Associative learning – Genetic modulation of extinction and reconsolidation and the effects of transcranial Direct Current Stimulation (tDCS)

[Assoziatives Lernen - Genetische Modulation der Auslöschung und Rückverfestigung und die Auswirkungen der transkraniellen Gleichstromstimulation (tDCS)]

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To my parents and my love

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ABBREVIATIONS

5HTTLPR	Serotonin Receptor
ADSk	Allgemeine Depression Scale
ASI3	Anxiety Sensitivity Index
BDNF	Brain Derived Neurotrophic factor
BISBAS	Behavior Inhibition / Approach System
CBT	Cognitive Behavioral therapy
COMT	Catechol O-Methytransferase
CR	Conditioned Response
CS	Conditioned Stimulus
DCS	D-cycloserine
DLPFC	Dorsolateral Prefrontal Cortex
ECS	Electroconvulsive Shock
EEG	Electrocephalography
FMC	Fear Memory Consolidation
FQ	Fear Questionnaire
GAD	Generalized Anxiety Disorder
IADS	International Auditory Database system
IL	Infralimbic
ISI	Inter-stimulus-interval
LA	Lateral Amygdala
LTD	Long-term Depression
LTM	Long-tern Memory
LTP	Long-term Potentiation
MTL	Medial Temporal Lobe
NACL	Sodium Chloride
NMDA	N-Methyl -Aspartate
OCD	Obsessive compulsive disorder
ODN	Oligonucleotides
PANAS	Positive Affect Negative Schedule
PD	Panic Disorder
PSQI	Schlafqualitäts-Fragebogen
PSWQ	Penn State Worry Questionnaire
PTSD	Post-traumatic Stress Disorder
RNA	Ribonucleic Acid
SCR	Skin Conductance Response
SEC	SECOND
STAI	State-Trait Anxiety Inventory
STM	Short –term Memory
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
TrkB	Tyrosine kinase B
UCS	Unconditioned Stimulus
vmPFC	Ventromedial Prefrontal Cortex
vs.	Versus
Zif268	Zinc Finger 268
CAPS	Clinican Administered PTSD Scale
PCL	PTSD Check List

ABSTRACT

Scientific surveys provide sufficient evidence that anxiety disorders are one of the most common psychiatric disorders in the world. The lifetime prevalence rate of anxiety disorder is 28.8% (Kessler, et al., 2005). The most widely studied anxiety disorders are as follows panic disorder (PD), post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), social phobia (or social anxiety disorder), specific phobias, and generalized anxiety disorder (GAD). (NIMH Article, 2009).

Classical conditioning is the stable paradigm used from the last one century to understand the neurobiology of fear learning. Neurobiological mechanism of fear learning is well documented with the conditioning studies. In the therapy of anxiety disorders, exposure based therapies are known to be the most effective approaches. Flooding is a form of exposure therapy in which a participant is exposed to the fear situation and kept in that situation until their fear dissipates. The exposure therapy is based on the phenomena of extinction; this means that a conditioned response diminishes if the conditioned stimulus (CS) is repeatedly presented without an unconditioned stimulus (UCS). One problem with extinction as well as with exposure-based therapy is the problem of fear return (for e.g. renewal, spontaneous recovery and reinstatement) after successful extinction. Therefore, extinction does not delete the fear memory trace.

It has been well documented that memory processes can be modulated or disrupted using several scientific paradigms such as behavioral (for e.g. exposure therapy), pharmacological (for e.g. drug manipulation), non-invasive stimulation (for e.g. non-invasive stimulation such as electroconvulsive shock (ECS), transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), etc. However, modulation of memory processes after reactivation or via non-invasive stimulation is still not clear, which is the focus of the current study. In addition, study of genetic variant suggests that genetic differences play a vital role in the psychiatric disorder especially in fear learning. Hence, it is also one of the concerns of the current dissertation to investigate the interaction between gene and reconsolidation of memory.

With respect to fear-conditioning, there are three findings in the current dissertation, which are as follows: (i) In the first study we investigated that non-invasive weak electrical stimulation interferes with the consolidation process and disrupts the fear consolidation to attain stable form. This might offer an

effective treatment in the pathological memories, for e.g. PTSD, PD, etc. (ii) In the second study we demonstrated whether a brief single presentation of the CS will inhibit the fear recovery. Like earlier studies we also found that reactivation followed by reconsolidation douses fear return. Attenuation of fear recovery was observed in the reminder group compared to the no-reminder group. (iii) Finally, in our third study we found a statistically significant role of brain derived neurotrophic factor (BDNF) polymorphism in reconsolidation. Results of the third study affirm the involvement of BDNF variants (*Met vs. Val*) in the modulation of conditioned fear memory after its reactivation.

In summary, we were able to show in the current thesis modulation of associative learning and reconsolidation via transcranial direct current stimulation and genetic polymorphism.

ZUSAMMENFASSUNG

Mit einer lebenslangen Prävalenz von etwa 28% (Kessler Rc, 2005) stellen Angststörungen eine der häufigsten psychischen Störungen weltweit dar. Zu den am besten untersuchten Angststörungen gehören Panikstörungen (PD), posttraumatische Belastungsstörungen (PTSD), Zwangsstörungen (OCD), soziale Phobien (oder soziale Angststörungen), spezifische Phobien und generalisierte Angststörungen (GAD) (NIMH Artikel, 2009).

Die klassische Konditionierung ist das seit dem letzten Jahrhundert gültige Paradigma zur Erforschung der neurobiologischen Mechanismen des Angstlernens. Bei der Behandlung von Angststörungen haben sich Konfrontationstherapien als äußerst wirksam herausgestellt. Reizüberflutung (Flooding) ist beispielsweise eine Form der Konfrontationstherapie, bei der der Teilnehmer einer furchteinflößenden Situation ausgesetzt und in ihr gehalten wird, bis seine Furcht vergeht. Die Konfrontationstherapie basiert auf dem Phänomen der Extinktion, also dem Rückgang eines konditionierten Verhaltens nach wiederholter Präsentation eines konditionierten Stimulus (CS) ohne einen unkonditionierten Stimulus (UCS). Ein Problem der Extinktion und der Konfrontationstherapien ist, dass das Furchtgefühl nach einer erfolgreichen Extinktion zurückkehren kann, was darauf hinweist, dass eine Extinktion nicht die Spuren des Angstgedächtnisses löscht.

Vieles deutet darauf, dass der Erinnerungsprozess mittels verschiedenener wissenschaftlicher Paradigmen moduliert oder unterbrochen werden kann. Hierzu gehören etwa behavioristische (z.B. Konfrontationstherapie), pharmakologische oder nicht-invasive Interventionen (z.B. Elektrokonvulsionstherapie (ECS), transkranielle Magnetstimulation (TMS) oder transkranielle Gleichstromstimulation (tDCS)). Da die Modulation von Erinnerungsprozessen nach einer Reaktivierung oder durch eine nicht-invasive Stimulation derzeit noch unzureichend erforscht ist, wurde der Schwerpunkt der vorliegenden Studie auf diese Thematik gelegt. Ein weiteres Ziel ist es, die Wechselwirkung bestimmter Gene mit der Rekonsolidierung des Gedächtnisses zu untersuchen, also Prozesse, denen eine entscheidende Rolle für Angststörungen im Allgemeinen und Furcht-Lernen im Speziellen zugeschrieben wird.

Die vorliegende Dissertation umfasst drei zentrale Ergebnisse zur konditionierten Angst: (i.) In der ersten Studie wurde herausgefunden, dass eine nicht-invasive, schwache Stimulation den Konsolidierungsprozess beeinflusst und verhindert, dass die Angstkonsolidierung eine stabile Form erreicht. Dies

könnte eine neue Möglichkeit darstellen, pathologische Gedächtnisinhalte, die z.B. bei Störungen wie PTSD oder PD vorkommen, effektiv zu behandeln. (ii.) Die zweite Studie untersuchte, ob eine kurze, einfache Präsentation des CS das Wiederaufkommen von Angst hemmen kann. Ähnlich wie in früheren Studien beschrieben, fanden auch wir, dass eine Reaktivierung gefolgt von einer Rekonsolidierung die Rückkehr der Angst unterbindet. Insbesondere wurde in der Gruppe, deren Teilnehmer erneut konfrontiert wurden (reminder), im Vergleich zur Kontroll-Gruppe (no-reminder) ein verringertes Wiederaufkommen von Angst beobachtet. (iii.) Die dritte Studie zeigte, dass ein Polymorphismus im BDNF-Gen (Met vs Val) eine signifikante Rolle für die Rekonsolidierung und die Modulation des konditionierten Angstgedächtnisses nach seiner Reaktivierung spielt.

Zusammenfassend konnte in dieser Thesis eine Modulierung von assoziativem Lernen und Rekonsolidierung durch *transkranielle* Gleichstromstimulation und einen genetischen Polymorphismus gezeigt werden.

CHAPTER 1

INTRODUCTION

This thesis addresses the effects of associative learning and reconsolidation and its modulation by transcranial current stimulation and genetic polymorphism. The thesis has been divided into three sections describing fear-conditioning, its modulation and genetic polymorphism. The purpose of the these sections are to give readers a better understanding about fear-conditioning, role of reactivation and memory reconsolidation in classical conditioning (i.e., encoding after retrieval), effects of stimulation on memory consolidation, and role of genes in fear-conditioning, extinction, and reconsolidation. Three different paradigms were employed in the following study: (i) stimulation study, (ii) behavioral study, and (iii) genetic study. The study focuses on the associative learning and its modulation by targeting the consolidation and reconsolidation. Further the genetic polymorphism for consolidation and reconsolidation of fear learning. Each section of fear-conditioning, modulation and genetic polymorphism has been further sub-divided to give the reader a broad perspective to understand the research topic and related fields.

MOTIVATION

Threatening stimuli are the positive elicitors of anxiety and its related disorders. Several studies have showed that the threatening value of the threatening stimulus can be changed overtime and prevent recovery (Monfils, Cowansage, Klann, & LeDoux, 2009; Schiller, et al., 2010). Exposure therapy is found to be an effective methodology in the treatment of anxiety disorders. It proves to be an effective tool in the disruption of fear recovery (Follette & Smith, 2005). Extinction process assumed underlying exposure therapy; it is the new learning about the CS-no UCS associations, which results into decrement in the CR from first trial until last trial. The strength of new learning determines the long-term change in the safety or threatening behavior in individuals. Extinction can be performed immediately or after some delay from learning. From animal studies, it is quite clear that immediate extinction has robust effect on disruption (Johnson, Escobar, & Kimble, 2010; Schiller, et al., 2008).

Recently, researchers have demonstrated that the brief exposure of the single threatening stimulus a few minutes before the extinction might attenuate the fear recovery in animals and humans within a

period of time (Agren, et al., 2012; Agren, Furmark, Eriksson, & Fredrikson, 2012; Golkar, Bellander, Olsson, & Ohman, 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010). The explanation for such an inhibition could be understood from the theory of memory consolidation. According to theory of memory consolidation when memory is retrieved, it becomes labile and during this phase, the old memory can be disrupted or enhanced (Dudai, 2004; Hardt, Einarsson, & Nader, 2010). This state of malleable/plastic memory is known as reconsolidation. Reconsolidation offers a methodology to study the maintenance and the update of memory consolidation (Gisquet-Verrier & Riccio, 2012). Moreover, it has been recently suggested for therapeutic procedures in the treatment of anxiety disorders (Monfils, et al., 2009; Schiller, et al., 2010) and addiction (Sorg, 2012). Monfils and co-workers (2009) demonstrated for the first time the attenuation of fear recovery after fear retrieval followed by extinction training within reconsolidation window. They explained that the CS+ (i.e., threatening cue) changes from inducer to inhibitor from the acquisition phase to the extinction phase. Furthermore, CS+, which was a threatening cue, during acquisition the same CS+ behaves as an inhibitor during the extinction phase; CS during extinction phase enhances the new learning. The interference, during the reconsolidation leads to destabilization of old fear memory followed by learning CS-no UCS association, i.e. new valence association with the CS at the time of update.

Translating this to human, Schiller and colleagues (2010) also targeted the reconsolidation and prevented the return of fear after memory re-activation. Extinction performed within the reconsolidation period prevented the spontaneous recovery and affected only the reactivated conditioned fear memory while the non-activated fear memory remained intact. They suggested that updating the fear memory with non-fearful information during the phase of reconsolidation leads to the blockade of previously learned fear responses and preventing the return of fearful memory. In contrast, to the earlier findings Chan and co-workers (2010) explained that fear is augmented after the fear retrieval. CS+ always acted and showed as a fear inducer irrespective to the learning phase and showed inhibition of fear recovery with change in context. They failed to show reconsolidation in their study and argued that the amount of unlearning or new learning during extinction is dependent upon reminder and context association. In addition, the failure to target reconsolidation was the result of associative learning differences during fear acquisition and extinction.

Some studies successfully showed that the reconsolidation phase had attenuated the fear recovery after the brief exposure of threatening cue (Monfils, et al., 2009; Schiller, et al., 2010). Equally, some studies failed to show reconsolidation and observed augmentation in the fear recovery (Chan, Leung, Westbrook, & McNally, 2010; Golkar, et al., 2012). It is still unclear whether brief retrieval of a threatening cue before the extinction will augment or attenuate the fear recovery. If brief exposure really successfully blocks the fear recovery, then it can be used in the clinical settings for the treatment of anxiety

and related disorders. Memory research provides evidence that consolidation and reconsolidation process can be modulated either by pharmacological (Jarvik, 1972) or by stimulation processes (Nielson, 1968). The stored/consolidated memory becomes labile after its retrieval and when it is followed by drug intervention or cranial stimulation it is possible to disrupt/modify the old memory. This experimental amnesia could be long lasting, depending upon the blocker and the process involved in disruption. Drugs employed soon after memory retrieval have shown a robust effect on memory disruption and also have been found to be an effective methodology in the treatment of high anxiety. Despite the effectiveness of drug therapy in pathological memories it is not suitable for two reasons: (i) the drug might lead to discontinuity of memory and (ii) its role in humans is unpredictable.

Cranial stimulation has long been used now as an effective methodology in the treatment of high anxious individuals. ECS is one of the most famous and popular techniques among researchers in order to suppress the conditioned fear response in animals. The biggest drawback of ECS is that chances of amnesia are very high leading into a more deteriorate situation of fear memory consolidation (Geller & Brady, 1961; Misanin, Miller, & Lewis, 1968). Recently, weak electrical cranial stimulation has been introduced in the field of psychiatry and psychology for the treatment of depression (Palm, et al., 2009), drug cravings (Fregni, et al., 2008), and migraine (Chadaide, et al., 2007). It has also been reported that this weak current stimulation influences the cortical excitability and modulates the learning and memory process (Albert, 1966a, 1966b). Since the last decades this methodology has been used to target psychiatric disorders such as depression, anxiety, etc.

Furthermore, many converging lines of evidence suggest the involvement of genes in the mechanism of fear learning and extinction (Lonsdorf & Kalisch, 2011). Genes might eventually be better able to predict which individuals might be more susceptible to develop anxiety or related disorders. Genetic studies gives us an understanding how gene influences the development and functions of the brain in psychiatric disorders. Combined with the interaction of our environment, changes in the genetic pattern give an early onset or early detection of particular psychiatric disease. Past simple models and genetic studies motivated and lay foundation for future studies. Research in this area shows a great potential to have a deeper insight into the pathological circuitry and root cause of psychiatric disorders, which will help us develop a better prevention and treatment important for human beings.

