ROLE OF HISTONE ACETYLATION & DEACETYLATION DURING FEAR CONSOLIDATION AND EXTINCTION

SUMMARY OF THESIS

SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

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प्रज्ञा शील करुणा

ESTABLISHED 1996

FOR THE DEGREE OF

Doctor Of Philosophy

IN

BIOTECHNOLOGY

Submitted by

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Enrolment No. 467/09

2016

SUMMARY

The society worldwide has seen an enormous increase in the incidences of crime, terrorism, natural disasters and war. This has lead to an increased number of patients of posttraumatic stress disorders (PTSD) and other fear related anxiety disorders. However, it is a matter of research that why not all, but only some of the victims develop these disorders. Pavlov's classical conditioning based fear conditioning is extensively used by the neuroscientists to study the fear leaning processes. The current focus of research in field of neurobiology of fear is to decipher cellular, molecular mechanism operative during different phases of fear learning processes, in order to find out better treatment strategies for people afflicted by these fear related disorders.

The stabilization of the fear associations into long-term memory is referred to as fear consolidation (Abel & Lattal, 2001; Tonegawa *et al.*, 2003; Nader, 2003). Extinction of fear memory is another stage of memory during which part of memory loses its ability to recall, in other words memory of events weakens with time. Some psychological theories have described extinction as an "unlearning" process (Rescorla, 1967) but recently extinction has been found to be new inhibitory learning which also depends on gene expression, protein synthesis and synaptic plasticity (Bruel-Jungerman *et al*., 2007; Alberini, 2009) as in process of consolidation; and does not erase the registered association between conditioned stimulus (CS) and unconditioned stimulus (US), instead fear extinction forms a new memory that inhibits expression of conditioned response.

Lately some studies have focused on the role of histone acetylation in amygdala, hippocampus and PFC towards fear memory consolidation and extinction. Reduced histone acetylation at H3K14, H4K5 & H4K12, induced by isoflurane, in CA1 of hippocampus is associated with reduced fear response (Zhong *et al*, 2014). The increased histone H4 acetylation in PFC has been associated with strong extinction while increased acetylation at H3 is found only in conditioned mice but not in extinction group (Bredy *et al*, 2007). HDAC inhibition with NaB infused within mPFC resulted in increased histone acetylation in IL but not PL, which was correlated with enhanced extinction (Stafford et al, 2012). Activity of p300, a HAT, in ILPFC constrain synaptic plasticity and reduction in its' function is required for formation of extinction memory. The activity of PCAF; p300/CBP associated factor, in IL region of PFC is reported as necessary for fear extinction memory formation and interfere with reconsolidation of original memory trace (Breddy & Barad, 2008).

The present work was done to find out epigenetic correlates, which regulate the fear circuit in amygdala, hippocampus and prefrontal cortex (PFC) cued model of fear conditioning. Behavioral training for fear conditioning and subsequently for fear extinction was performed in S D rats by a protocol similar to Chang *et al*., 2009. Neuronal activity at the protein level was checked using immunohistochemistry (IHC), in amygdala, hippocampus and PFC. Level of histone acetylation was also explored to find out the epigenetic correlation, if any, for regulation of selected molecular expressions in fear circuit during fear memory consolidation and extinction of memory.

Table1: Represents the status of CREB, ERK, ARC, H3K9, H4K5, CBP following fear conditioning and extinction in the PFC, Hippocampus and Amygdala of rats

		LA	**BA**	**CeL**	**CeM**	**CA1**	**CA3**	**DG**	**PL**	**IL**
CREB/p-CREB	Cond.	++		- -	+++	++++			++	
	Ext.	+				++++	++++	++++		++
ERK/p-ERK	Cond.	++++	++	+++	+++	+++	+	- -	+++	
	Ext.	+					+++	- - -		+++
ARC	Cond.	+++		++	++	+++	+++	++	++++	- - -
	Ext.	++		++		+++			++++	
H3K9	Cond.	+++	+++	+	+++	++	++	+++	++	
	Ext.	+++	+++	+	+			+++		+++
H4K5	Cond.	++			+++	++	++	++	++++	- - -
	Ext.	++		+				++		+
CBP	Cond.	+++		- - -	++	++	+++		++++	- - -
	Ext.	+++	++	-				- - -		

The differential phosphorylation of ERK,CREB was found the sub regions of amygdala, PFC and Hippocampus along with the expression of immediate early gene (IEG) ARC. These changes were parallel to the changes in the acetylation of H3 and H4, as well as expression of CBP, a histone acetyl transferase, following fear conditioning and extinction.Overall the behavioral changes correlated to the changes in the CREB, ERK and ARC and the changes in the expression of these genes were under the control of Epigenetic changes as evident increased/ decreased acetylation of Histones (H3 & H4) in different brain regions.

