 1030.
- Orsini, C. A., & Maren, S. (2012). Neuroscience and Biobehavioral Reviews Neural and cellular mechanisms of fear and extinction memory formation. Neuroscience and Biobehavioral Reviews, 36(7), 1773– 1802.
- Pare D. (2004). New Vistas on Amygdala Networks in Conditioned Fear. J.

Neurophysiol. 92, 1 - 9.

- Pare, D., & Duvarci, S. (2012). Amygdala microcircuits mediating fear expression and extinction. Current Opinion in Neurobiology, 22(4), 717–723.
- Paré, D., Royer, S., Smith, Y., & Lang, E. J. (2003). Contextual inhibitory gating of impulse traffic in the intra-amygdaloid network. Annals of the New York Academy of Sciences, 985, 78–91. Retrieved from
- Park, J., & Choi, J.-S. (2010). Long-term synaptic changes in two input pathways into the lateral nucleus of the amygdala underlie fear extinction. Learning & Memory (Cold Spring Harbor, N.Y.), 17(1), 23–34.
- Parkel S, Lopez-Atalaya JP, Barco A. (2013). Histone H3 lysine methylation in cognition and intellectual disability disorders. Learn Mem. 20, 570-579.
- Patton, M. H., Bizup, B. T., & Grace, A. A. (2013). The Infralimbic Cortex Bidirectionally Modulates Mesolimbic Dopamine Neuron Activity via Distinct Neural Pathways. Journal of Neuroscience, 33(43), 16865–16873.
- Pavlov, & P., I. (1927). Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex. Oxford Univ. Press.
- Peixoto, L., & Abel, T. (2012). The Role of Histone Acetylation in Memory Formation and Cognitive Impairments. Neuropsychopharmacology, 38(1), 62–76.
- Peixoto, L., & Abel, T. (2013). The role of histone acetylation in memory formation and cognitive impairments. Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology, 38(1), 62–76.
- Pelloux, Y., Murray, J. E., & Everitt, B. J. (2013). Differential roles of the prefrontal cortical subregions and basolateral amygdala in compulsive cocaine seeking and relapse after voluntary abstinence in rats. The European Journal of Neuroscience, 38(7), 3018–26.
- Penke TJ, McKay DJ, Strahl BD, Matera AG, Duronio RJ. (2016). Direct interrogation of the role of H3K9 in metazoan heterochromatin function. Genes Dev. 30, 1866 - 1880.
- Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. Learning & Memory (Cold Spring Harbor, N.Y.), 16(5), 279–88.
- Peterson, G. M., & Naunton, M. (2005). Valproate: a simple chemical with so

much to offer. Journal of Clinical Pharmacy and Therapeutics, 30(5), 417–421.

- Petrovich GD and Swanson LW (1997). Projections from the lateral part of the central amygdalar nucleus to the postulated fear condi- tioning circuit. Brain. Res. 763, 247-254.
- Pikkarainen, M., Rönkkö, S., Savander, V., Insausti, R., & Pitkänen, A. (1999). Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. The Journal of Comparative Neurology, 403(2), 229–60.
- Ploski, J. E., Pierre, V. J., Smucny, J., Park, K., Monsey, M. S., Overeem, K. A., & Schafe, G. E. (2008). The Activity-Regulated CytoskeletalAssociated Protein (Arc/Arg3.1) Is Required for Memory Consolidation of Pavlovian Fear Conditioning in the Lateral Amygdala. Journal of Neuroscience, 28(47), 12383– 12395.
- Preston, A. R., & Eichenbaum, H. (2013). Interplay of hippocampus and prefrontal cortex in memory. Current Biology : CB, 23(17), R764- 73.
- Qing, H., He, G., Ly, P. T. T., Fox, C. J., Staufenbiel, M., Cai, F., … Song, W. (2008). Valproic acid inhibits Aβ production, neuritic plaque formation, and behavioral deficits in Alzheimer's disease mouse models. The Journal of Experimental Medicine, 205(12), 2781– 2789.
- Quirk, G. J. (2002). Memory for extinction of conditioned fear is long-lasting and persists following spontaneous recovery. Learning & Memory (Cold Spring Harbor, N.Y.), 9(6), 402–7.
- Quirk, G. J., & Beer, J. S. (2006). Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. Current Opinion in Neurobiology, 16(6), 723–727.
- Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology, 33(1), 56–72.
- Quirk, G.J., Russo, G.K., Barron, J.L., Lebron, K., 2000. The role of ventromedial prefrontal cortex in the recovery of extinguished fear. J. Neurosci. 20, 6225–6231. https://doi.org/20/16/6225 [pii]
- Ranjan, V., Singh, S., Siddiqui, S. A., Khan, M. Y., & Prakash, A. (2015). Differential histone acetylation in the Amygdala leads to fear memory