Henceforth, the investigation of novel method in the modulation and treatment of pathological memories motivated the current dissertation. Therefore, the current dissertation has been designed to investigate the modulatory effects of tDCS in consolidation of memory, effect of reminder in pathological memory reconsolidation and extinction process and the efficacy of genetic polymorphism in the modulation of associative learning.

SECTION I: FEAR LEARNING AND EXTINCTION

1.1.1 FEAR

Fear is the most basic and primitive emotion, which helps an animal to anticipate danger and organize the desired defensive response (Hamm & Weike, 2005). It helps an animal to distinguish the threatening and non-threatening stimuli in the existing environment. Threatening stimuli are the positive elicitors of fear and anxiety. It is deeply wired in the human brain (e.g., spiders and snakes). People get confused most of the time with fear and anxiety, fear is an immediate response to the threat while anxiety is the anticipation of the threat in the absence of the threatening stimuli e.g., spider picture alone elicits the fear in spider phobic (Gordon & Hen, 2004). Fear is also learned with the presence of various stimuli in novel environment to adapt the situation (Kim & Jung, 2006). Hence, excessive fear learning results into pathological memories and give birth to fear and anxiety disorders such as PD or PTSD (Sehlmeyer, et al., 2009). In routine activity, we are bombarded with thousands of stimuli and just a single stimulus is sufficient to evoke fear in an individual (Bush, Schafe, & LeDoux, 2009). The biggest challenge for the anxiety related research is to block the fear from returning. Every individual has some kind of fear during life and the abrupt fear recall could lead to the anxiety disorders (Parsons & Ressler, 2013). The aim of the current anxiety research field is to prevent fear return, and to offer an effective method in the treatment of anxiety and related disorders.

1.1.2 FEAR CONDITIONING

Pavlovian or Classical conditioning is used as a basic model, which has gained clinical relevance in the investigation of anxiety and related disorders (Lissek, et al., 2005). It is the stable paradigm used from decades to understand the neurobiology of the fear learning. It also offers to understand the fear and anxiety at behavioral, cellular, molecular, and genetic level (Johansen, Cain, Ostroff, & LeDoux, 2011; Schafe, Nader, Blair, & LeDoux, 2001). In classical conditioning, a neutral stimulus (non-threatening) is paired with the threatening stimulus (shock, tone, pressure, etc.). After repeated pairing individuals learn about the association and assign the threatening value of the threatening stimulus (UCS) to the neutral stimulus (CS) (Bush, et al., 2009). Due to this associative learning the neutral stimulus predicts threat and elicits a response, this response is the conditioned response (CR). CR is elicited at every

presentation of the reinforced CS with or without UCS. CRs for conditioned stimulus (CS+) and non-conditioned (CS-) will justify the strength of fear learning.

CR is an autonomic indicator such as skin response (Milad, Orr, Pitman, & Rauch, 2005), startle (Lonsdorf, et al., 2009), heart rate (Stiedl, Tovote, Ogren, & Meyer, 2004), freezing (Johansen, et al., 2011), etc. in fear-conditioning. These autonomic indicators go up with the presence of the emotional or salient stimuli. The reason these autonomic indicators go up is because the response is needed to move the blood in those parts of the body, which require energy. As a result of exaggeration of fear we feel chilly because blood, which is flowing in the body, is not cooling down. In simple fear-conditioning paradigm these autonomic indicators or psychophysiological responses indicate the level of fear learning before and after the fear learning. These autonomic indicators give an insight to understand the mechanism of fear learning. In mice or rats freezing is the most common studied fear response while in human skin conductance response and startle are the most common indexes.

1.1.3 FEAR CONSOLIDATION

Pavlovian conditioning is a simple learning about the association between the CS-UCS. It has helped in having a better insight into the cellular and molecular mechanism of fear, anxiety and related disorders. In this paradigm an animal learns about the CS-UCS association and stores this information for future prospects. Hence, whenever this animal encounters CS or UCS it prepares against the threat. From animal studies, it is clear that every time when we form a memory protein synthesis is required (Nader, Schafe, & Le Doux, 2000; Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999). LeDoux and group (1999, 2000) suggested that whenever fear memory is recalled or in the presentation of CS or UCS, the protein formed during first learning comes back to the synapse and strengthens the connections of CS-UCS association. However, when new learning takes place CS-no UCS association it forms a new memory. Both these memories compete with each and the one, which succeeds leaves the impression in the brain. Consolidation of fear memory is the processes of storage of information for future reference. This stored information is used in the future to encounter threat (Schafe, et al., 2001). Initially formed memory is labile in nature and overtime it undergoes consolidation. This early-formed memory is sensitive to any kind of disruption such as drug manipulation (McGaugh, 1973), ECS, etc. (Jarvik, 1972). However, from animal studies it is clear that the memory consolidation process requires the protein synthesis. When protein synthesis is disrupted or interfered with protein blocker soon after fear learning, it blocks the memory consolidation process resulting into the disruption of the fear memory storage for future reference (Nader, Schafe, & LeDoux, 2000; Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999). This later leads to the poor recall of fear in animals. When the initially formed memory is left untouched it consolidates and moves to a more stable form.

1.1.4 FEAR EXTINCTION (RECIPROCAL INHIBITION)

Pavlovian Extinction refers to the new learning rather than unlearning of initial learning. From animal studies, it is clear that it is context-dependent. It is a procedure in which previously fear CS is presented repeatedly in absence of UCS. This repeated presentation of CS leads to the suppression of the conditioned fear responses. Over time extinction leads to the dampening of the excitatory association between CS and UCS. It has been controversial whether extinction is unlearning or new learning. From animal studies, it is clear that extinction destructs the old learning and has inhibitory effects on initial learning. However, after successful extinction the trace of initial learning still exists. Extinction learning is the inhibitory association, i.e. presentation of the CS now does not predict the UCS. It is the loss of fear response overtime. Timing is crucial for extinction; depending on timing extinction is of two types (i) short-term extinction and (ii) long-term extinction. It has been a prime interest for researchers to understand whether extinction is unlearning or new learning of CS- no UCS association. Recent advancement in neuroscience reveals that extinction is a new learning instead of unlearning. Studies affirm that extinction builds up a new memory, i.e. CS-no UCS association, which competes with the old memory CS-UCS association. This means that extinction does not delete the old trace of fear memory; instead it forms a new memory with new expression. Furthermore, both the old and new memory compete with each other for expression. The memory, which succeeds in competition, leaves an impression in the brain.

In addition, extinction of fear is a context dependent which means that if context is changed it prevents extinction memory recall (Herry, et al., 2010). Older theories suggest that extinction changes the meaning of the CS-UCS association. Hence, while extinction training i.e. change in context provides some new cues to rodents, which they reclassify during extinction this develops a new state, and this simultaneously preserves an old memory association. In contrast, if an animal does not reclassify the cues into a new state then it cannot preserve the old memory association (Chan, et al., 2010). Hence, the reclassification of cues into a new state decides the preservation of old associative memory, which further decides the renewal and reinstatement of fear. Albeit extinction offers a good fear-inhibition, one still fears a relapse. Fear of relapse can happen under three basic conditions: (i) reinstatement; (ii) renewal, and (iii) spontaneous recovery (Rescorla, 2004). These basic conditions of fear relapse suggest whether fear has been erased or inhibited.

1.1.5 REINSTATEMENT

Ample studies have shown the reemergence of fear on presentation of unsignaled UCS after successful extinction. This reemergence of fear is known as reinstatement; in this process UCS triggers the associa-

tion of previously learned fear CS-UCS in a clinical set-up. It activates the fear network and brings back the fear in an individual. It offers an approach to understand the recall of fear in an individual having pathological memories e.g., PTSD, PD, etc. Some studies show the reinstatement effect after 24-hours UCS presentation, suggesting the effects of UCS-context conditioning. However, when UCS is presented off context then no-reinstatement is observed. In contrast, if extensive extinction is performed, exposure to the UCS context does not bring fear back. This suggests the UCS-context dissociative learning. Bouton (2002, 2004) suggested in his review article that reinstatement is context dependent, i.e. reinstatement should occur in the same context as of fear acquisition.

1.1.6 RENEWAL

Renewal refers to a reemergence of fear when there is change in context after successful extinction. In rodents' reduction of CRs, i.e. less freezing is associated with the unlearning of the fear context. During Pavlovian fear-conditioning animal not only learns about the CS-UCS association, but also about the context. From animal studies, we understand that an animal also learns about fear in a novel context followed by extinction training they stores the information about the context as well. When an animal is tested for fear in the original context compared to the novel context it shows augmentation in the conditioned fear response, i.e. CRs. Renewal of fear in animals has been tested with many simple basic paradigms such as ABA, ABC, & AAB. In such paradigms, fear learning is performed in context A, followed by fear extinction in context B and then fear testing in context A. While, when the paradigm is ABC, i.e. fear learning in A, fear extinction in B and fear test is in novel context C. Results of renewal effect suggest that an animal shows high elevation of fear in the original context compared to novel context. Similar findings have been reported with the AAB paradigm, in which fear acquisition and extinction performed in context A, but fear is tested in novel context B. This might be due to reclassification of new context with CS-UCS association. Bouton (2002, 2004) in his review article stated five facts about renewal and it is worth mentioning them here: (i) renewal can be seen in all types of condition, (ii) it also reemerges after strong extinction, (iii) role of context is different from the one we know from research studies. Generally, CS is considered context in conditioning paradigm. (iv) it is context-specific unlike the original conditioning. (v) there are several contexts responsible for the renewal.

1.1.7 SPONTANEOUS RECOVERY

Spontaneous recovery refers to reemergence of fear after sufficient time has been passed. CRs is observed when CS is tested, this suggests that fear trace is not erased completely and does not have any permanent effect (Rescorla, 2004). Spontaneous recovery occurs due to the failure in recall of the extinction. It is the result of failure in recalling cues associated with the extinction and extinction context.

When the cues of extinction is retrieved completely it attenuates spontaneous recovery. While when the cues of extinction is not retrieved completely it augments spontaneous recovery. It is noteworthy to mention that fear learning or CS-UCS association is more robust learning than the extinction and is also better in recall. Hence, the reemergence of fear is due to failure of retrieving extinction. It is important to have some more replication studies to have a better understanding of CS-UCS association at initial learning and CS-UCS association unlearning (Myers & Davis, 2006).

Decades of neuroscientific studies have revealed the insight about the mechanism of fear from storage-extinction-restoration. Different brain faculties have been involved from fear acquisition, extinction, modulation, and reconsolidation (Agren, et al., 2012). Advanced neuroscience provides an understanding of these processes at neural level. However, understanding the mechanism of anxiety and related disorders can give us a deeper insight about the pathological memory formation and degradation. In addition, it also provides an explanation for the neural circuitry of fear acquisition and extinction, which might help the clinical psychologist and psychotherapist in the treatment of anxiety disorders (Kim & Jung, 2006).

1.1.8 NEURAL CIRCUITRY OF FEAR EXPRESSION AND FEAR SUPPRESSION

Advancement in neuroscience supplies evidence and explanation of the neurobiology on the fear expression or fear learning. From lesion studies, amygdala, hippocampus and frontal cortex have turned out to be an important faculty for the detection of threat, its information storage and its cure or treatment. Amygdala has dense connections with the hippocampus and frontal cortex. From animal and human studies, we get evidence that it plays an important role in the emotional learning and memory modulation. In general, the whole amygdalae do not play a role in the detection of threat in the environment. Recent research on amygdala has revealed that substructures of amygdala play excitatory and inhibitory roles in the detection of threat (Bush, et al., 2009). The dysfunctioning of specific amygdala regions is responsible for the threat and anxiety disorders. The Amygdala is composed of 13 nuclei; each further has sub-nuclei, which is assumed collectively or partly play a role in fear learning. The Lateral amygdala (LA) is the major site for the acquisition of fear. It receives the CS-UCS sensory input via thalamus and strengthens the plasticity of the CS-UCS association. Lesion studies affirm that disruption or dysfunction of LA leads to the failure in fear expression. In contrast, condition strengthening the synaptic plasticity in LA promotes fear learning (Bush, et al., 2009; Hamm & Weike, 2005; Kim & Jung, 2006). With the available data, it is clear now that amygdala is the prime center for the fear learning. However, it is noteworthy that other responsible structures for fear learning in humans are the insular cortex, hippocampus, cerebellum, and perirhinal cortex (Kim & Jung, 2006). Hence, it is still debated

whether the amygdala alone is the site of fear storage and holds fear information for a longer period of time or if other brain structures are involved as well.

Another line of animal research provides evidence and explanation of the neurobiology of fear suppression or extinction. Neuronal mechanism of fear extinction has clinical importance in the treatment of anxiety and related disorders. Ivan Pavlov demonstrated in 1927 that when CS is presented without the reinforcer (UCS) it leads to the reduction in the CR suggesting a behavioral inhibition. This behavioral inhibition of CR is referred to as extinction learning, which further leads to the formation of short-term or long-term extinction memory.

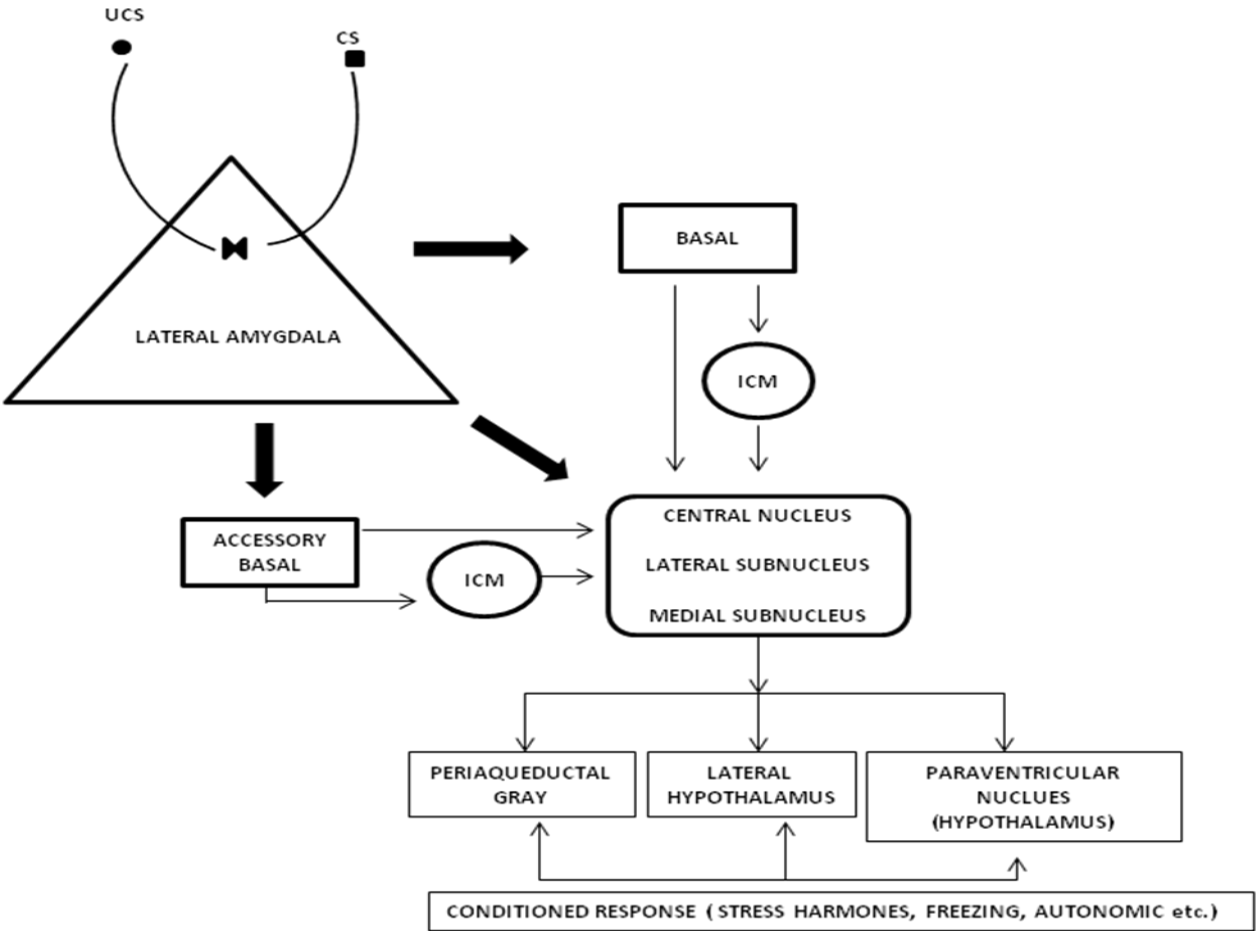


Figure 1: Depicting the schematic pathway of fear circuitry. This figure has been modified according to Johansen et al. (2011). CS-UCS association occurs in lateral amygdala (LA) LA sends projections directly and indirectly via basal (B), accessory basal (AB) and intercalated cell masses (ICM) to central nucleus (CE) CE has direct connections with hypothalamic and brainstem regions. Hence, CE gets involved in the expression of the conditioned fear responses such as stress hormones, freezing, autonomic response, etc.

