Role of Histone Methylation in Extinction of Fear Memory

SUMMARY

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SUMMARY

Fear is psychological and psycho physiological response to a perceived external threat that is consciously recognized as a danger. Fear response include increased alertness; concentration on the source of fear; attack and fightor-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) (VanElzakker 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 et al., 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 study 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 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. It was studied it was hypothesized that the role of histone modifications especially histone methylation was studied in correlation to ARC, CREB, 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 (VanElzakker 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 worked on fear related anxiety disorders (Maren, 2005, Pare et al., 2004). The model is well described 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 this in turn to known as ''immediate extinction deficit (IED)" (Maren, 2014, Stafford et al., 2013, Long and Fanselow, 2012, Archbold et al., 2010, Kim et al., 2010, Woods and Bouton, 2008). 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 all 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 which 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, Levenson et al., 2004). Increased histone methylation (H4) in neurons of IL-PFC is a well documented fact in the role to 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. 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 deficits in IE is mainly due to the sustained activation of IL because of it is associated with the changes involved in histone methylation.

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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) (VanElzakker 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 it is most importantly reported by Pavlovian based fear conditioning in rats (Maren, 2005, Pare et al., 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)

Aims and Objectives

1. To investigate whether extinction of fear memory is just inhibitory learning or it also has some erasure component.

2. To trace out the molecular events taking place during erasure or inhibitory of fear memory and compare it to inhibitory learning.

3. To find out whether the molecular events during erasure or inhibitory are assoiated with differential histone methylation in erasure or inhibitory of fear memory.

The current study focused on the role of histone methylation and the association of HDACs with the methylation during fear memory consolidation and extinction. The amygdala, PFC and hippocampus 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, BA, CeL and CeM showed differential activity during conditioning and extinction of fear. The LA, BA, CeL and CeM were active in conditioning while during extinction all the subregions were active except CeM. This might be the result of overlapping circuitries being engaged following fear and extinction learning. Likewise, the histone methylation was enhanced in LA, BA, CeL and CeM in conditioning while during

extinction the LA, BA, and CeL show enhanced histone acetylation. The result suggests that the histone methylation 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 deacetylases (HDACs) HDAC1 and HDAC2 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, BA and CeL the HDAC2 shows its association with the histone methylation during conditioning and extinction. A decreased HDAC2 expression was observed with increased histone methylation in LA, BA and CeL during conditioning and extinction. In CeM, both the HDAC1 and HDAC2 expression decreased during conditioning together with the increased histone acetylation. This suggests the role of both the HDACs in the regulation of histone methylation in CeM during conditioning. However during extinction, only the HDAC1 exhibited its association with the regulation of histone methylation as its expression increased in CeM together with the decreased expression of histone acetylation. So, from the result, it may be speculated that HDAC2 is associated mainly with the regulation of the histone methylation in LA, BA and CeL in conditioning and extinction while HDAC1 together with HDAC2 is associated with the regulation of histone methylation in CeM. In LA, BA and CeL the enhanced HDAC1 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 HDAC1 expression decreased in PL and IL, following conditioning and extinction respectively. HDAC2 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 HDACs, HDAC1 and HDAC2 together regulate the expression of histone methylation in conditioning while HDAC2 alone regulates the histone methylation in PL following extinction. But on the other hand the HDAC1 in IL might be associated alone with the regulation of histone methylation during extinction.

The hippocampus CA1, CA3 and DG exhibited enhanced histone methylation during conditioning and extinction of fear learning. This suggests the role of histone methylation in the hippocampus for regulation of consolidation and extinction of fear. HDAC1 expression decreased in the hippocampus following conditioning while HDAC2 expression decreased following extinction. The result suggests the association of HDAC1 in the regulation of histone methylation during conditioning while HDAC2 exhibits its regulation of histone methylation during extinction learning. The enhanced HDAC1 expression in hippocampus in extinction might be associated with the inhibition of the fear memory component to enhance extinction learning.

Overall the above mentioned results suggest that HDAC1 and HDAC2 are involved in the regulation of histone methylation either alone or in combination in different brain regions during fear memory consolidation and extinction. Furthermore, the use of HDAC inhibitor was found to inhibit the expression of HDAC2 but not HDAC1 leading to enhanced conditioning and extinction learning. HDAC inhibitor enhanced the learning for fear memory consolidation and extinction in a weak learning paradigm which mostly targets HDAC2 to enhance histone acetylation. So it may be speculated that targeting specifically HDAC2 might be important to enhance the conditioning and extinction learning. Further research using HDAC subtype specific HDAC inhibitor may promise to clarify the mechanism associated with conditioning and extinction of fear as well as in therapeutic application in the treatment of behavioral disorders like anxiety and trauma.