consolidation and extinction. International Journal of Science, Technology & Society, 1(1).

- Repa, J. C., Muller, J., Apergis, J., Desrochers, T. M., Zhou, Y., & LeDoux, J. E. (2001). Two different lateral amygdala cell populations contribute to the initiation and storage of memory. Nature Neuroscience, 4(7), 724–731.
- Riga, D., Matos, M. R., Glas, A., Smit, A. B., Spijker, S., & Van den Oever, M.C. (2014). Optogenetic dissection of medial prefrontal cortex circuitry. Frontiers in Systems Neuroscience, 8, 230.
- Rodrigues, S. M., LeDoux, J. E., & Sapolsky, R. M. (2009). The Influence of Stress Hormones on Fear Circuitry. Annual Review of Neuroscience, 32(1), 289–313.
- Rodriguez, B. I., Craske, M. G., Mineka, S., & Hladek, D. (1999). Context-specificity of relapse: effects of therapist and environmental context on return of fear. Behaviour Research and Therapy, 37(9), 845–862.
- Rosen, J.B., and Schulkin, J., 1998. From Normal Fear to Pathological Anxiety. Psychol. Rev. https://doi.org/10.1037/0033-295X.105.2.325
- Roth, S.Y., Denu, J.M., Allis, C.D., 2001. Histone Acetyltransferases. Annu. Rev. Biochem. 70, 81–120. https://doi.org/10.1146/annurev.biochem.70.1.81
- Rothbaum BO and Davis M. (2003). Applying Learning Principles to the Treatment of Post- Trauma Reactions, in: Annals of the New York Academy of Sciences. pp. 112–121.
- Rothbaum, B. O., & Schwartz, A. C. (2002). Exposure therapy for posttraumatic stress disorder. American Journal of Psychotherapy, 56(1), 59–75.
- Ruthenburg, A. J., Li, H., Patel, D. J., & Allis, C. D. (2007). Multivalent engagement of chromatin modifications by linked binding modules. Nature Reviews. Molecular Cell Biology, 8(12), 983–94.
- Saffari, R., Teng, Z., Zhang, M., Kravchenko, M., Hohoff, C., Ambrée, O., & Zhang, W. (2016). NPY+-, but not PV+-GABAergic neurons mediated long-range inhibition from infra- to prelimbic cortex. Translational Psychiatry, 6(2), e736.
- Sah, P., Faber, E. S. L., Lopez De Armentia, M., & Power, J. (2003). The amygdaloid complex: anatomy and physiology. Physiological Reviews, 83(3), 803–34.