Current research gives us an advantage to understand the circuitry of fear extinction. Animal researches provide evidence, which affirms the importance of frontal cortex in the behavioral inhibition of fear CRs. Animal work in the early 1990s showed that extinction learning is a form of inhibition process. The major breakthrough came with the findings of Morgan *et al*. (1993) who demonstrated that pre-training lesion of ventromedial prefrontal cortex (vmPFC) leaves fear learning intact, but impairs the extinction learning suggesting it has major role in extinction. Later Quirk and colleagues (2000) lesioned infralimbic (IL) of vmPFC focusing its role in fear extinction. They lesioned the IL and observed that rats easily acquired the extinction memory and successfully extinguished the fear response. This suggests that the prefrontal cortex is not involved in the acquisition of fear. However, on the following day of fear extinction during testing sessions animals failed to retrieve the extinction memory. The Author then argued that IL plays an important role in the fear retention further suggesting the distinction between the acquisition of extinction and retrieval of extinction.

The last several decades of animal research provide evidence about the important role of amygdala in fear acquisition, hippocampus in consolidation and storage information, and frontal cortex especially DLPFC in fear extinction. So far, animal studies suggest that acquisition, retrieval, and consolidations enhance an understanding of neural circuitry involved in fear learning and extinction (Milad & Quirk, 2012). Furthermore, researchers have argued that lesion studies alone are not so much reliable, hence additional methodology such as single cell recording, imaging, genetics; stimulation methods, etc. might offer a deeper understanding in the treatment of fear and anxiety disorder. Therefore, in the near future research studies must be designed to combine various measurements and methodologies with each other.

SECTION II: CONSOLIDATION, RECONSOLIDATION, AND MODULATION OF MEMORY

In the late 18th century, Theodule-Armed Ribot reported the problems of memory and stated that amnesic patients have an unusual pattern of memory process. Their memory fades over time while leaving some traces alive. This further suggests that memory is a time-dependent process (Hardt, et al., 2010). However, memory research was revolutionized when researchers Müller and Pilzezecker hypothesized to understand the complex phenomenon of memory. They first coined the term "consolidirung" which means consolidation, and proposed a hypothesis that new memories undergo consolidation overtime (Lechner, Squire, & Byrne, 1999). Initially the memory traces are labile and are susceptible to disruption. When memory is left alone it then undergoes a stabilization state to become stable and get stored for future reference (Dudai, 2004). In 1900, Müller and Pilzecker performed a series of experiments to understand the nature of the memory consolidation process. Findings of their research suggested that when two memories are formed closely in reference to time, they interfere with each other resulting into a poor recall of memory. In addition, this interference between two memories is independent of stimuli nature (Lechner, et al., 1999). Lewis (1979) argued that the retroactive inhibition proposed by Müller and Pilzecker might involve different forgetting neural circuitry than proactive interference. At that time it was hard for the researchers to understand the findings of the study and neurological concept.

The early animal model of human memory investigated the mechanism involved in the behavioral, molecular, and cellular level of memory consolidation process. These findings gave birth to the two-memory model system: (i) synaptic consolidation & (ii) system consolidation (Dudai, 2004; Hardt, et al., 2010). Basically the classification of two-memory model is based upon how much time consolidation is taking. Earlier it was highlighted that initial learning changes the synaptic strength along with the importance of cellular process in consolidation. Hence, the early consolidation processes are referred to as cellular or synaptic consolidation (Dudai, 2004). Hardt and colleagues stated (2009) synaptic (or cellular) consolidation depends upon the interaction between synapse, cell body and nucleus. It takes minutes to hours for memory stabilization; it is specifically involved in stabilization not in the reorganization (system consolidation).

According to Dudai (2004), system consolidation refers to the reorganization of the memory information with respect to time. At this state memory becomes independent of hippocampus and takes weeks, months, and years to form. Reorganization means synaptic consolidation and the modification of synaptic network. Modification or the promotion of the synaptic network requires longer time resulting into slow consolidation. Hence, slow consolidation would refer to system consolidation and fast consolidation process would refer to synaptic consolidation. In an excellent review of the active and inactive memory, Lewis (1979) argued that when memory is active it is susceptible to modification or disruption, while inactive memory is not. It is not clear whether active memories occupies or require brain regions. He also stated that short-term memory (STM) and long-term memory (LTM) are initiated simultaneously with learning and STM holds the memory template until memory is permanently formed (LTM). Further, memory can be retrieved at any level irrespective to time (STM, LTM or time of shift from STM to LTM). Different active memories will have different pattern and densities of neuronal firing.

From Hebbian learning we receive the explanation that neural connectivity is necessary for the representation of the new experience (see figure 2). This new experience is nothing other than the short-term memory. If it is left undisturbed, then the synaptic connectivity will transform itself accordingly and becomes independent of hippocampus, also known as system consolidation. The reason that memory undergoes consolidation is because we need information for future reference in case we encounter the same situation again.

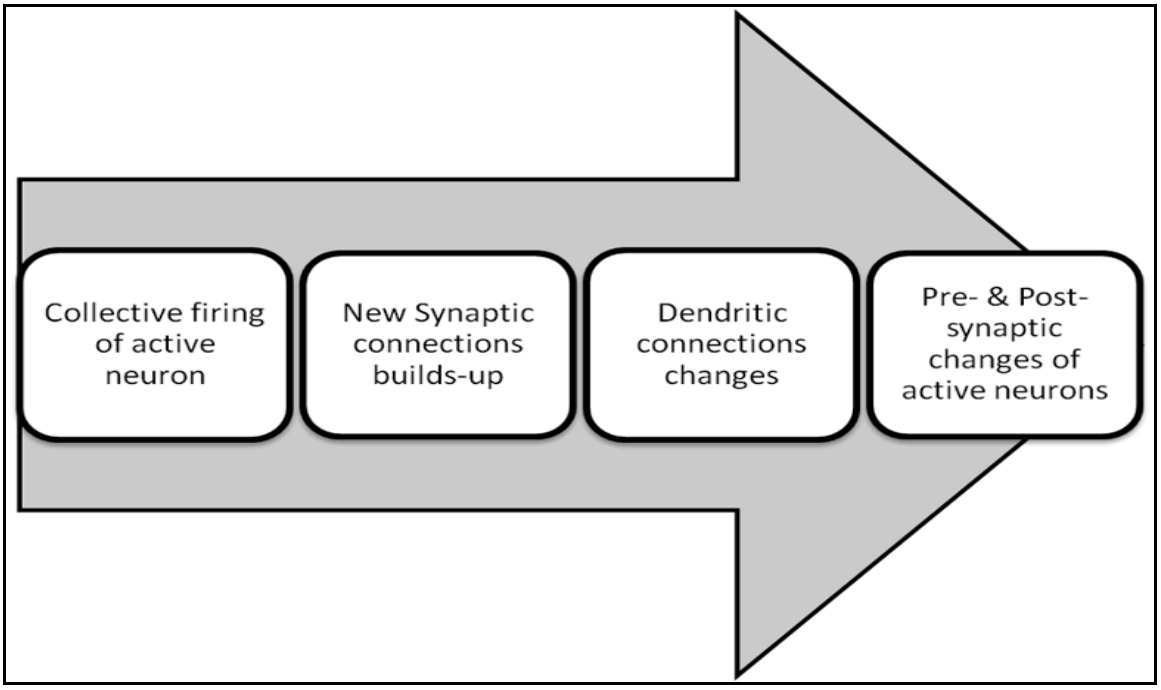


Figure 2: Building up of Neuronal Connections

1.2.1 SYNAPTIC OR CELLULAR CONSOLIDATION

Decades of memory research imply that all species undergo synaptic or cellular consolidation (see figure 3). Initial trace of memory consolidation is dependent upon the synapses, cell connectivity, and protein synthesis, and is a time-dependent process (McGaugh, 2000). Early phase of memory is very important for the memory to undergo consolidation. And, any kind of early memory interference might influence the storage process and lead to disruption of storage (Hardt, et al., 2010). From Hebbian learning we understand that cellular network is required for the successful synaptic connections (Spatz, 1996). Each neuron has a certain electrical potential this is due to the ion in-out flow of the cell. When the neuron's electrical potential reaches a threshold, it fires an electrical signal to the other neuron, and this process is repeated again and again (see figure 2). When many neurons fire an electrical signal, it becomes an electrical wave. This electrical wave is responsible for all mental processes such as memory, attention, intelligence, and emotion. These waves can be recorded by various means such as EEG and studied offline to understand consolidation process. These electrical waves are important for strengthening the old neuronal network and for building up new synaptic connections (see figure 2).

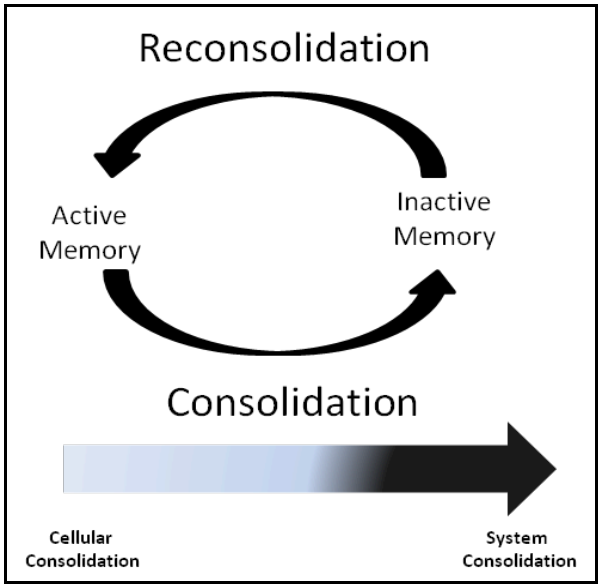


Figure 3: Depicting the pathway of consolidation and reconsolidation of memory

Several studies have been performed targeting the synaptic consolidation process. The aim was to investigate the nature of synaptic consolidation. Findings suggested as follows: (i) firstly, the early treatment of memory with ECS or protein synthesis blockers disrupts consolidation processes; (ii) post-treatment of learning is a time dependent process; (iii) post-treatment of learning targets long-

term memory formation leaving short-term intact (Dudai, 2004). These findings suggested that the immediate treatment of new learning influences the synaptic consolidation, however, delayed treatment leaves consolidation intact (Hardt, et al., 2010; McGaugh, 2000).

1.2.2 SYSTEM CONSOLIDATION

Unlike synaptic consolidation system consolidation is independent of the hippocampus (Dudai, 2004). The interesting case of memory impairment was patient HM, which gives an insight about the processes of system consolidation. HM underwent a brain surgery for the treatment of epilepsy, however, as a result he developed a retrograde amnesia which means his remote memory was intact, but his recent memory was impaired. He was unable to form new memories (Squire, 2009). This made the neuroscientist curious to understand how memory persists in HM after the loss of the key structures and its associated connections responsible for memory formation. HM was having similar findings with the patients with medial-temporal lobe (MTL) damage. Their remote memory was fine; however, new memory was impaired, suggesting that the memory had not undergone consolidation. Corkin (2002) suggested that the memory impairment in HM is not in encoding or in storage, but it is in consolidation or in retrieval. Besides having some similarity in the memory formation hippocampal and MTL damaged patients have dissimilarity in temporal gradient. This discrepancy might be due to the size and location of the lesion. If damage is limited to the hippocampus, then the memory impairment is not so mild howbeit if the damage is in the MTL, the memory impairment might be severe.

Evidence from human neuroimaging studies affirms the role of the MTL and the hippocampus. A number of human studies have shown higher activation in the hippocampus during memory formation. They affirmed the role of the hippocampus in the formation of new memory. Furthermore, the hippocampus has a dense connection with the other three lobes: frontal, occipital, and parietal. Recent imaging study by Smith & Squire investigated the role of memory faculties in recent and remote memories. They showed that the MTL shows less activation than other lobes during the retrieval of memories. This finding supports the model of system consolidation of memory with the passage of time memory becoming independent of the hippocampus (Corkin, 2002; Squire, 2009).

1.2.3 RECONSOLIDATION

Reactivation of consolidated memory makes its labile or unstable, which undergoes reorganization or restabilization. This overall process of memory from reactivation-to-restabilization is known as reconsolidation (Alberini, 2011). Misanin and colleagues (1968) findings showed the memory loss after retrieval is followed by ECS. Animals were made to lick from a drinking bottle and later animals under-

went conditioning (CS served as tone and US served as a shock). CR was the licking rate from the drinking water. They reported that the group, which underwent conditioning, followed by ECS observed impaired memory consolidation. Furthermore, they observed memory loss also when animals received activation followed by ECS. In contrast, they did not observe an amnesic effect when animals received only ECS, but no CS. This finding suggested that ECS interferes in similar fashion after reactivation of memory just like after early memory formation.

