Abstract of the Thesis

To study the role of different subsets of HDACs (Histone Deacetylases) in Fear memory Consolidation and Extinction

Thesis

Submitted To

Babasaheb Bhimrao Ambedkar University

Lucknow

For the Award of Degree

DOCTOR OF PHILOSOPHY

IN

BIOTECHNOLOGY

Under the Supervision of

Dr. Anand Prakash

(Assistant Professor)

Submitted by

SARFRAJ AHMAD SIDDIQUI

Department of Biotechnology

School for Biosciences and Biotechnology

Babasaheb Bhimrao Ambedkar University

(A Central University, NACC 'A' GRADE)

Vidya vihar, Raebareli road, Lucknow-226025

Enrolment No. 006/12

2018

Summary of Ph.D. Thesis

Preamble

The traumatic conditions such as PTSD, creates a strong impact on human life, the result of which is the development of psychiatric disorders including the characteristic symptoms of fear, shock, guilt and anxiety (Zoellner et al, 2011; Desmedt et al, 2015). The basic approaches for the therapeutics of PTSD involve combined behavioral therapy (CBT) which includes behavioral and pharmacological applications together (Morrison, 2009; Kar N, 2011). To understand the mechanism associated with PTSD the Pavlovian fear conditioning paradigm has been extensively used, in which animals are trained with paired conditioned stimulus (CS) such as tone or context with an unconditioned stimulus (US) such as shock in an experimental condition (Tarpley et al, 2010; Curzon et al, 2009). In fear conditioning animal learns to predict the occurrence of an US on presentation of CS whereas in extinction, the previously acquired fear learning is reversed by training the animal with un-paired CS in absence of shock or US (Calandreau et al, 2007).

The fear and extinction learning involve different brain areas that function in a synchronized manner (Ehrlich et al, 2009). Amygdala, PFC and Hippocampus are the functional participant in the consolidation of fear and extinction memory. In fear memory consolidation and extinction the amygdala, PFC and Hippocampus with their interconnected network, function in modulation of fear circuitry (Peters et al, 2009; Marek et al, 2013).

The role of epigenetics in regulation of chromatin activity is amongst one of the most important molecular mechanisms associated with learning and memory. This includes various types of modifications such as histone acetylation, methylation, phosphorylation, sumoylation and DNA methylation (Bannister and Kouzarides, 2011; Sultan and Day, 2011; Kim and Kaang, 2017). The histone acetylation is one of the most important mechanisms involved in learning and memory processes (Peixoto and Abel, 2013). The use of HDAC inhibitors (TSA, SAHA) further affects these epigenetic mechanisms by enhancing the histone acetylation in both contextual and cued fear memory (Itzhak et al, 2012, 2013). The involvement of histone acetylation in amygdala, hippocampus and prefrontal cortex has been found to be associated with the fear memory consolidation and extinction (Ranjan et al, 2015; Siddiqui et al, 2017; Kritman and Maroun 2013, Debiec et al., 2010; Duvarci et al. 2005; Herry et al. 2008; Zimmerman and Maren 2010).

Histone acetylation at various N-terminal residues plays an important role in gene regulation through alteration in expression of genes involved in fear memory consolidation and extinction (Bannister and Kouzarides, 2011; Kouzarides, 2007). These histone modifications are controlled by the activity of HDACs (Histone deacetylases) to regulate the expression of memory related genes (Seto and Yoshida, 2014). Out of all four classes of HDACs the class I HDACs is known for its extensive involvement in learning and memory (Whittle and Singewald, 2014). However, HDAC1 and HDAC2 are extensively studied for their role in learning and memory (Bahari jawan et al, 2012; Zhou et al, 2009). Number of studies have so far confirmed the role of HDACs mainly as a negative regulator of fear memory consolidation and extinction (Valiati et al, 2017). Though the HDAC1 subtype is found to be mainly associated with the promotion of fear extinction (Bahari-jawan et al, 2012), other HDACs and more specifically the HDAC2 subtypes mainly function in the suppression of fear extinction (Morris et al, 2013; Whittle and Singewald, 2014). These studies prove the potential role of different subsets of HDACs with the fear memory extinction.

In the present study, the involvement of histone acetylation as well as the HDAC subtypes was studied and mainly focused on the molecular events associated with fear memory consolidation and its extinction. The result from this study will enhance our understanding about the neuronal network and molecular mechanisms that control PFC, amygdala and hippocampus in a coordinated manner resulting in the behavioral outcomes of fear and extinction learning.

Result summary

The study sheds light on the epigenetic regulatory mechanism operative following fear and extinction learning. The role of histone acetylation and histone deacetylases (HDACs) in fear learning and extinction was chalked out. It was observed that differential spatial histone acetylation and HDACs expression was associated with the fear learning and extinction in different brain subregions of the amygdala. mPFC and hippocampus.