- Sanders, M. J., Wiltgen, B. J., & Fanselow, M. S. (2003). The place of the hippocampus in fear conditioning. European Journal of Pharmacology, 463(1–3), 217–23.
- Saunders, R. C., Rosene, D. L., & Van Hoesen, G. W. (1988). Comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: II. Reciprocal and non-reciprocal connections. Journal of Comparative Neurology, 271(2), 185–207.
- Schafe, G. E., & LeDoux, J. E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 20(18), RC96.
- Schlenger, W. E., Caddell, J. M., Ebert, L., Jordan, B. K., Rourke, K. M., Wilson, D., … Kulka, R. A. (2002). Psychological reactions to terrorist attacks: findings from the National Study of Americans' Reactions to September 11. JAMA, 288(5), 581–8.
- Seto, E., & Yoshida, M. (2014). Erasers of histone acetylation: the histone deacetylase enzymes. Cold Spring Harbor Perspectives in Biology, 6(4), a018713.
- Siddiqui, S. A., Singh, S., Ranjan, V., Ugale, R., Saha, S., & Prakash, A. (2017). Enhanced Histone Acetylation in the Infralimbic Prefrontal Cortex is Associated with Fear Extinction. Cellular and Molecular Neurobiology, 37(7).
- Siddiqui, S. V., Chatterjee, U., Kumar, D., Siddiqui, A., & Goyal, N. (2008). Neuropsychology of prefrontal cortex. Indian Journal of Psychiatry, 50(3), 202–8.
- Sierra-Mercado, D., Padilla-Coreano, N., & Quirk, G. J. (2011). Dissociable Roles of Prelimbic and Infralimbic Cortices, Ventral Hippocampus, and Basolateral Amygdala in the Expression and Extinction of Conditioned Fear. Neuropsychopharmacology, 36(2), 529–538.
- Silva, A. J. (2003). Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. Journal of Neurobiology, 54(1), 224–237.
- Sintoni, S., Kurtys, E., Scandaglia, M., Contestabile, A., Monti, B., 2013. Chronic valproic acid administration impairs contextual memory and dysregulates hippocampal GSK- 3β in rats. Pharmacol. Biochem. Behav. 106, 8–15. https://doi.org/10.1016/j.pbb.2013.02.013

- Sokolowski, K. and Corbin, J., G. (2012). Wired for behaviours from development to function of innate limbic system circuitry. Frontiers in Molecular neurosciences, 5(55), 1-15.
- Sotres-Bayon, F., & Quirk, G. J. (2010). Prefrontal control of fear: more than just extinction. Current Opinion in Neurobiology, 20(2), 231–235.
- Sotres-Bayon, F., Cain, C.K., LeDoux, J.E., 2006. Brain Mechanisms of Fear Extinction: Historical Perspectives on the Contribution of Prefrontal Cortex.
- Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2005.10.012
- Stafford, J. M., Raybuck, J. D., Ryabinin, A. E., & Lattal, K. M. (2012). Increasing Histone Acetylation in the Hippocampus-Infralimbic Network Enhances Fear Extinction. Biological Psychiatry, 72(1), 25–33.
- Stefanko, D. P., Barrett, R. M., Ly, A. R., Reolon, G. K., & Wood, M. A. (2009). Modulation of long-term memory for object recognition via HDAC inhibition. Proceedings of the National Academy of Sciences of the United States of America, 106(23), 9447–52.
- Strekalova, T., Zorner, B., Zacher, C., Sadovska, G., Herdegen, T., & Gass, P. (2003). Memory retrieval after contextual fear conditioning induces cFos and JunB expression in CA1 hippocampus. Genes, Brain and Behavior, 2(1), 3–10.
- Sultan, F. A., & Day, J. J. (2011). Epigenetic mechanisms in memory and synaptic function. Epigenomics, 3(2), 157–81.
- Thompson BM, Baratta MV, Biedenkapp JC, Rudy JW, Watkins LR, Maier SF. (2010). Activation of the infralimbic cortex in a fear context enhances extinction learning. Learn. Mem. 17, 591 - 599.
- Tronson, N. C., Schrick, C., Guzman, Y. F., Huh, K. H., Srivastava, D. P., Penzes, P., … Radulovic, J. (2009). Segregated populations of hippocampal principal CA1 neurons mediating conditioning and extinction of contextual fear. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 29(11), 3387– 94.
- Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat. Neurosci. 9, 519 - 525.
- Turner BH and Herkenham M. (1991). Thalamoamygdaloid projections in the rat: a test of the amygdala's role in sensory processing. J. Comp. Neurol. 313, 295 - 325.