Ample evidence suggests the requirement of protein synthesis in memory consolidation. Inhibition of protein synthesis soon after learning impairs the LTM formation leaving the STM intact (Schafe, et al., 1999). Nader and colleagues (2000) showed that inhibition of protein synthesis soon after the memory reactivation induces amnesia. They also commented that experimental amnesia after reactivation is irrespective of time of reactivation (i.e., reactivation after 1 day or 14 days from training). Reactivation of consolidated memory can render the memory labile at any time. It is noteworthy that reconsolidation is also a time-dependent process like memory consolidation; delay in memory interference, i.e. 6 hours from reactivation does not induce amnesia. Their findings affirmed that reactivation makes consolidated memory labile and also makes it RNA and protein synthesis dependent for stabilization. The reconsolidation has been investigated in many species and has earned positive results. However, it is still a controversial process on some ground such as: Do all memories undergo reconsolidation after retrieval? Can it be used in the treatment of pathological memories such as PTSD? (Alberini, 2011)

1.2.4 RECONSOLIDATION AND TIME

It has been documented that memory consolidation is a time dependent phenomenon (Dudai, 2004; McGaugh, 2000). Similarly, numbers of studies have shown that reconsolidation is also time dependent (Inda, Muravieva, & Alberini, 2011; Nader, 2003). Memory retrieval makes old memory activation very quick, (few seconds) but the activated memory requires more time to become labile for an update. This means that memory requires some time for destabilization (Stickgold & Walker, 2005). It has been shown with an animal model that memory becomes labile after 30 seconds of re-exposure (Nader, Schafe, & LeDoux, 2000). In contrast, some researchers argued and showed that more than 10 minutes of re-exposure is required (Suzuki, et al., 2004). This discrepancy in memory retrieval depends upon the strength of memory, i.e. reinforcement level. This means that 38%-reinforced memory will undergo destabilization slower compared to 100% reinforced memory. The findings are similar to the initial consolidation interference, which is also dependent upon the strength of memory (Jarvik, 1972).

Time has been an important factor in the processes of reactivation-reconsolidation-restoration, but still there are some missing links in understanding destabilization of memory after retrieval and time. A number of studies have been published showing that the memory reconsolidation window is active from minutes especially after 10 minutes of retrieval few hours, i.e. up to six hours. It has been observed that after a 6-hour window memory does not undergo reconsolidation (Monfils, et al., 2009; Schiller, et al., 2010). This suggests that destabilization of memory after retrieval depends upon the reconsolidation window. In addition, destabilized memory beyond the reconsolidation window becomes stabilized, and after 24 hours, any form of destabilized memory becomes stabilized. However, it is important to understand why do we need reconsolidation? There are two important reasons why reconsolidation is necessary and important for us: (i) for the updating of old stored information overtime and (ii) retrieval makes an old memory robust and prevents forgetting (Alberini, 2011; Lewis, 1979). However, it is also important to understand that not all memories undergo reconsolidation (Nader & Hardt, 2009).

1.2.5 CLINICAL CONSIDERATION OF REACTIVATION AND RECONSOLIDATION

Single brief exposure of non-reinforced conditioned stimulus (CS) presented just before the extinction regulates the new learning (extinction). This reminder (single CS before the extinction) activated consolidated memory becomes labile and undergoes re-consolidation. (Agren, et al., 2012; Dudai, 2006; Kindt, Soeter, & Vervliet, 2009; Kwak, Choi, Bakes, Lee, & Kaang, 2012; Lee, 2009; Monfils, Cowansage, Klann, & LeDoux, 2009; Nader & Einarsson, 2010; Nader & Hardt, 2009; Oyarzun, et al., 2012; Schiller, et al., 2010) In theory the re-consolidation process means that consolidated memory can return to a labile state, in which it can be restored with some modification, changes or even erased (Alberini, 2007, 2011; Dudai, 2004; Inda, et al., 2011; Nader & Hardt, 2009). Studies have potentially targeted the re-consolidation mechanism in the treatment of anxiety disorders along with drug addiction (Lee, Di Ciano, Thomas, & Everitt, 2005). Furthermore, some studies failed to target the re-consolidation (Chan, et al., 2010; Golkar, et al., 2012; Sevenster, Beckers, & Kindt, 2012). Regarding explanation why reminder trial within the re-consolidation period attenuates fear recall is still debated.

Recently, there has been a resurgence of interest in the disruption of fear memory after its reactivation by targeting the re-consolidation mechanism (Gisquet-Verrier & Riccio, 2012; Kindt, Soeter, & Vervliet, 2009; Riccio, Millin, & Bogart, 2006; Schafe, et al., 1999). Evidence shows that blocking the cellular and protein synthesis after memory reactivation is an effective way in blocking the fear return (Alberini, 2011; Diaz-Mataix, Debiec, LeDoux, & Doyere, 2011; Doyere, Debiec, Monfils, Schafe, & LeDoux, 2007; Kaang & Choi, 2011; Kindt, et al., 2009; Kwak, et al., 2012; Lewis, 1979). Some studies have used drugs such as propranolol (Kindt, et al., 2009), D-Cycloserine (DCS) (Lee, Gardner, Butler, & Everitt, 2009),

and NMDA antagonist MK-801 (Sara, 2000) which affirmed the effectiveness of drugs in the treatment of anxiety and related disorders. Albeit drug manipulation enhances the effect of extinction training. However, its effect on humans is still unclear (Kindt, et al., 2009; Monfils, et al., 2009; Schiller, et al., 2010).

In contrast, using exposure therapy during the re-consolidation window offers a drug-free pathway in blocking the fear return (Agren, et al., 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010). Activation of old fearful memory with reminder followed by exposure therapy within the re-consolidation period leads to new learning and updating an old memory (CS-US) with new information (CS-no US) (Monfils, et al., 2009; Schiller, et al., 2010). The aim of the present study is to investigate whether reminder and re-consolidation can effectively block the fear return. Like earlier studies we propose that a reminder will attenuate fear and will make an old fear memory sensitive to restoration (Agren, et al., 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010).

1.2.6 PATHOLOGICAL MEMORIES (IMPAIRED EMOTIONAL MEMORY)

Brain surgery of HM laid the foundation of the new era of memory research in the early 1960s. The HM case study made researchers curious to understand the role of brain structures in memory processes especially emotional memory. Does the amygdala have any contribution in the memory formation and consolidation? Squire (2009) stated in his report that the hippocampus was removed along with the uncus (an anterior extremity of the parahippocampal gyrus involved in the origin of seizures) and amygdala in HM's. In the past few decades ample studies have been conducted to understand the emotional memory from acquisition-retrieval-reacquisition (Anderson, Wais, & Gabrieli, 2006; Przybyslawski, Roullet, & Sara, 1999). Emotional memory research gives an insight about the impairment and development of pathological memories

Emotion is one of the leading areas in the field of psychology and psychiatry. Decades of neuroscience research on emotion have revealed that the root cause for the development of pathological memories is dysfunctioning of the neural system (LeDoux, 2012; Shin & Liberzon, 2010). In addition, it has been evidently shown that emotion plays a vital role in the formation of memory. It can either boost or douse the consolidation processes depending upon the nature of the event itself (Anderson, et al., 2006). Primitive animal research is the basic model for the study of emotions and its processes. However, over the past few decades researchers have focused their attention to investigate how emotional network works underlying emotions in humans and animals. Translational studies have provided relevant insight about the underlying mechanism of emotion (Todd & Anderson, 2009). Recent emotional research has been oriented towards clinical disorders and their treatment. It has been proven that

arousal (emotional state varying from calm-to-excitement) and valence (emotional state varying from unpleasant-to-pleasant) influences different forms of memory such as declarative and non-declarative memory (Anderson, et al., 2006; Todd & Anderson, 2009).

Sharot and Phelps (2004) showed with their study that aroused words were remembered better in the delay recall compared with neutral words. They argued that arousal influences the memory formation targeting perception and attention processes. They successfully showed that arousal decreases the rate of forgetting and improves the consolidation processes. Results of their findings suggest that better memory recall was the result of arousal on less decrement of memory. Furthermore, they suggested that memory is superior for arousing stimuli compared to neutral stimuli, and arousal stimuli promote the consolidation. Clinical studies so far have suggested that patients with amygdala damage do not have a problem in the description of the arousing event. This suggests that perhaps the problem is in the emotion-cognition interaction rather than in the understanding of emotion. In an excellent review by Cabeza and LaBar (2006) the authors explained that emotional information promotes the memory processes. Emotional circuitry in the brain is indulged from initial storage to consolidation to long-term reactivation. They discussed that amygdala-damaged patients easily discriminate between the emotional stimuli and the neutral ones. This suggests that these patients are using a network connection between amygdala, MTL and PFC. Advancement in neuroscience has revealed the significant interaction between emotion and memory in humans (Anderson, et al., 2006; McGaugh, 2006). Now there is a need for some translational research unfolding the involvement of neural circuitry of emotion in affective disorders especially fear and anxiety.

1.2.7 MEMORY MODULATION AND FEAR DISORDERS

Millions of people are affected by the memory dysfunction, by life trauma or by pathological memories. Best way to treat the pathological way is to modulate the memory by means of stimulation, drug or by behavioral method such as exposure therapy. Memory modulation can offer a method of treatment of pathological memories. Modulation of memory consolidation might be helpful to those who suffer from pathological memories for, e.g. PTSD, PD, etc. According to memory, research three major findings laid the foundation of memory modulation:

- Initial trace of memory is soft and over time, it consolidates and is preserved for future preference. However, if this initial memory is treated with drug injection or electrical stimulation (ECS) it will influence the consolidation processes.
- A Number of studies have shown that post-treatment of memory might enhance or disrupt the memory consolidation. The excitatory or inhibitory effect of post-treatment of memory depends upon the experimental condition.

- Post-training treatment (e.g. injection of sympathetic stress hormones) affects the brain circuitry resulting in a modulatory effect on the brain.

The early 60s of memory research targeted the pathological memories and have revealed that behavioral and neural circuitry of fear is well conserved across species. Clinical studies highlight that the level of fear and trauma decides the development of the pathological memories. As mentioned earlier memory is a time-dependent process. If initial memory is left untouched, then it will undergo normal consolidation and become stored. Many studies have demonstrated pharmacological manipulations (Alberini, 2011; Dudai, 2006; Nader & Einarsson, 2010; Nader & Hardt, 2009; Schafe, et al., 1999), electrical stimulation (e.g., ECS, TMS & tDCS) and behavioral (e.g., exposure therapy) (Agren, et al., 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010) methods in the treatment of anxiety and related disorders. The goal of such methods is to dampen the expression of fear and to help an individual to recover from the threat (Agren, et al., 2012; Alberini, 2011; Chan, et al., 2010; Golkar, et al., 2012; Inda, et al., 2011; Kindt & Soeter, 2011; Kwak, et al., 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010)

1.2.8.1 PHARMACOLOGICAL METHOD IN CONSOLIDATION AND RECONSOLIDATION

1.2.8.1.1 CONSOLIDATION AND PHARMACOTHERAPY

Studies have shown that the use of drugs such as propranolol (Przybyslawski, et al., 1999), D-Cycloserine (DCS) (Hofmann Sg & et al., 2006; Hofmann, Pollack, & Otto, 2006; Kalisch, et al., 2009; Ressler, et al., 2004), etc. are helpful to modulate the memory. It might enhance or disrupt the consolidation processes. In addition, it has different effects on short-term or long-term memory processes. The facilitation or inhibition effects of drug on memory are studied by observing animal's behavior. In simple fear-conditioning paradigm CR defined the facilitatory or inhibitory effect of drugs on memory consolidation.

McGaugh's finding in 1950s that certain drugs enhance the memory in rodents laid the foundation of pharmacological method in consolidation and later in reconsolidation of memory. To investigate the effects of drugs on memory McGaugh administered the drug after the training to rule out its effect on training performance. Some research findings provide an evidence of drug facilitation of learning and memory. A number of drugs have influenced either through enhancement or disruption of memory consolidation such as strychnine, amphetamine, nicotine, etc. (McGaugh, 1973) Extensive animal research provides evidence that styrene boosts learning. When given before training it attenuates the CR enhancing the habituation rate in mice. In addition, memory enhancement is observed in maze train-

ing, discrimination tasks, and inhibitory avoidance tasks when strychnine is injected after 1-hour of training (McGaugh, 1973). Howbeit, strychnine showed some negative findings as well, i.e. impairment of learning. This impairment of learning depends upon the time-interval between two trials or when the animal is returned in its home-cage. This suggests that post-training conditions influence the enhancing effect of strychnine resulting into impairment. Researchers affirmed that drugs promoted the storage process. Enhancements of learning and memory findings are similar to the ECS findings.

Kalisch and colleagues (2009) found that NMDA receptor promotes fear memory consolidation in fear-conditioning paradigm. To test the proposed hypotheses two groups underwent fear-conditioning (Day 1) followed by the injection of a drug or placebo. A recall test was performed on Day 2 (after 72 hours from fear acquisition) showing the results that DCS has a better fear recall than the placebo. Findings were irrespective of context this means that the DCS group always showed higher fear recall than placebo. This finding suggests that the NMDA promotes the conditions required for fear consolidation. In addition, imaging data from the prior study shows the enhanced facilitation in the posterior hippocampus/collateral sulcus region and in the medial prefrontal cortex during recall test.

Recently, there has been a resurgence of using drugs with psychotherapy. Psychotherapy has showed the treatment of pathological memories. Drug manipulation might enhance the treatment effect of psychotherapy. It increases the effectiveness of psychotherapy and probably helps delete the fear memory traces. Ressler and colleagues (2004) used the D-Cycloserine to improve the fear extinction in acrophobics. A double-blind study was designed in which all participants underwent two sessions of behavioral therapy using virtual a reality system. D-Cycloserine or placebo was given before the beginning of the session. Later participants returned for the post-treatment and testing of the acrophobia. Their results suggest that participants who took D-Cycloserine showed a significant reduction in the SCR and other dependent measures compared to the placebo group. This finding suggests DCS is very effective when combined with the exposure-based psychotherapy in the treatment of anxiety related disorders.

Similarly, Hoffman and colleagues (2006) showed the effects of DCS in social anxiety disorder (SAD). Twenty-seven participants with public speaking anxiety were recruited in a double-blind study. Participants underwent 5-therapy sessions and were divided into two groups: the experimental group received DCS with exposure therapy, while the control group received a placebo with exposure therapy. Researchers reported that the experimental group showed significant attenuation over five sessions compared to control group. The result suggests the combined effect of DCS and exposure therapy as a novel therapeutic methodology in the facilitation of fear extinction. Although DCS increases the efficacy of exposure therapy, to our knowledge DCS effects have been observed only under laboratory con-

ditions. We still do not know if DCS will work outside the laboratory. But we hope that future studies will bring more clarity and shed some more light on efficacy of DCS on exposure therapy.

1.2.8.1.2 RECONSOLIDATION AND PHARMACOTHERAPY

Successful modulation of memory consolidation using drug and exposure therapy brought interest in post-reactivation reconsolidation. Progress in the psychotherapy and pharmacotherapy suggest a novel method in the impairment or disruption of pathological memory partially or completely. Post-reactivation makes an old memory labile and susceptible to disruption, if left untouched it becomes stored with an old expression. Sara (2000) stated that consolidated memory upon reactivation becomes sensitive to the application of amnesic agents such as ECS, NMDA receptor antagonists, etc. In addition, memory becomes active every time when old consolidated memory is retrieved or recalled by a reminder. In a study, Sara and colleagues (1997) showed that upon retrieval memory triggers cellular processes, which are dependent on NMDA receptor. Animals were trained on a maze task and the post-treatment of learning via NMDA receptor antagonist (MK-801) disrupted the well-trained performance. Rats with post-treatment after two hours or more showed normal retention after 24 hours, compared to the rats whose post-treatment was done less than 2-hours from reactivation. Their result suggests the important role of NMDA receptors in the consolidation of memory, and that every time when memory is reactivated it undergoes a consolidation process for, e.g. reconsolidation.