Aim 1: Histone acetylation and HDAC expression in consolidation and extinction of fear
Histone acetylation was found to be associated with the activity of the brain regions involved in the consolidation and extinction. Expression of c-fos, an immediate early gene which is also a neuronal activity marker, was found to be increased differentially in LA, BA, CeL and CeM following conditioning and extinction. The LA, BA, CeL and CeM activity was associated with the conditioning while LA, BA and CeM activity was associated with the extinction learning. The histone acetylation was enhanced together with the activity in LA, BA, CeL and CeM following conditioning and extinction. This result suggests that the activity of these brain regions might be controlled by the increased histone acetylation following conditioning and extinction. The major inhibitory mechanism over histone acetylation was exhibited by the HDAC2 expression in amygdala following conditioning and extinction, as its expression decreased in conditioning and extinction. The HDAC1 expression shows its inhibitory role only for CeM activity in combination with the HDAC2 expression during conditioning, while in extinction the key inhibition by HDAC1 expression regulates the CeM activity. However, the HDAC1 expression shows differential expression in amygdala following conditioning and extinction. In LA, BA and CeL the enhanced HDAC1 expression was observed in conditioning and extinction both the stages of learning. From this, it may be concluded that this enhanced HDAC1 expression might be associated with the regulation of inhibitory mechanism over conditioning and extinction circuit during conditioning and extinction, respectively.

In PFC, the PL activity which is associated with conditioning (Knapska and Maren, 2009) exhibited an enhanced c-fos expression following conditioning while in IL, whose activity is associated with the extinction (Knapska and Maren, 2009), the expression increased following extinction. Moreover, the histone acetylation increased in PL following conditioning and in IL following extinction learning. The result suggest that increased histone acetylation in PL is associated with the enhanced activity of PL following conditioning and in IL following extinction. The HDAC1 and HDAC2 expression in PL decreased following conditioning while in IL, the HDAC2 expression decreased following conditioning. The HDAC2 expression increased in PL following extinction while HDAC1 expression decreased in IL following extinction. The result suggests that in PL both the HDAC1 and HDAC2 regulate histone acetylation in conditioning while HDAC2 alone regulates the activity of PL in extinction. Furthermore, it may be speculated that in IL the HDAC1 alone regulates the acetylation and activity for extinction learning.

In hippocampus, the c-fos expression analysis suggests the role of CA1, CA3 and DG in conditioning and extinction. The result showed an enhanced activity of CA1, CA3 and DG following conditioning and extinction learning. The histone acetylation increased in CA1, CA3 and DG following conditioning and extinction. So, it may be concluded from the observation that histone acetylation is associated with the activity of CA1, CA3 and DG in conditioning and extinction of fear. The HDAC1 expression exhibited different expression in hippocampus. The HDAC1 expression decreased in CA1 and CA3 following conditioning which might be the main regulator for histone acetylation in hippocampus during conditioning. HDAC2 expression in hippocampus might be associated with the regulation of histone acetylation during extinction, as HDAC2 expression decreased in CA1, CA3 and DG following extinction together with an increased histone acetylation in hippocampus following extinction. The increased HDAC1 expression in hippocampus following extinction might be associated with the suppression of inhibitory machinery having suppression over extinction learning.

Overall, the results suggested that the differential spatial expression of histone acetylation in amygdala, PFC and hippocampus is associated with the conditioning and extinction learning, and the histone acetylation enhanced the activity of different nuclei of amygdala, PFC and hippocampus to regulate the circuit of fear during conditioning and extinction learning. Furthermore, the HDAC1 and HDAC2 function alone or in combination with each other to regulate histone acetylation during fear memory consolidation and extinction.

Aim 2: HDAC inhibitors effect in consolidation and extinction

The global HDAC inhibitor valproic acid when introduced 2 hours prior to the conditioning and extinction, it was observed that valproic acid enhanced the conditioning and extinction learning respectively, in a weak behavioral training paradigm of conditioning and extinction. Furthermore, the enhanced conditioning and extinction learning was followed by the change in the expression of histone acetylation as well as with other molecular mechanisms. The valproic acid enhanced the activity of amygdala, PFC and hippocampus following conditioning and extinction learning. Likewise, the histone acetylation was enhanced by the HDAC inhibitor in these regions following conditioning and extinction learning. The HDACs exhibited different association with the HDAC inhibition as HDAC inhibitor targets mainly HDAC2 expression and the HDAC1 expression was unaffected by the HDAC inhibition. HDAC inhibition especially suppressed the expression of HDAC2 in amygdala, PFC and hippocampus for enhancing conditioning and extinction learning, while HDAC1 expression was indirectly modulated in conditioning and extinction learning. It may also be concluded that HDAC2 is the main target molecule for enhancement of conditioning and extinction learning.

Concluding Remarks

The findings from the research study have lead to the following final concluding remarks:

- The differential activity of the subregion within amygdala, PFC and hippocampus exhibited an overlapping activity for conditioning and extinction learning which suggests an overlapping fear and extinction circuitry in terms of molecular mechanism.
- The histone acetylation expression is found to be associated with the activity of amygdala, PFC and hippocampus following fear memory consolidation and extinction.

