Summary on

"HISTONE ACETYLATION RELATED ENHANCED EXPRESSION OF CREB TARGETED GENES MAY UNDERLIE ERASURE OF FEAR MEMORY LEADING TO EXTINCTION"

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Introduction:

With the increasing instances of traumatic events, stress related disorders like post-traumatic stress disorder (PTSD) develops (Ranjan et al; 2017, Siddiqui et al; 2017, 2019, Singh et al; 2018). However, the occurrence of PTSD was reported only in 10% of individuals (Kessler et al, 1995; Yehuda and LeDoux, 2007) and it might be due to the persistence of fear memory associated with a traumatic event for longer duration of time in such individuals. PTSD symptoms include nightmares or flashbacks, insomnia etc. and the avoidance of places or activities that remind the traumatic event. So there is great need to develop some psychological therapies or pharmacological intervention that can help in the treatment of PTSD. Along with the treatment it is also equally important to know the nature of the cause of PTSD. It is found that memory associated with the traumatic experience like—the sights, sounds, smells, and context of the trauma plays a key role in both the development and expression of the disorder (Holmes and Singewald, 2013; Maren et al, 2013). The disorder also affects the individuals mood functioning. Thus the brain system is involved in learning and memory has an essential role in the development and expression of the disorder. several studies are there that reported the involvement of the hippocampus, prefrontal cortex and amygdala (Singh et al., 2018, Ranjan et al., 2017, Knapska and Maren, 2009) In the laboratory the model for PTSD developed by Pavlovian fear conditioning and extinction paradigm was used.

After the fear conditioning session, a fear memory is generated that represents the associative learning between CS-US pairing. Extinction is a popular behavioral paradigm that can block the recurring traumatic memories by generating a non-associative learning (inhibitory memory) between CS-US (unpairing) that was previously paired during conditioning. The produced inhibitory memory competes with the initially generated fear memory and response will be produced accordingly. Recent studies have shown that extinction may also take place by the reversal or erasure of the original fear memory and is dependent on the time period between conditioning and extinction session initiation (Peterson and Laniel, 2004; Gupta et al., 2010). However after the successful inhibition of fear memory by the extinction training, fear can return via three process- with the change in context (renewal), with the presentation of unsignaled shock (reinstatement) and with the passage of time (spontaneous recovery) (Pavlov, 1927; Rescorla and Heth, 1975; Bouton and Bolles, 1979).

Result

Aim1: Under this aim the extinction memory was analyzed on the basis of time that is immediate extinction (10 min) and delayed extinction (24 hrs) along with two different contexts. To conclude whether extinction of fear memory is "erasure" or "inhibitory" component, the fear consolidation was performed in context A and extinction training was delivered in two different context that is context A and context B. Later the retention test was performed in context A to assess the recovery of fear memory. In the same context i.e. AAA, the immediate extinction group possessed more freezing during retention test due to lesser time for extinction memory to be framed while delayed extinction group possessed reduced level of freezing as they had more time for extinction memory to be framed. While during change in the context i.e. ABA, the immediate extinction group showed less freezing than the delayed extinction group because animals placed in the delayed extinction group aversed the context for a longer duration and thus they possess more freezing than the immediate extinction group.

On comparing the behavior for same and different context, the differences were evident only in the retention test. No significant difference in the freezing response was observed in the immediate extinction of same and different contexts which suggests that there was no recovery of fear memory and thus immediate extinction may be said to possess "erasure" component. The delayed extinction group exhibited decreased freezing response in the same context when compared to the different context. It can be said that the fear response reappears when delayed extinction training was delivered in a novel context explaining that delayed extinction showed inhibitory learning.

In reinstatement test analysis, similar results were observed as observed during renewal. When the immediate extinction group was compared with immediate reinstatement test group, no significant change in the expression of fear response was observed suggested that immediate extinction exhibited 'erasure'. While the delayed extinction group when compared with the delayed reinstatement test group, recovery of fear memory was observed in delayed reinstatement group suggested the inhibitory learning.

The behavioral results thus observed from renewal and reinstatement tests were in line with the Myers et al., 2006, they also suggested that the extinction of fear memory can be inhibitory learning and 'erasure' of previously acquired memory depending whether the context is the same or different and also on the time lag between the conditioning and extinction.

Aim 2 :

After the behavioral analysis, PFC, Hippocampus and Amygdala regions of the brain were isolated and immunostained for p-CREB and its target gene ARC. Firstly the expression of c-fos was measured. c-fos along with ARC is also an immediate early gene (IEG). c-fos represents the activation of neurons in a particular brain region while the ARC is reported to be involved in neuronal plasticity. For all the brain regions, it was found that the expression of c-fos was similar to the expression pattern of p-CREB and its target gene ARC. The results thus obtained after immunohistochemistry (IHC) are summarised in the table given below.

Aim 3:

As behavioral results suggested extinction is both the inhibitory learning as well as it also has some 'erasure' components. To know whether histone acetylation played any role during these processes the brain sections were immunostained with the acetyl H3K9 and acetyl H4K5 and the observed results are summarised in the table below.

Expression pattern for c-fos, p-CREB, ARC, acetyl H3K9 and acetyl H4K5 in same context

Amygdala

	Control	Immediate extinction	Delayed extinction
LA	**	**	*
BA	**	****	***
CeL	**	***	****
CeM	**	****	**

Hippocampus

	Control	Immediate extinction	Delayed extinction
CA1	**	**	***
CA3	**	**	***
DG	**	**	***

Prefrontal cortex (PFC)

	Control	Immediate extinction	Delayed extinction
PL	**	***	**
IL	**	**	***

Expression pattern for c-fos, p-CREB, ARC, acetyl H3K9 and acetyl H4K5 indifferent context

Amygdala

	Control	Immediate extinction	Delayed extinction
LA	**	***	**
BA	**	****	***
CeL	**	***	****
CeM	**	*****	***

Hippocampus

	Control	Immediate extinction	Delayed extinction
CA1	**	**	**
CA3	**	**	**
DG	**	**	**

Prefrontal cortex (PFC)

	Control	Immediate extinction	Delayed extinction
PL	**	***	***
IL	**	***	****

* Represents the number of positive neurons in particular brain region

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