A second line of evidence comes from studies of the effects of propranolol after memory reactivation. Przybyslawski and colleagues (1999) argued about the other intracellular pathway, it is thought to be involved in the memory reactivation followed by reconsolidation. In a series of experiments, they affirmed the role of blockers of β-adrenergic receptor antagonist propranolol in the treatment of emotional and non-emotional memories. They reported that control rats performed better in retention tests followed by reactivation, while the rats that received propranolol after five minutes followed by reactivation showed significantly more errors. The results were intermediary when propranolol was given 2-hours after reactivation. Animals showed significantly less error after 5-hours instead of five minutes post-treatment of propranolol. This finding clearly suggests that blockage of β-adrenergic receptor antagonist propranolol after reactivation induces amnesia in rats.

Similarly, Kindt *et al.* (2009) have shown the administration of β-adrenergic receptor antagonist propranolol in humans before the memory reactivation prevents the return of fear. They employed a fear-conditioning paradigm: day one all participants learned about fear, a day later they underwent extinction training followed by 10-minute break after the memory reactivation. Just before the memory reactivation all participants were divided into three groups: one group administered with propranolol

with reactivation; a second group received propranolol without reactivation; and a third group received placebo with reactivation. A day later all three groups underwent a retention test the group that received propranolol with reactivation prevented the return of fear whereas the groups that received propranolol without reactivation and placebo with reactivation showed significantly higher fear-potentiated startle responses, confirming the spontaneous recovery. With this discovery researchers concluded, that the β-adrenergic receptor antagonist propranolol plays an important role in memory reconsolidation. Further suggesting that propranolol may offer a treatment to people suffering from emotional disorders with relapse of fear.

Pharmacological manipulation is very effective in the treatment of pathological memories, but due to its invasiveness and its after effects on humans can be unpredictable. (Inda, et al., 2011; Kindt & Soeter, 2011; Schiller, et al., 2010) It is worth mentioning here that no drugs specifically target memory leaving psychological processes such as motivation, arousal, attention, sleep, and motor activity undisturbed (McGaugh, 1973). The findings of the animal and human studies indicate that consolidation and reconsolidation of memory can be facilitated. This facilitation and attenuation of memory consolidation and reconsolidation suggest that memory is a time-dependent process (Dudai, 2004; Lee, Everitt, & Thomas, 2004). Finally, we conclude that facilitatory effect of drugs on learning and memory has served many principles and provided many answer in the treatment of pathological memories.

1.2.8.2 NON-PHARMACOLOGICAL METHODS IN CONSOLIDATION & RECONSOLIDATION

1.2.8.2.1 EXPOSURE THERAPY (BEHAVIORAL INHIBITION)

These days' pharmacological treatments are offering an effective treatment of psychiatric disorders especially anxiety memories. The aim of all pharmacological intervention is to inhibit the fearful response leaving fear memory intact in patients. Nevertheless, they observed a relapse after a successful treatment of pathological memory. Over the last one-century exposure therapy has been thoroughly investigated by the researchers. A number of studies have shown it has potential in the treatment of psychiatric illnesses especially anxiety and related disorders (Follette & Smith, 2005). Exposure sessions involve two processes (other than psychoeducative sessions explaining in detail the rationale of exposure therapy): (i) confrontation of the patients with the threatening stimuli or situation and (ii) engaging the patients not to withdraw their attention of the threatening stimuli or situation until fear significantly attenuates (Muller, 2012).

Wolpe's (1968) findings of systematic desensitization established the concept of exposure therapy in the treatment of anxiety and related disorders. Over several decades now it has been used as an effective treatment for fear and anxiety. Exposure therapy changes the subjective anxiety, behavioral and physiological response pre-and post-training (Foa, 2009). Currently, researchers have started using exposure therapy along with counter conditioning in order to suppress the conditioned fear response. In a simple conditioning paradigm, participants first learned about the CS-UCS association and developed CR. Later participants were exposed to the non-reinforced CS repeatedly until the CR dissipated. This process is known as extinction training, which builds a new learning or association about CS - no UCS (Myers & Davis, 2006). Extinction training is a form of exposure therapy, e.g., flooding or implosive therapy. Cognitive behavioral therapy (CBT) and Pharmacotherapy shows a good potential in the treatment of pathological memories (Foa, 2009; McNally, 2007). This offers a drug-free paradigm and gives the idea of combining the psychotherapy with pharmacology. Today we know a lot about drug manipulation and its influence on memory consolidation. Combining psychotherapy with pharmacology might give us a new dimension of treatment of pathological memories.

1.2.8.3 NON-INVASIVE BRAIN STIMULATION IN MEMORY DESTABILIZATION

1.2.8.3.1 ELECTROVONVULSIVE SHOCK (ECS)

Early 1960s ECS memory-impairment actions led to the foundation of memory interference after initial learning. An immediate test (i.e., 3 hours) of memory after ECS application showed memory impairment compared to the delayed test (i.e., 3 weeks) of memory. This suggests that ECS leads to temporary memory-impairment. In addition, unilateral ECS produces less amnesia compared to bilateral ECS. The effects show that bilateral effects are superior to unilateral ECS. From human findings, it became clear that amnesic treatment disrupts learning, which further impairs the consolidation. Limitation of investigation of retrograde amnesia is difficult in humans compared to animals. Findings of memory-impairment in humans' were similar in animals and offered more freedom to researchers to investigate every aspect of retrograde amnesia. As stated by Jarvik (1972) animal research during that time tried to answer whether amnesia due to ECS in animal is as a result of the impaired consolidation of LTM or is the result of the increased sensitivity of interference.

Animal studies affirmed the efficacy of ECS on learning, which is measured by animal performance. Attenuation in animal performance suggested the retention deficit, which was termed as RA. There were various methods to induce RA in animal such as drug, cortical depression, localized brain stimulation, etc. It is evident that not all memories undergo reconsolidation of impairment. These memories are resistant to any kind of interference also to ECS. The problem with the ECS application was that the

neural electrical vistas are not well defined. ECS is hard to localize howbeit one might expect the area under the montage is under higher influence. Jarvic (1972) explained that ECS amnesia was specific to certain regions such as the stimulation of dorsal hippocampus and amygdale induction of amnesia The septum, ventral hippocampus or fornix do not have any influence on memory. Due to the high usage of current, high amnesic effects and localization effects are unpredictable. It is still used in the treatment of psychiatric disorders such as depression, mania, etc. as it offers a faster and more effective treatment in prior mentioned disorders. In future, we think combined results of ECS and lesions may offer a better understanding of amnesic effects, and might offer a treatment for anxiety and related disorders.

1.2.8.3.2 TRANSCRANIAL MAGNETIC STIMULATION (TMS)

Amnesic effects of ECS on memory initiated the investigation of novel methods in the treatment of pathological memories. In the past two decades there has been a growing optimism about the transcranial magnetic stimulation (TMS). It is a non-invasive method that has been used to understand the underlying mechanism of psychiatric disorders. TMS works on the principle of Faraday's law of electromagnetic induction. This means that the magnetic field aligns the electrical activity generated in the brain tissue. As a result, an electric current is induced which depolarizes the neurons. TMS has potential to increase or decrease the neuronal activity of the specific brain region. rTMS have been used as a modulatory tool in the treatment of psychiatric disorders. In addition, it can offer a novel method in the treatment of anxiety disorders (Machado, et al., 2012). Some recent small sample studies have shown the potential effects of TMS on anxiety disorders. Researchers have argued that TMS application over the left and right-DLPFC shows a clinical relevance.

A recent study performed by Watts and colleagues (2012) showed the efficacy of 1-Hz rTMS over the right DLPF in PTSD. In the study, 20 PTSD volunteers were divided into two groups. One group received 10 times 1-Hz rTMS while the other group received sham rTMS over right DLPFC. Volunteers in the 1-Hz active group significantly scored less in the questionnaires clinician administered PTSD scale (CAPS) and PTSD check list (PCL) compared with the sham TMS. This finding suggests potential of therapeutic effects of TMS in PTSD. In addition, they argued that the study might not shed light on machinery of PTSD. Howbeit, suggested that PTSD is the dysfunction of the neural circuitry at the right DLPFC and, TMS might be the good modulatory tool in improving the dysfunctional circuitry of psychiatric disorders especially anxiety disorders. Currently, researchers are skeptical about implementing TMS in the clinical set-up for the treatment of anxiety or related disorders. Because of some discrepancy in the earlier findings, this might be due to the experimental difference in factors such as location, frequency, intensity, and duration. Another problem with the TMS is that often the sham condition attributes high placebo effect (Machado, et al., 2012). We think that more replication and translational

studies are needed to understand the effects of TMS. Furthermore, both imaging and TMS measurements together might give a deeper insight about anxiety and TMS.

1.2.8.3.3 TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS)

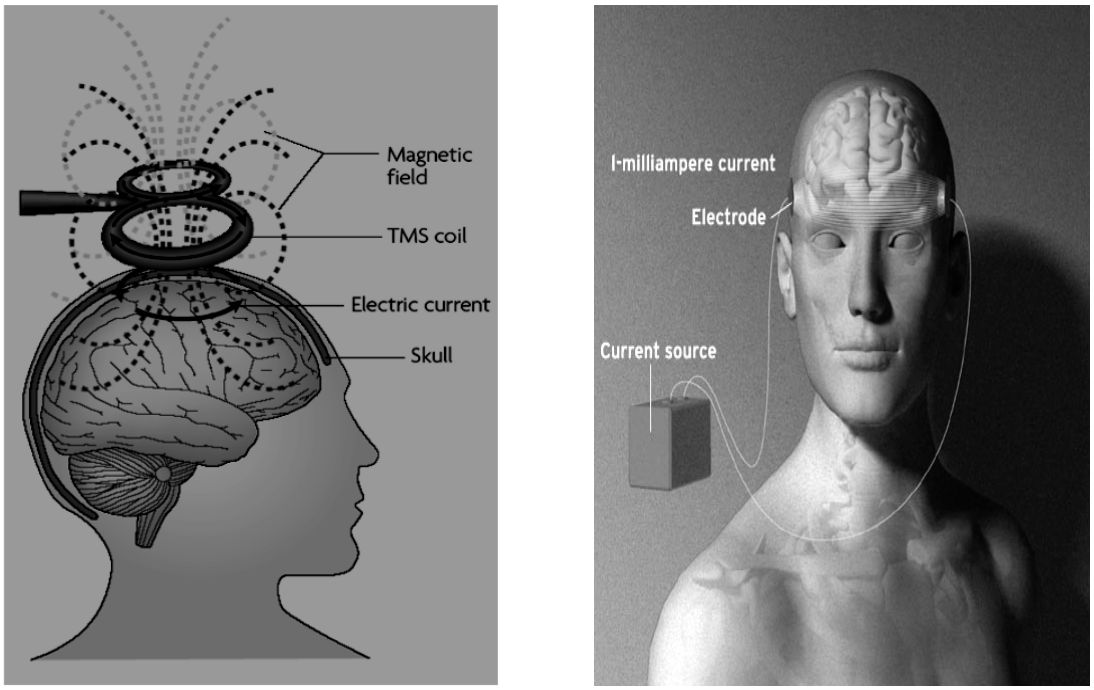
Brief period of polarizing current on the animal cortex changes the cortical activity, which lasts for several minutes (Bindman, Lippold, & Redfearn, 1964). Today ample experimental data from animal and human studies is available suggesting the efficacy of weak DC on the excitability of cortex. It has potential to modulate cortical excitability of the cortex. Due to its longer aftereffects it has attracted researchers to study motor learning, memory, attention, craving, etc.

Electrophysiology is a primitive methodology used in ancient period around 43 – 48 AD. A roman physician named Scribonius Largus observed and demonstrated the modulatory effect of electric fish in the treatment of pain patients (Priori, 2003; Utz, Dimova, Oppenländer, & Kerkhoff, 2010). A series of experiments performed by Galvani's and Volta during the late 18^{th} century started the modern era of transcranial direct current stimulation (tDCS) in clinical application. However, discovery of ECS during the same period pushed the techniques of tDCS into the dark for over a half-century. Early 1960s studies brought tDCS into attention of researchers (Priori, 2003). Bindman and colleagues (1962, 1964) demonstrated the long-lasting effects of surface-positive or negative polarization. Their findings showed for the first time alteration of brain activity using direct current. They suggested that the artificially induced DC potential in the brain cortex has either depolarizing or hyperpolarizing effect. The polarizing effect of the tDCS depends upon the polarity of the charge flows.

In tDCS methodology, the weak DC is applied over the scalp using two electrodes via a 9-volt driven battery. The electrodes are made from rubber and are either put in NaCl (Elmer, Burkard, Renz, Meyer, & Jancke, 2009)-soaked synthetic sponges or in an EEG electrode cream (Asthana, et al., under review). These agents prevent any kind of direct contact of electrode to the skin in order to avoid any kind of chemical reaction (Utz, et al., 2010). There is a variation in electrode sizes used so far in the different studies. Few standard sizes of electrodes are 25, 35, and 100 cm^2, used with current 1-2mA which facilitates current density 0.01-0.08 milli-Ampere (mA) / cm^2. To increase the effectiveness of the stimulation researchers prefer electrodes of smaller sizes and maintain 6-cm distance between the two electrodes to avoid any kind of seizure effect due to stimulation.

Side effects of tDCS are not noxious; however, it needs sincere attention when applied over the scalp. Types of stimulation, site of application, duration of application are an important concern regarding the safety issues. Research studies have reported some online and offline effects of stimulation as fol-

lows: (i) skin burns or redness, (ii) headache, and (iii) discomfort and dizziness. Poriesz and colleagues (2007) demonstrated the safety aspects of tDCS in humans and patients. They showed that application of tDCS over the motor and non-motor area has negligible aftereffects. Some common tDCS aftereffects are a tingling and burning sensation. Some other aftereffects were also observed such as nausea and insomnia. In rats, Bindman and colleagues (1962, 1964) reported that anodal (surface-positive) polarization increases the firing rate of the neurons, which were inactive earlier. While cathodal (surface-negative) polarization decreases or inhibits the firing rate of the neurons. They argued that when positive polarization is applied, spontaneous firing and prolonged facilitation is observed in the cortex. The surface positive facilitation lasted a few hours after the stimulation. In contrast, cathodal polarization leads to diminish cortical excitability. Their findings highlighted that polarization of the cortex induces a long lasting alteration in the cortical evoked potentials.



(a) **(b)**

Figure 4: Showing the image of (a) Transcranial magnetic stimulation (TMS) & (b) Transcranial direct current stimulation (tDCS) **[Source: http://www.brainclinics.com/rtms and http://www.biotele.com/tdcs.html]**

Similarly, Albert (1966a) suggested that the first few minutes are crucial for memory to undergo consolidation, but this initial consolidation of memory requires electrical potential. Any interference or disturbances in the early few minutes in the cortex after acquisition will decrease or reverse the process of consolidation. He demonstrated that cathodal polarization spreads depression over the cortex

and impairs consolidation. It has dissimilar effects than anodal polarization, which has a tendency to improve the retention when applied after training. A basic concept was that after acquisition potential at the cortex is required to build network connections for the consolidation. In a subsequent study, Albert (1966b) showed that anodal polarization promotes the conditions required for consolidation, and has positive effects compared to cathodal polarization. Anodal polarization when applied after the acquisition enhances the consolidation processes. He argued that early acquisition is sensitive to electrical potential and either speeds-up or slows-down the consolidation processes depending upon the polarization. In addition, the enhancement or diminution of consolidation is due to the disturbance of intracellular potentials or the change in distribution of ions.