- In general the enhanced histone acetylation was found to be associated with the promotion of fear and extinction learning.
- The HDAC subtypes acts in combination or alone in regulation of histone acetylation during fear and extinction learning.
- The HDAC inhibitor (VA- valproic acid) mainly targets HDAC2 subtypes for enhancement of the fear and extinction learning.
- In amygdala, PFC and hippocampus the HDAC2 might be functioning as a strong negative regulator of histone acetylation for conditioning and extinction learning, while HDAC1 functions in some region for the regulation of activity.
- The enhanced HDAC1 expression together with histone acetylation in amygdala and hippocampus might be associated to suppress the inhibitory mechanism over corresponding learning paradigm.

Publications

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. doi: 10.1007/s10571-017-0464-6.

Ranjan, V., Singh, S., **Siddiqui, S. A.**, Tripathi, S., Khan, M. Y., & Prakash, A. (2017). Differential histone acetylation in sub-regions of bed nucleus of the stria terminalis underlies fear consolidation and extinction. Psychiatry Investigation, 14(3).

Ranjan V., Singh S., **Siddiqui S. A.**, Khan M. Y. and Prakash A. (2015) Differential histone acetylation in the Amygdala leads to fear memory consolidation and extinction. International Journal of Science, Technology & Society. Vol. 1 (1); Jan-June 2015, 43-50.

References

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.

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. Cell Research, 21(3), 381–95.

Calandreau, L., Jaffard, R., & Desmedt, A. (2007). Dissociated roles for the lateral and medial septum in elemental and contextual fear conditioning. Learning & Memory (Cold Spring Harbor, N.Y.), 14(6), 422–9.

Curzon, P., Rustay, N. R., & Browman, K. E. (2009). Cued and Contextual Fear Conditioning for Rodents. Methods of Behavior Analysis in Neuroscience. CRC Press/Taylor & Francis.

Debiec, J., LeDoux, J. E., & Nader, K. (2002). Cellular and systems reconsolidation in the hippocampus. Neuron, 36(3), 527–38.

Desmedt, A., Marighetto, A., & Piazza, P.-V. (2015). Abnormal Fear Memory as a Model for Posttraumatic Stress Disorder. Biological Psychiatry, 78(5), 290–297.

Duvarci, S., Nader, K., & LeDoux, J. E. (2005). Activation of extracellular signal-regulated kinase- mitogen-activated protein kinase cascade in the amygdala is required for memory reconsolidation of auditory fear conditioning. European Journal of Neuroscience, 21(1), 283–289.

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.

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.

Hoffman, G. E., Smith, M. S., & Verbalis, J. G. (1993). c-Fos and Related Immediate Early Gene Products as Markers of Activity in Neuroendocrine Systems. Frontiers in Neuroendocrinology, 14(3), 173–213.

Itzhak, Y., Anderson, K. L., Kelley, J. B., & Petkov, M. (2012). Histone acetylation rescues contextual fear conditioning in nNOS KO mice and accelerates extinction of cued fear conditioning in wild type mice. Neurobiology of Learning and Memory, 97(4), 409–17.

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.

Kar, N. (2011). Cognitive behavioral therapy for the treatment of post-traumatic stress disorder: a review. Neuropsychiatric Disease and Treatment, 7, 167–81.

Kim, S., & Kaang, B.-K. (2017). Epigenetic regulation and chromatin remodeling in learning and memory. Experimental & Molecular Medicine, 49(1), e281.

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. http://doi.org/10.1101/lm.1463909

Kouzarides, T. (2007). Chromatin Modifications and Their Function. Cell, 128(4), 693–705.

Kritman, M., & Maroun, M. (2013). Inhibition of the PI3 kinase cascade in corticolimbic circuit: temporal and differential effects on contextual fear and extinction. The International Journal of Neuropsychopharmacology, 16(4), 825–833.

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.

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.

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.

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.

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).

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).

Sultan, F. A., & Day, J. J. (2011). Epigenetic mechanisms in memory and synaptic function. Epigenomics, 3(2), 157–81.

Tarpley, J. W., Shlifer, I. G., Halladay, L. R., & Blair, H. T. (2010). Conditioned turning behavior: a Pavlovian fear response expressed during the post-encounter period following aversive stimulation. Neuroscience, 169(4), 1689–704.

Valiati, F. E., Vasconcelos, M., Lichtenfels, M., Petry, F. S., de Almeida, R. M. M., Schwartsmann, G., … Roesler, R. (2017). Administration of a Histone Deacetylase Inhibitor into the Basolateral Amygdala Enhances Memory Consolidation, Delays Extinction, and Increases Hippocampal BDNF Levels. Frontiers in Pharmacology, 8, 415.

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.

Zhou, Y., Won, J., Karlsson, M. G., Zhou, M., Rogerson, T., Balaji, J., … Silva, A. 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).

Zoellner, L. A., Feeny, N. C., Bittinger, J. N., Bedard-Gilligan, M. A., Slagle, D. M., Post, L. M., & Chen, J. A. (2011). Teaching Trauma-Focused Exposure Therapy for PTSD: Critical Clinical Lessons for Novice Exposure Therapists. Psychological Trauma : Theory, Research, Practice and Policy, 3(3), 300–308.