There are no studies till date, which give the idea about the dynamics of the molecular mechanism operative in the fear related brain areas (amygdala, hippocampus & PFC) at the same time in the presence/ absence of cues associated with the fear. The findings of this study would be helpful to understand and develop potential behavioral as well as novel drug therapeutic targets for PTSDs. Further research would be required towards better understanding of these changes gauged at the protein level changes. Looking at the changes at the promoter level of the genes implicated in fear behavior will further support our notion and would be helpful in finding newer molecular targets for setting better treatment regimes for the fear related disorders.

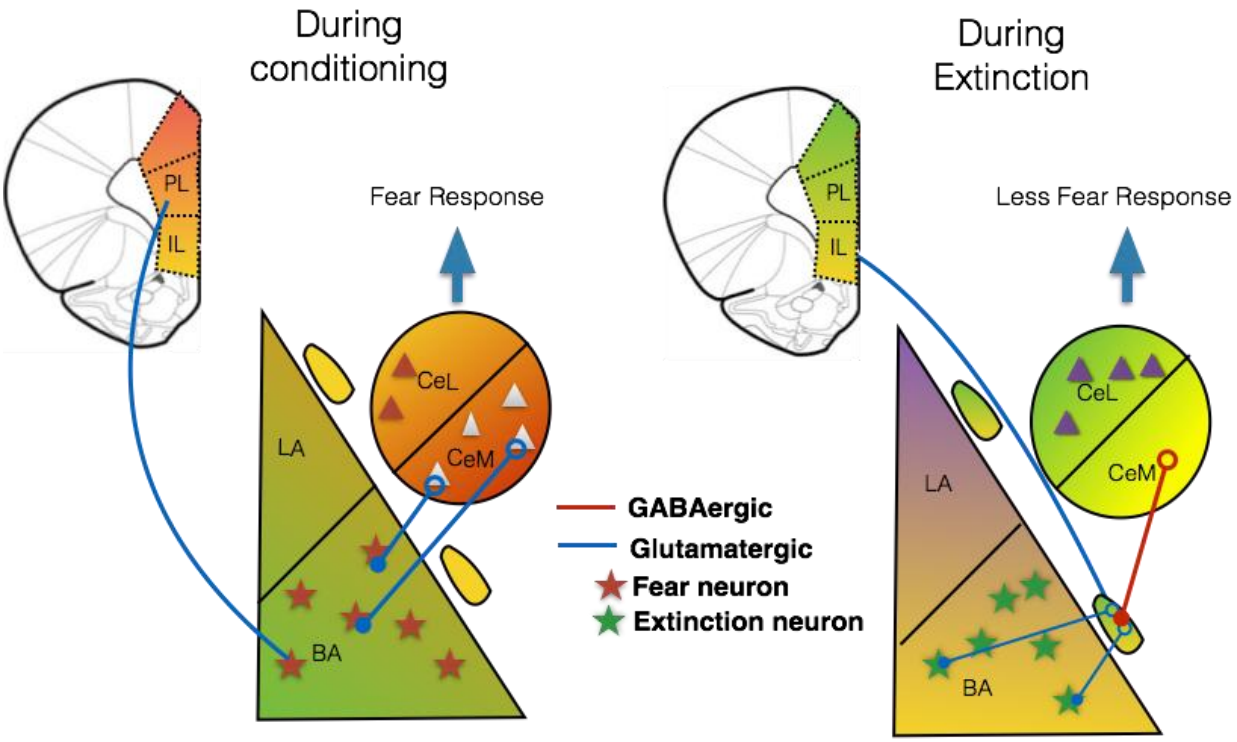

Figure 1: Represents the overall findings in the study. The first panel shows the activation pattern during fear conditioning. The PL, which was active during conditioning as evident by the phosphorylation of ERK and CREB and increased level of ARC an IEG and also increased histone acetylation, through its innervations on the fear neurons of BA activated CeM resulting in fear outcomes. The second panel shows neuronal activation pattern in different sub regions of PFC and Amygdala during extinction. The IL, which was active during conditioning as evident by the phosphorylation of ERK and CREB and increased level of ARC an IEG and also increased histone acetylation, through its innervations on extinction neurons in BA as well as direct innervation on mITCs inhibits CeM and thus very less fear or no fear was observed following extinction.

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