Earlier evidence has been considered which supports the idea that declarative memory consolidation can also be modulated by transcranial brain stimulation. Marshal and colleagues (2004) designed an experiment where participants learned a declarative (word-pair association) and procedural (finger tapping) task. After the acquisition, all participants went to sleep and were tested on day 2. All participants were applied active or sham stimulation. The electrode was placed over the bilateral frontal cortex and mastoid. Stimulation was applied during SWS-rich sleep. All participants were tested for the declarative and procedural memory. tDCS specifically enhanced the declarative memory consolidation leaving procedural memory unaffected. EEG data of the same study revealed that active simulation significantly enhanced the frontal spindle activity compared with sham. Spindle activity plays an important role in the consolidation of memory and helps the unstable memory to attain the stable form. Findings of the study suggested that consolidation of memory requires the cortical potential change for improvement.

Recently, Marquez-Ruiz and colleagues (2012) showed the modulatory effect of tDCS in associative learning in rabbits. Classical eyeblink conditioning was employed to investigate the tDCS effects on associative learning. After the CS-US association experimental group received the 3-blocks of anodal and cathodal tDCS over the right somatosensory cortex. Findings from the study suggest that tDCS modulates the associative learning, and anodal shows excitatory effect while cathodal seems to have inhibitory effect on the rate of conditioned response during the session. The findings affirm the earlier finding of Bindman (1962) and Albert (1966) and suggest the modulatory effect of tDCS in the associative learning task. A role for the prefrontal cortex and tDCS in memory has been well documented; however the prefrontal lateralization of direct current has been a debate. The understanding of lateralized frontal DC will give additional information about the tDCS process. Recent findings of Elmeri *et al.* (2009) demonstrated the effectiveness of tDCS over left DLPFC in a short-term verbal learning task. Cathodal tDCS over the left DLPFC leads to decreased performance in short-term verbal learning compared with the placebo stimulation (sham) condition. Their findings suggested the importance of tDCS

over the left DLPFC. Furthermore, complex cognitive processes such as verbal memory might also be influenced and modulated via weak DC stimulation. Penolazzi and colleagues (2010) showed that tDCS could also modulate emotional memory. In detail, they reported that a combination of right anodal and left cathodal stimulation of the DLPFC facilitates the recall of pleasant compared to unpleasant and neutral images, whereas left anodal / right cathodal stimulation shows contrasting results. This double dissociation effect of tDCS adds evidence to the hypothesis of hemispheric specialisation with regard to emotional processes.

Recently, researchers have reported the effects of weak DC stimulation on cognitive processes such as attention (Stone & Tesche, 2009; Weiss & Lavidor, 2012), categorization (Lupyan, Mirman, Hamilton, & Thompson-Schill, 2012), deception (Priori, et al., 2008) etc. Lupyan and colleague (2012) designed an experiment to demonstrate the role of left prefrontal cortex DC stimulation in categorization of items. They observed that when tDCS is applied over the left frontal cortex it affects categorization of similar items. They explained that anodal stimulation seems to promote the category-relevant feature while inhibiting the category-irrelevant feature. In contrast, cathodal stimulation shows reverse effects compared to anodal stimulation. They suggested that the difference in anodal and cathodal stimulation might be due to distinct neural signatures. In addition, they are not sure if DC stimulation of any other brain region will also give similar results. In future, tDCS might be helpful to understand the relationship between cognitive control and language.

Stone and Tesche (2009) reported the effects of tDCS on global / local attentional task. Participants were asked to attend the local or global relevant feature of the stimuli ignoring the irrelevant feature. The first time they reported the attentional shift via tDCS. However, they did not investigate the different stimulation effect on attentional shift in humans. Similarly, Weiss and Lavidor (2012) reported that cathodal tDCS facilitates cognitive performance. To test the proposed hypothesis researchers used the flanker task and observed that cathodal tDCS enhanced the attentional capacity compared with the sham. They showed that cathodal tDCS seems to promote the conditions required to enhance cognition, which is in contrast to earlier findings. Like earlier findings (Stone & Tesche, 2009) they propose that replication is needed to investigate the relation between attention and stimulation polarity. Still one can conclude that the diminution effect induced by cathodal tDCS might be sufficient and essential conditions to influence the neural cortical excitability associated with the cognitions, which might further influence the synaptic plasticity too. However, we cannot ignore the fact that anodal tDCS have shown similar effects in the learning and memory (Marshall, Molle, Hallschmid, & Born, 2004). These findings suggests that tDCS has a potential to improve the dysfunctional neural circuitry in the various psychiatric disorders (Rosenkranz, Nitsche, Tergau, & Paulus, 2000).

Furthermore, Priori and group (2008) successfully manipulated the brain functions via DC frontal stimulation. They showed that tDCS potentially modulates experimental deception in humans brains by decreasing the cortical excitability. They also explained that anodal tDCS over DLPFC seems to influence deception via targeting working memory. Findings suggest the modulation of experimental lies, also cannot deny from influencing working memory itself. Their study demonstrated the efficacy of frontal DC stimulation in lie production under laboratory conditions. In addition, they suggested that deception involves higher cognitive functions and engages several brain regions. The findings have several ethical issues, but show the possibility to influence mental functions targeting frontal DC stimulation. Moreover, recent studies show that tDCS modulates attention (Stone & Tesche, 2009), working memory processes (Elmer, et al., 2009; Fregni, et al., 2005; Marshall, et al., 2004; Marshall, Molle, Siebner, & Born, 2005) and behavioural inhibition (i.e., difficulty to inhibit response) (Jacobson, Ezra, Berger, & Lavidor, 2012). Jacobson and colleagues (2012) found that anodal tDCS to the right inferior parietal gyrus improves behavioural inhibition suggesting that tDCS modulates cognitive control in healthy individuals. Balconi and Vitaloni (2012) demonstrated that cathodal tDCS over the left DLPFC and anodal tDCS over the right supraorbital area attenuates the cognitive load in the incongruent processing task and limits the incongruence effect generated by semantic anomaly.

Thus, converging evidences points to tDCS being a successful neuromodulator tool. Earlier findings robustly suggest tDCS interference with memory consolidation processes (Elmer, et al., 2009; Fregni, et al., 2005; Marshall, et al., 2004; Marshall, et al., 2005). With respect to conditioned fear, we expect an enhancement of fear consolidation by anodal stimulation and attenuation by cathodal stimulation. In an excellent review on tDCS Jacobson and colleagues (2012) suggests that so far tDCS studies have targeted motor or cognitive functions. Depending upon the electrode's polarity, i.e. anode or cathode different results have been reported. They commented that cathodal tDCS failed to show inhibitory effects in cognition while anodal tDCS successfully aimed cognition. However, motor studies have reported equally excitatory effects through anodal tDCS and inhibitory effects of through cathodal tDCS.

Today although we know so much about the polarity and application of tDCS over brain areas, the mechanism is still unknown and we do not know whether stimulation in one part of the brain is not influencing the other areas of the brain as well. According to Hebbian learning (Spatz, 1996) we know multiple neurons as well as millions of synapses are connected with each other. Every now and then there are electrical signals generated in the brain, which put millions of synapse to work. We propose that some more replication and translational studies are required in order to fully understand the underlying mechanism of tDCS and therefore it should only be used in a controlled environment such as a clinical set-up.

SECTION III: GENES IN RECONSOLIDATION

1.3.1 BACKGROUND

In the late 1960s, it was shown that post-treatment of consolidation processes makes memory susceptible to disruption (Jarvik, 1972; Misanin, 1968). The old consolidated memory becomes labile upon reactivation and retrieval (Sara, 2000; Tronson & Taylor, 2007). This reactivated consolidated memory or reminder undergoes consolidation again via a cue. This stabilization of an unstable reminder activated old memory is known as reconsolidation (Dudai, 2004; McGaugh, 2000). Reconsolidation serves two purposes: first it updates old information with some recent information (i.e., updation) and secondly it maintains memory for a longer period (i.e., maintenance) (Tronson & Taylor, 2007). In recent years it has been shown that the process to update memory mechanisms can offer an effective methodology in the treatment of pathogenic memories (Gisquet-Verrier & Riccio, 2012; Nadel & Land, 2000; Nader, et al., 2000; Riccio, et al., 2006). It has been affirmed that consolidation and reconsolidation are time dependent processes and govern distinct mechanisms (Lee, et al., 2004; Lee & Hynds, 2013). Memory formation at cellular level affirms that every time memory is formed it requires protein synthesis (Nader, et al., 2000; Schafe & LeDoux, 2000; Schafe, et al., 1999). The proteins formed during the learning are stored in the neuron and upon reactivation again come to the synapse and strengthen the old synaptic connections (Schafe & LeDoux, 2000; Schafe, et al., 1999).

As it has been well documented in memory research every time memory is retrieved it undergoes reconsolidation and if it remains untouched or untreated, then it undergoes consolidation with the same impression (Lewis, 1979; Tronson & Taylor, 2007). However, if it is interrupted or interfered, then the memory becomes stored with some new traces and the old memory is updated with the new information (Gisquet-Verrier & Riccio, 2012; Riccio, et al., 2006; Sara, 2000). These findings suggested that reconsolidation might offer a therapeutic procedure in the treatment of pathological memories (see above). In the late 90s, Schafe and co-workers (1999) demonstrated two things: (a) post-treatment of memory leaves the STM intact while it impairs the LTM and (b) protein synthesis is required for the persistence of memory. This finding suggested that the LTM is protein synthesis dependent while the STM is not. Soon after this discovery multiple studies were published targeting the pre- or post-treatment of memory (Kindt, et al., 2009; Przybyslawski, et al., 1999; Przybyslawski & Sara, 1997).

Drug manipulations offer an effective methodology in blocking pathological memory, but its side effects in humans are still unclear and doubtful. Hence, there is a need of drug-free paradigms such as exposure based therapies, CBT, etc. However, the problem with such methods is that fear recovery is observed. Recently, researchers had demonstrated a drug free non-invasive method to treat the pathological memories using extinction and reconsolidation together in humans (Schiller, et al., 2010) and animals (Monfils, et al., 2009). Their findings affirmed that reconsolidation could be an effective method to update an old pathological memory trace.

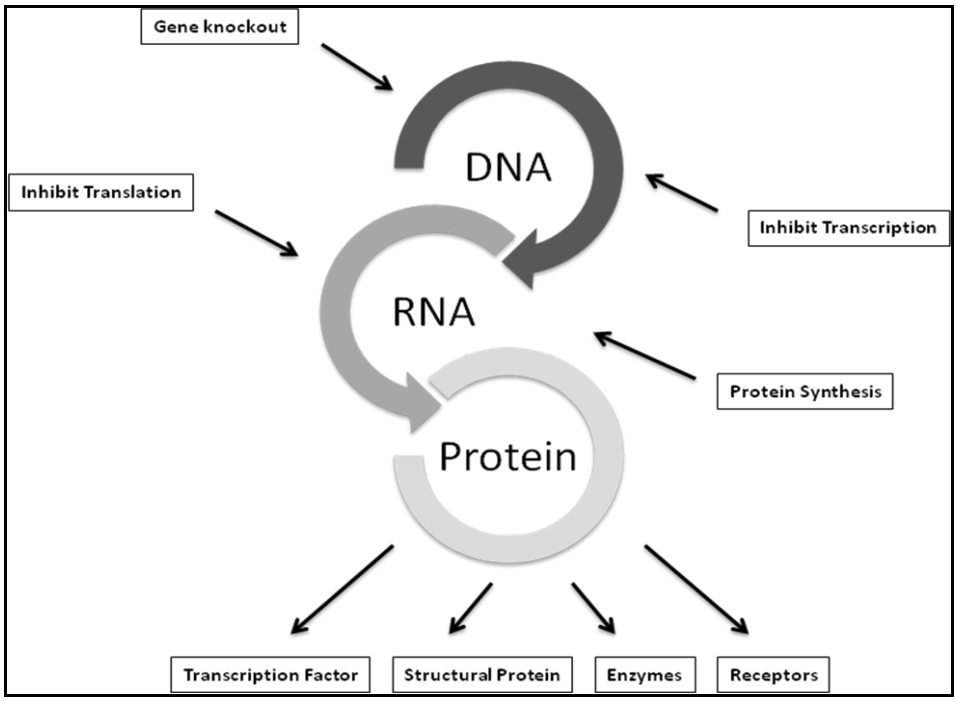


Figure 5: Depicting protein synthesis

In contrast, few studies failed to target the reconsolidation (Chan, et al., 2010; Kindt & Soeter, 2013). The reason for such discrepancy is unknown; however, researchers have argued that it might be due to several reasons such as: (i) small differences in exact procedure, (ii) boundary conditions of memory, or (iii) individual differences reflected by genetic variations (Chan, et al., 2010; Golkar, et al., 2012; Schiller & Phelps, 2011). However, Lewis (1979) in his excellent review has argued that reactivated memories and new memory, i.e. extinction learning might be governing distinct mechanisms. Also, it is important for the memory to be in an active form during modification. An inactive form of memory does not respond to the inhibitors such as ECS or protein blockers. In addition, Tronson and Taylor (2007) explained that reconsolidation is a highly specific process and it consists of two characteristics: (i) in the update of old memories (i.e., updation) and (ii) memory enhancement after reactivation (i.e., maintenance). They also suggested the boundary conditions of memory as follows: (i) age of memory, (ii)

strength of memory, and (iii) length of reactivation trial. Pavlovian conditioning is often implemented to investigate the consolidation and reconsolidation processes of pathological memories. Furthermore, it has also been shown that a number of genes are associated with pathological memory in humans, which suggests an influence of genetic heritability in simple paradigms like fear conditioning and extinction (Lonsdorf & Kalisch, 2011). The reason that genes might influence fear learning and extinction can be understood with the help of biological mechanisms.

For decades researchers have been using the Pavlovian fear-conditioning paradigm to investigate the biological mechanism of anxiety and related disorders (Hamm & Weike, 2005; Kim & Jung, 2006). It helps in understanding the mechanism of consolidation and reconsolidation of pathological memories. It is a simple method used in laboratories to make individuals learn about fear followed by safety learning (extinction). Everyone learns about fear learning over a period of time, while not everyone learns about safety learning and shows extinction. This suggests the effect of subjective nature of individuals. Some are slow learners while some are fast learners. or perhaps some individuals are non-learners, or deniers of safety learning (Myers & Davis, 2006). Differences in the behavioural response advocate the role of individualism and hint the role of genes in individual behaviours (Mahan & Ressler, 2012). Genetic studies give us an understanding of psychiatric diseases at a deeper level. They help in the understanding of fear and its extinction circuitry in rodents and humans (Gordon & Hen, 2004; Lonsdorf, et al., 2009). The interaction between genes and behaviour has been well affirmed in earlier studies (Canli, Ferri, & Duman, 2009; Hariri & Weinberger, 2003). In the case of fear learning and extinction genetic studies can give us an insight about the biological mechanisms involved. It can give a better understanding why some acquire fear rapidly while others do not. And, further why some can suppress their fear more effectively than others (Lonsdorf & Kalisch, 2011).