- Tzeng TY, Lee CH, Chan LW, Shen CKJ. (2007). Epigenetic regulation of the Drosophila chromosome 4 by the histone H3K9 methyltransferase dSETDB1. PNAS. 104, 12691 – 12696.
- VanElzakker MB, Kathryn Dahlgren M, Caroline Davis F, Dubois S, Shin LM (2014). From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders. Neurobiol. Learn. Mem. 113, 3-18.
- Vecsey, C. G., Hawk, J. D., Lattal, K. M., Stein, J. M., Fabian, S. A., Attner, M. A., … Wood, M. A. (2007). Histone Deacetylase Inhibitors Enhance Memory and Synaptic Plasticity via CREB: CBP- Dependent Transcriptional Activation. Journal of Neuroscience, 27(23), 6128–6140.
- Veening JG, Swanson LW, Sawchenko PE. (1984). The organization of projections from the central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: a combined retrograde transport-immunohistochemical study. Brain. Res. 303, 337 - 357.
- Vianna, D. M., Landeira-Fernandez, J., & Brandão, M. L. (2001). Dorsolateral and ventral regions of the periaqueductal gray matter are involved in distinct types of fear. Neuroscience and Biobehavioral Reviews, 25(7–8), 711–9.
- Vlachos, I., Herry, C., Lüthi, A., Aertsen, A., & Kumar, A. (2011). Context-Dependent Encoding of Fear and Extinction Memories in a Large- Scale Network Model of the Basal Amygdala. PLoS Computational Biology, 7(3), e1001104.
- Wei, W., Coelho, C. M., Li, X., Marek, R., Yan, S., Anderson, S., … Bredy, T.W. (2012). p300/CBP-associated factor selectively regulates the extinction of conditioned fear. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 32(35), 11930–41.
- Wessa M and Flor H. (2007). Failure of extinction of fear responses in posttraumatic stress disorder: Evidence from second-order conditioning. Am. J. Psychiatry 164, 1684 - 1692.
- Whittle, N., & Singewald, N. (2014). HDAC inhibitors as cognitive enhancers in fear, anxiety and trauma therapy: where do we stand? Biochemical Society Transactions, 42(2), 569–581.
- Wilensky, A. E., Schafe, G. E., Kristensen, M. P., & LeDoux, J. E. (2006). Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning.

The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 26(48), 12387– 12396.

- Yasuda, S., Liang, M.-H., Marinova, Z., Yahyavi, A., & Chuang, D.-M. (2009). The mood stabilizers lithium and valproate selectively activate the promoter IV of brain-derived neurotrophic factor in neurons. Molecular Psychiatry, 14(1), 51–59.
- Yeh, S.-H., Mao, S.-C., Lin, H.-C., & Gean, P.-W. (2005). Synaptic expression of glutamate receptor after encoding of fear memory in the rat amygdala. Molecular Pharmacology, 69(1), 299–308.
- Young, S. L., Bohenek, D. L., & Fanselow, M. S. (1994). NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: immunization against amnesia by context preexposure. Behavioral Neuroscience, 108(1), 19–29.
- Zhou, Y., Won, J., Karlsson, M. G., Zhou, M., Rogerson, T., Balaji, J., … Silva, J. (2009). CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nature Neuroscience, 12(11), 1438–1443.
- Zimmerman, J. M., & Maren, S. (2010). NMDA receptor antagonism in the basolateral but not central amygdala blocks the extinction of Pavlovian fear conditioning in rats. European Journal of Neuroscience, 31(9), no-no.
- Zimmerman, J. M., & Maren, S. (2011). The bed nucleus of the stria terminalis is required for the expression of contextual but not auditory freezing in rats with basolateral amygdala lesions. Neurobiology of Learning and Memory, 95(2), 199– 205.

PUBLICATIONS

(2018)

Siddiqui S.A .; Singh S.; Ugale R.; Ranjan V.; Kanojia R.; Saha S.; Tripathy S.; Kumar S.; Mehrotra S.; Modi D R.; and Prakash A.; 2019. "Regualtion of hdac1 and hdac2 during Consolidation and Extinction of fear memory". Journal of Brain Research Bulletin, 17(150):86-101. (Impact Factor -3.3404)

(2019)

Kanojia R..; Modi D.R.; Jaiswal K.; 2019. "Behavioral acclimatization of fear memory and its relation with histone methylation in correlation with accurate nucleus of the hypothalamus ,c AMP response element –binding protein and its target gene". Journal of Emerging Technologies and Enovative Research (JETIR) ISSN -2349-5162 (impact factor 5.87)