Hariri and colleagues (2003) explained that three-fourth of total genes are expressed in the human brain. And, the genetic background of an individual is as important as his living environment. In the recent past the importance of *5HTTLPR* and *COMT* in fear and safety learning have been shown (Lonsdorf, et al., 2009). In the past decades researchers have investigated several novel genes, which might be responsible for the emotion regulation and threat (Canli, et al., 2009; Canli & Lesch, 2007; Lonsdorf & Kalisch, 2011). Evidence suggests that more than one gene is responsible for fear and safety learning. Recent emerging data from animal and human studies suggests the interaction between several genes, several synapse and several neurons leads to individualism (Mahan & Ressler, 2012). Such findings might give us a deeper insight about neural circuitry and genetics of anxiety and related disorders.

To understand the role of genes in fear memory consolidation and reconsolidation the current study was conducted. The Selection of candidate genes is based on their role in fear learning and memory consolidation. It has been well-documented gene-expression in the brain regions and association with the anxiety related psychiatric disorders. It has been shown in earlier scientific studies that the brain derived neurotrophic factor (BDNF) plays an important role in the conditioned fear and its maintenance (Mahan & Ressler, 2012; Monfils, Cowansage, & LeDoux, 2007). Hence, we hypothesize that it also plays a role in the reconsolidation of fear memory.

1.3.2 BRAIN-DERIVED NEUROTROPHIC FACTOR (BNDF)

BDNF is a family member of the neurotrophins highly expressed in the hippocampus. Neurotrophins are the class of proteins involved in the growth, the development, and in the functions of neurons. They are the chemicals that help in the stimulation and in the control of neurogenesis (Bekinschtein, Cammarota, Izquierdo, & Medina, 2008; Monfils, et al., 2007). It has been well documented that BDNF regulates the neuronal circuitry throughout life; it plays an important role in synaptic plasticity in the hippocampus (Ninan, et al., 2010). Both human and animal studies show that BDNF and its receptor tyrosine kinase B (TrkB) have direct co-relation in fear-conditioning, extinction and reciprocal inhibition (Mahan & Ressler, 2012; Monfils, et al., 2007). From animal studies we receive evidence that BDNF-TrkB signalling is necessary in the amygdala, frontal cortex and hippocampus (Monfils, et al., 2007). Bekinschtein and colleague (2007) demonstrated an increase in BDNF expression 12-hours after post-fear training. They suggested that this increase in BDNF expression is necessary for the memory maintenance, which promotes LTM. However, when BDNF transcription is blocked after 12-hours, LTM was intact after 2-days, but when tested again after 7-days LTM was disrupted. This suggested that BDNF expression after 12hr posttraining has a gradual disruption; and is important for the persistence, but not for its formation. Furthermore, they also argued that the finding suggests that endogenous BDNF activity is required for memory maintenance.

Similarly, Liu and co-workers (2004) demonstrated that BDNF depleted animals showed impaired contextual fear-conditioning compared to wild-knockouts. They injected the BDNF directly into the hippocampus and observed some improvement in the contextual fear-conditioning. They argued that hippocampal BDNF activity is necessary during fear-conditioning. In addition, they observed that a low-level of BDNF results in fear impairment in the contextual conditioning not in the tone-conditioning paradigm. They suggested that this might be because context is hippocampal dependent while tone is independent of hippocampus.

Recent finding have highlighted that BDNF's role is not limited to the plasticity in amygdala and hippocampus, but also plays an important role in the frontal cortex (Monfils, et al., 2007). The frontal cortex (FC) as a whole is not responsible for fear expression, but it plays a main role in the extinction of conditioned fear. FC as a whole does not participate in the suppression of fear, but its sub regions, i.e. PL play an important in the fear expression (Choi, et al., 2010), while IL is involved in the fear suppression (Peters, Dieppa-Perea, Melendez, & Quirk, 2010). However, Choi and colleagues (2010) demonstrated that BDNF expression in PL is important for the consolidation of fear expression. But the BDNF expression in PL is independent for innate fear and in extinction of fear. They observed that BDNF deficient animals failed to consolidate learned fear. However, the animals were not having any problem in acquiring the fear. This behaviour of the animals suggests that prelimbic BDNF is necessary for the LTM consolidation of conditioned fear.

Similarly, Peters and co-workers (2010) showed that a BDNF injection in IL promotes extinction up to 24-hours in absence of extinction training. It has been well documented in animal studies that IL plays an important role in the extinction of conditioned fear memories. Their finding suggests that infralimbic BDNF expression is required for fear extinction. Low-level of infralimbic BDNF is observed in animals who failed to learn about extinction. Findings of Choi and colleagues (2010) and Peters and colleagues (2010) suggest the double dissociation of BDNF expression in different regions of medial prefrontal cortex (mPFC) i.e. PL and IL respectively. This finding gives us an insight about the dysfunctional memory processes in anxiety or related disorders especially PTSD or PD and the level of BDNF expression. Notwithstanding, there are some discrepancies regarding the BDNF expression in the brain (Lonsdorf & Kalisch, 2011). Many studies showed that a low-level of BDNF in the frontal cortex results in fear memory enhancement (Choi, et al., 2010) or impairment (Peters, et al., 2010). However, in contrast, Cunha and colleagues (2009) reported that expression of BDNF in the forebrain impairs and disrupts both the short-term and long-term memory process. Their findings were in contrast to the earlier reports that BDNF expression is involved in memory formation and synaptic plasticity. They observed the memory impairment in instrumental and spatial memory tasks with the moderate level of BDNF expression in rodents' forebrain.

Incredible research on BDNF polymorphism has been documented with animals and humans in the last decade (Bekinschtein, et al., 2008; Lonsdorf & Kalisch, 2011; Monfils, et al., 2007). It has been successfully established that Met-carriers of *BDNF* show irregular BDNF secretion and are characterized as highly anxious compared with the *Val-carriers*. In addition, *Met-carriers are* also characterized through reduced hippocampal structure and function. Hajack and colleagues (2009) demonstrated that *Met-carriers* showed impairment in fear-conditioning. They findings suggest that dysfunctioning to elicit defensive response by met-carrier individuals is similar to depressives or PTSD.

Montag and co-workers (2008) provided the evidence of imaging genetics in humans. They showed the role of BDNF polymorphism in the processing of emotional stimuli. Met-carrier of BDNF showed higher amygdala activation compared to *Val66Val and Val66Met*. Their results showed that *Met-carrier*s have smaller hippocampi and observed higher amygdala activation compared to the Val66Val *or Val66Met*. Further, higher activation of amygdala in *Met-carriers* suggests the dysfunctional neural mechanism. Also, they are associated with high trait anxiety. They do not observe any differential activation in hippocampus for *Met (Met66Met and Val66Met) - and Non-met (Val66Val) carriers*. Researchers proposed to investigate the role of BDNF polymorphism in higher cognitive functions. This might give a clear picture of BDNF involvement with structures such as amygdala, hippocampus, and mPFC.

Decades of memory research have revealed that memory processes have four distinct stages: (i) acquisition (encoding), (ii) consolidation (formation), (iii) storage, and (iv) retrieval (Wang, Hu, & Tsien, 2006). These processes govern the highly specific molecular and cellular stream of processes. The last phase of memory, i.e. retrieval has a very distinct characteristic and has been further sub-divided into two: (a) extinction and (b) reconsolidation. Recent animal findings with memory reactivation, i.e. memory retrieval, have suggested the involvement of hippocampal activity in the persistence of memory (Bekinschtein, et al., 2007; Bekinschtein, et al., 2008). Hippocampus has dense connections with the neo-cortex and amygdala (Monfils, et al., 2007) and, it is suggested to have been involved in the LTM storage. Later LTM becomes independent of hippocampus, but at the earlier stage when memory moves from STM to LTM it depends upon the hippocampal circuitry (Dudai, 2004; Hardt, et al., 2010).

However, it is still unknown whether BDNF plays any role after memory retrieval or reactivation and reconsolidation in humans. Albeit we know that memory upon retrieval becomes labile and is a protein dependent process (Nader, et al., 2000; Schafe, et al., 1999) and, any kind of interference (e.g., amnesic agent) after acquisition disrupts the memory undergoing reconsolidation (Jarvik, 1972; Nader, et al., 2000). Lee and colleagues (2004) have demonstrated the double dissociation between consolidation and reconsolidation of memory processes. They suggested that consolidation requires BDNF while reconsolidation requires the *Zif268* transcription factor. To answer the proposed hypothesis animals were injected with the BDNF protein blocker, e.g. oligodeoxynucleotides (ODN) 90-minutes before the training. The STM was intact; however, LTM was impaired in the group that received the anti-sense ODN. BDNF was reduced in the hippocampus after the 90-min pre-training infusion of ODN in the dorsal hippocampus. This shows the importance of BDNF activity in the hippocampus during consolidation process. In contrast, the *Zif268* transcription factor was tested to understand its role in reconsolidation in the contextual fear memory. Animals were infused with Zif268 90-minutes pre-activation of contextual memory and then compared with control (Zif268missense ODN). As a result

animal infusion with Zif268 left the STM intact while it impaired the PR-LTM. Researchers concluded that consolidation and reconsolidation have different chemical and molecular signatures just like the two have different temporal synchrony for stabilization.

It has been clear so far that the last several decades of research on BDNF polymorphism have cracked down on its role in memory consolidation especially LTM and L-LTP. Although due to the lack of BDNF literature on reconsolidation it would be too early to comment on its role in memory retrieval and reconsolidation. Hence, in the current dissertation we tried to investigate the role of BDNF in reactivation and reconsolidation of memory in humans.

CHAPTER 2

STUDY I: EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) IN THE MODULATION OF CONDITIONED FEAR CONSOLIDATION

2.1.1 Introduction

The present study examined the effects of transcranial direct current stimulation (tDCS) over the dorsolateral prefrontal cortex (DLPFC). The present study shows the efficacy of tDCS on emotional memory consolidation, especially conditioned fear, i.e. associative learning (see figure 6). To answer the proposed hypothesis an auditory fear-conditioning paradigm was employed, in which two differently coloured squares (blue and yellow) were presented as conditioned stimuli (CS) and an auditory stimulus as an unconditioned stimulus (UCS). Dependent measures included the self-report, questionnaires, and physiological indices. Sixty-nine participants were recruited and randomly assigned into three groups: anodal, cathodal and sham stimulation. The participants of the two active groups (i.e., anodal & cathodal) received tDCS over the left DLPFC for 12 minutes after fear-conditioning. Cathodal stimulation of the left DLPFC interferes with the consolidation processes and attenuates condition fear. Explanation of this finding can be understood through the effects of tDCS on declarative memory and sleep research.

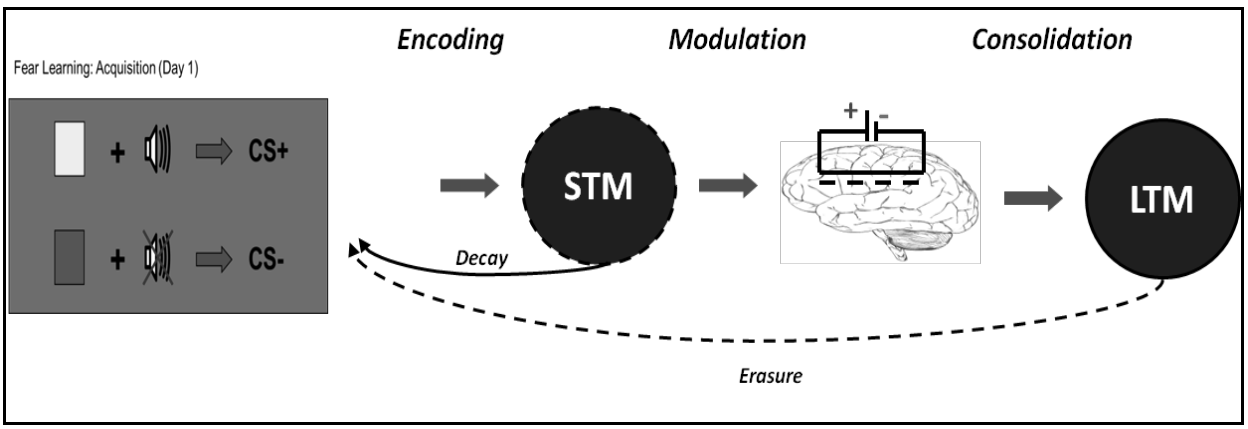


Figure 6: Schematic presentation of modulation of fear learning and its consolidation

2.1.2 Methods

2.1.2.1 Participants

Sixty-nine healthy participants were recruited by advertisements to participate in the present study. Participants were eligible for inclusion if they met the following criteria: (i) right handed; (ii) age 18-30 years; (iii) German native; (iv) females taking contraceptives during the study period. Participants were excluded from the trial if they met the following criteria: (i) any metal object or implant in brain, skull, scalp, or neck; (ii) implantable devices, including cardiac pacemakers and defibrillators; (iii) any neurological or psychiatric illnesses; (iv) pregnancy. Information about this criteria was obtained by questionnaires (see table 2). After data analysis (see below), 6 participants were excluded due to artefacts (i.e., origin of response to stimuli before a baseline and regarded as null-responses of the CRs and also if CRs < 0.01). Nine participants were excluded because they do not show a conditioned response [CS+> CS- during acquisition and also CS+ (Habituation) > CS+ (Acquisition)]. Two participants did not volunteer to participate on extinction training (24 hours later: day 2). Three participants were rejected due to high-impedance (i.e., 20 kΩ). One participant was rejected as it was identified with depressive symptoms and one participant was recruited twice by mistake so only the first recorded data was considered in the current study. The demo-graphical data of the remaining 49 participants is shown in Table 1. The study was in accordance with the declaration of Helsinki in their latest version from 2008 and has been approved by the Ethics Committee of the University of Würzburg. All participants gave written informed consent to participate in the study.

Table 1: Demographics of participants included in Study I

	Anodal tDCS	**Cathodal tDCS**	**Sham**
Nr. of Participants	16	18	15
Age Range	22.18 ± 2.26	23.11 ± 2.08	22.46 ± 2.38
Female / Male	6 /10	11 / 7	8 / 7

2.1.2.2 Stimulus

Blue and yellow colored squares were used as conditioned stimuli. One woman's scream of code number 276 adapted from the International Affective Digital Sounds (IADS) for 2-sec of 102-db loudness served as an unconditioned stimulus.

2.1.2.3 DC Polarization

tDCS is a non-invasive method, in which direct current is applied over the scalp to modulate human brain excitability. The exact mechanism of tDCS is unclear, but it is assumed that anodal tDCS excites the neurons below the stimulated region and leads to the depolarization, while cathodal tDCS has an inhibitory effect, i.e. it reduces the neuronal firing resulting in hyper-polarization. tDCS was delivered by a battery-driven stimulator (DC-Stimulator-Plus, NeuroConn GmbH, Ilmenau, Germany) approved for use in humans. A pair of conductive-rubber electrodes (size 5 cm x 7 cm = 35 cm^2) coated with Ten20 cream conductive paste (Waever and Company, Colorado, USA) was positioned over the left DLPFC (electrode position F3) and left mastoid (reference electrode) according to the international 10-20 EEG-system (Jasper, 1958)(see figure 7).

Figure 7: Image showing the attachment of montage on the scalp

For active tDCS, a constant current of 1mA was applied for 12 min (current density: 0.0286 m-A/cm^2). The current was ramped up or down over the first and last 10 sec of stimulation, respectively. The stimulator, always below 20 kΩ, controlled the impedance. During sham condition the constant current was ramped up for the first 10s once the DC had reached a current flow of 1 mA the current ramped down for 10s. Therefore, the sham stimulation leads to the same sensation in the participants, but has no long lasting effects. This means that sham tDCS turned off automatically after 10s of current ramping up and 10s of ramping down.