(2019)

Kanojia R.;Modi D.R.; Mishra S.: Jaiswal K.;2019. "Histone acetylation befuddledness in the infralimbic pre-frontal cortex and their association with extinction memory" journal of International Journal of Research and Review (E-ISSN: 2349-9788; P-ISSN: 2454-2237)

CONFERENCES

Best Paper Presentation Award

Best Paper Presentation Award (Oral presentation)
Entitled on **" Role of Histone Mehtylation in specific brain region amygdale and pre- frontal cortex (PFC) in extinction of fear memory "** Animal science congress in the : horizons in zoological studies organized by department of Zoology ,University of Kashmir associated with ZSI ,Gaya and JAKAS Kashmir " held on 4th to 6th of August 2018 University of Kashmir

International Conferences:

Oral presentation on "Small Metabolites As a Target Molecule For Cancer Therapy" International Conference on Updates in Cancer Prevention and Research (ICUCPR 2017) and Satellite Conference on Translational Pharmaceutical Research : Trends and Implication (IPC) 14TH to 16th February 2017 ICUCPR & IPCBBAU-2017

Baba saheb Bhimrao Ambedkar University Lucknow. U.P (India).
103th The Indian Science Congress Association 14, Dr.Biresh Guha Street, Kolkata - 700017 held on 3 to 7, 2016 University of Mysore. Krishna raja Boulevard Road, K.G Koppal, Mysuru, Karnataka 570006 (India).

Workshops and Conference's Attended:

Best presentation award (oral presentation) Entitled on " Role of Histone Methylation In Extinction of Fear Memory" animal science congress in the :horizons in zoological studies organized by department of zoology ,university of Kashmir associated with **ZSI,GAYA AND JAKAS Kashmir**" held on 4 to 6 august 2018 university of Kashmir Oral presentation on Entiled as " Expression of CREB Target Gene May Underlie Erasure of Fear Memory Leading To Extinction" National science day celebration and seminar "foresting scientific temper for welfare of society and surroundings" on 27-28 February 2018 zoological society of India, Bodhgaya .

National science day three day seminar on the theme Science And Technology for Specially Abled Persons" on February 28-02 march 2017 Department of Applied Animal Sciences Babasaheb Bhimrao Ambedkar University Lucknow U.P (India)

Three day conference on Entiled "Status of India's Under Changing Climate Conditions with Special Refrence To Semiarid Zone of Bundelkhand Region for Upliftment of Rural Economy And Women Empowerment" held on March 04 to 06, 2016 Bundelkhand, Jhashi ,U.P (India)

103th The Indian Science Congress Association 14, Dr.Biresh Guha Street, Kolkata - 700017 held on 3 to 7, 2016 University of Mysore. Mysore

26th All India Congress of Zoology and "International Symposium On Innovation In Animal Science For Food Security, Helath Security And Livehood " On October 29 to 31 2015 Department of Applied Animal Sciences Babasaheb Bhimrao Ambedkar University Lucknow U.P (india)

3rd Luscon science congress on “Science For Society : An Interdisciplinary Approach” on 31 october 2015 Department of Biotechnology Babasaheb Bhimrao Ambedkar University Lucknow U.P (India)

2nd Luscon science congress “Leveraging Science And Innovative For Development” march 27-28-2104 Department of Biotechnology Babasaheb Bhimrao Ambedkar University Lucknow U.P (India)

INDO –US Symposium on “Impact Of Early Life Adversity On Developing Brain” organized by biochemistry foundation and Indian academy of neuroscience (IAN) . Held on October 29, 2013 Department of Biotechnology Babasaheb Bhimrao Ambedkar University Lucknow U.P (India)

Patent workshop “placement and employment prospects in india patent offices and hands on training for patenting the research work organized by university placement cell” on 18 march 2013 Babasaheb Bhimrao Ambedkar University Lucknow U.P (India).

Life Time Membership:

Zoological society of India (Estd-1938) Registered Under Society Registration Act 21,1860; Regd. No.302/2002-2003

Section: Animal, Veterinary and Fishery’s Sciences

Membership No: SLM 1580