2.1.2.4 Procedure

The present study was conducted on two consecutive days maintaining the time difference between two sessions from 20 to 26 hours. The study involved three stages: habituation, acquisition, and extinction. The first session (day 1) consisted of the habituation and acquisition stages, in which participants learned the association of conditioned (CS) and unconditioned stimuli (UCS). Blue or yellow colored squares (presented at a 16 degree visual angle) were presented to the participants in randomized order on a monitor for 4 sec with the inter-stimulus-interval (ISI) of 20-22 sec. using Presentation® Version 13.0 software (Neurobehavioral Systems, Inc., Albany, Calif., USA). Along with one of the colored squares, a women's scream adapted from the International Affective Digital Sounds (IADS) (Bradley, 1999) was presented for 2 sec (2 sec after CS+ onset), while the other square served as CS-. Sixteen CS+ and CS- were presented. Only 12 of the 16 CS+ trials were reinforced (75% reinforcement rate). On day 1, the experiment had two phases: (i) the habituation phase, in which 8 trials with squares of each color were presented on the monitor to reach a stable response to the stimuli and (ii) the acquisition phase, during which 16 trials with squares of each color were presented. During the acquisition phase, one of the colored squares was paired with the scream; after 10-20 minutes of fear acquisition, electrodes for electrical stimulation were applied. As previous studies showed, the consolidation process occurs during the time window from a few minutes to 6 hours. Hence, in the current study all participants were stimulated after 10 minutes. The variable time frame of 10-20 minutes depended on the fitting of the tDCS electrodes. During the second day (Day 2), all participants underwent extinction training. During extinction training, 16 CS+ and CS- were repeatedly presented in absence of the UCS (see figure 8).

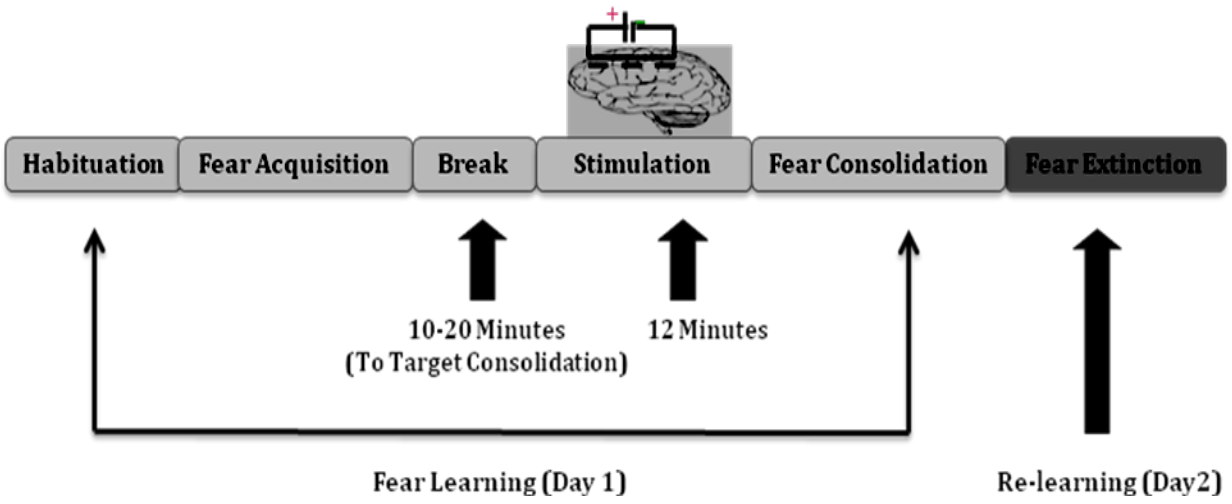


Figure 8: Schematic diagram depicting the experimental procedure

2.1.2.4 Measurement and Analysis

To measure the physiological response, SCRs were recorded from the volar surfaces on medial phalanges of the participants' non-dominant palm (Dawson, Schell, Filion, & Berntson, 2007). SCRs were recorded by a V-Amp 16 (Brain Products GmbH, Gilching, Germany) 16-channel DC amplifier system using the BrainVision Recorder Software (V-Amp Edition 1.10, Brain Products GmbH, Gilching, Germany) at a sampling rate of 1000Hz. All data was filtered with a 50Hz notch filter during recording and 1Hz high was filtered out during the offline analysis. SCRs were recorded by using two Ag/AgCl electrodes (diameter = 13 mm) filled with non-hydrating gel. Participants were excluded from the analysis when they did not show conditioning (CS+ < CS- during acquisition) or had more than 50% artifacts (i.e. ≥ 8 trials in CS+ or CS-) in the raw data of SCR. Participants were also excluded when the tDCS electrodes read impedances higher than 20kΩ during the active and sham condition. For the statistical analysis, the first CS+ and CS- trial were disregarded due to the orienting response at the beginning of the session in all phases. The remaining trials were used to produce average normalized SCR scores (CS+ and CS-) within subjects. Raw SCR scores were square root transformed to normalize distributions. To test the fear memory trace, after tDCS all trials of the acquisition phase (i.e. trials 2-16) and the first five trials of the extinction phase (i.e. trials 2-6) were considered. Conditioning effects were calculated by using two-way ANOVA with main effects of time (habituation & acquisition) and stimuli (CS+ & CS-). The effect of stimulation on fear memory consolidation was analysed by a three-factor ANOVA with stimuli (CS+ & CS-), time (acquisition (day 1) extinction (day 2) and group (anodal, cathodal and sham) as the main factors.

2.1.3 Results

2.1.3.1 Questionnaires Ratings

Table 2 : Summary of the t-test of mean (s.d.) of the reported anxiety sensitivity and fear questionnaire for the participants' selection between groups (anodal, cathodal, and sham)

	Groups [Mean (S.D.)]	t-value	P-value	df
ASI3	Anodal [0.93 (0.50)] vs. Cathodal [0.90 (0.43)]	0.21	0.84	32
	Anodal [0.93 (0.50)] vs. Sham [0.91 (0.55)]	0.02	0.89	29
	Cathodal [0.90 (0.43)] vs. Sham [0.91 (0.55)]	-0.04	0.97	31
FQ	Anodal [1.11 (0.56)] vs. Cathodal [1.18 (0.95)]	-0.25	0.81	32
	Anodal [1.11 (0.56)] vs. Sham [1.31 (0.81)]	-0.79	0.44	29
	Cathodal [1.18 (0.95)] vs. Sham [1.31 (0.81)]	-0.42	0.68	31

2.1.3.2 Skin Conductance Response (SCR) Recording

An analysis of variance (ANOVA) was performed on SCR data to access conditioning. Conditioning was effective leading to a significant main effect of time ($F [1, 48] = 97.12$; $p < 0.01$), stimuli ($F [1, 48] = 106.23$; $p < 0.01$), and an interaction effect time x stimuli ($F [1, 48] = 99.01$; $p < 0.01$). A post hoc t-test revealed that the SCRs in response to the CS+ were higher compared to the CS- ($t_{48} = 11.47$; $p < 0.01$), but only during acquisition, not during habitation. Both CS+ ($t_{48} = -10.87$; $p < 0.01$) and CS- ($t_{48} = -2.19$; $p < 0.05$) increased from habitation to acquisition, but the increase for the CS+ was higher as compared to the CS- ($t_{48} = 9.95$; $p < 0.01$). Means and standard deviations for SCRs in response to the conditioned stimulus are shown in (see table 3).

To evaluate the effect of stimulation on fear memory consolidation, a three-factor ANOVA was calculated showing a significant main effect of stimuli ($F [1, 46] = 106.32$; $p < 0.01$), time ($F [1, 46] = 32.73$; $p < 0.01$), and interaction effects stimuli x time ($F [1, 46] = 109.97$; $p < 0.01$) and stimuli x time x group ($F [1, 46] = 5.03$; $p < 0.01$) (see figure 9). A follow-up t-test was used to compare the anodal and cathodal group with regard to the amount of attenuation between the acquisition and the extinction phase for the differential values between CS+ and CS- (see figure 9), which differed, significantly ($t_{32} = 2.34$ $p < 0.01$) with lower values for the cathodal group. In a similar way, the cathodal group also displayed diminished differential values compared to the sham group ($t_{31} = -2.88$; $p < 0.01$). This might indicate that cathodal stimulation has an inhibitory effect on fear consolidation, while anodal stimulation and sham do not differ significantly from each other ($t_{29} = -0.32$; $p > 0.05$) (see figure 10). Chi-square test revealed non-significant differences in the gender distribution among the 3 groups (anodal, cathodal, and sham) ($chi^2 = 1.94$, $df = 2$, $p = 0.38$.)

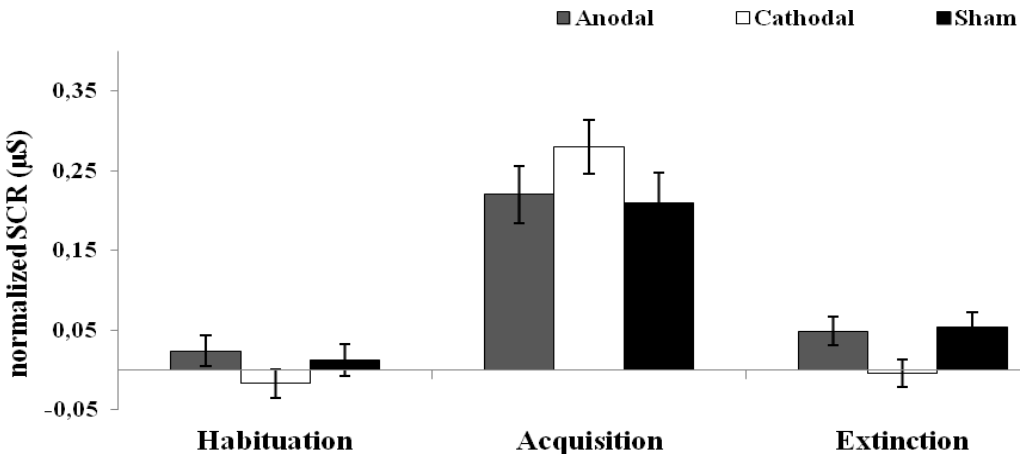


Figure 9: Depicts the differential SCRs (CS+ minus CS-) during acquisition and extinction phase for all three groups (anodal, cathodal, and sham)

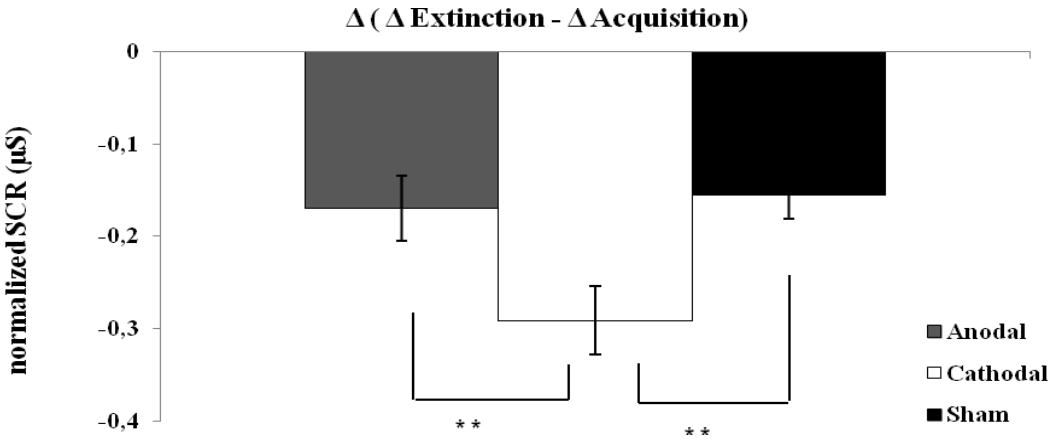


Figure 10: Plot show the post- to pre- differential SCRs (Δ Extinction vs. Δ Acquisition) values and comparison between the anodal, cathodal, and sham. Note: n.s. denotes non-significant level and ** denotes significant level at < 0.01

Table 3: Mean (S.D.) SCRs (CS+ and CS-) during habituation, acquisition, and extinction for all three groups (anodal, cathodal, and sham)

	Anodal tDCS		Cathodal tDCS		Sham	
	CS+	CS-	CS+	CS-	CS+	CS-
Habituation	0.10 (0.11)	0.08 (0.10)	0.06 (0.08)	0.08 (0.11)	0.05 (0.08)	0.04 (0.07)
Acquisition	0.33 (0.23)	0.11 (0.09)	0.38 (0.22)	0.09 (0.09)	0.28 (0.14)	0.07 (0.05)
Extinction	0.12 (0.17)	0.07 (0.10)	0.08 (0.09)	0.08 (0.10)	0.10 (0.14)	0.05 (0.09)

2.1.4 Discussion

In our study, we found that application of tDCS over-left DLPFC attenuates the fear consolidation process. The finding suggests that cathodal tDCS disrupts fear memory consolidation after fear-conditioning. Participants were made to learn about the threat and no-threat situation in the laboratory. To measure the threat level against the CS+ and CS- SCRs were recorded from the electrodes attached to the finger of the non-dominant hand. The higher SCRs values of CS+ compared to CS- suggest that participants were conditioned to fear. After the fear-conditioning we applied left DLPFC stimulation to all participants for duration of 12 minutes. The finding suggests the efficacy of tDCS on the fear consolidation process. Several studies have shown the importance of DLPFC in memory and learning processes (Elmer, et al., 2009; Fregni, et al., 2005). Marshall and colleagues (2004) examined the role

of tDCS on declarative memory. They have shown that application of tDCS during non-rem sleep improves retention of declarative memory. In addition, it supplies the explanation that tDCS modulates the cortex of the brain. This facilitates the brain waves such as alpha and delta, which promotes the conditions required for memory consolidation.

Studies using a N-back task (Fregni, et al., 2005) or verbal-memory task (Elmer, et al., 2009), respectively, showed improvement in memory after stimulating the left DLPFC, indicating the efficiency of tDCS in modulating DLPFC activity (Marshall, et al., 2004; Zaehle, Sandmann, Thorne, Jancke, & Herrmann, 2011). Anodal stimulation increases the neural firing resulting in the improvement of memory. However, cathodal stimulation showed a reciprocal effect (Zaehle, et al., 2011). In an electroencephalographic (EEG) study, Zaehle and colleagues (2011) observed reduced accuracy in a working memory task along with tDCS of the DLPFC. The main finding of the present study was an impaired fear memory consolidation after left DLPFC stimulation, which further supports the earlier findings, the role of DLPFC stimulation in memory consolidation (Elmer, et al., 2009; Fregni, et al., 2005; Marshall, Helgadottir, Molle, & Born, 2006; Marshall, et al., 2004; Marshall, et al., 2005; Penolazzi, et al., 2010). In synopsis evidence from the existing literature and the present results show a crucial impact of tDCS over the left DLPFC on fear memory consolidation processes.

There is resurgence to understand the mechanism of tDCS works on the principle of changing the intracellular potential via influencing the flow of ions such as sodium or calcium (Marshall, et al., 2004) Artificially generated negative potential in the neo-cortex generates a spindle-form activity, which is responsible for the Ca^{2+} ion influx (Marshall, et al., 2006). tDCS can have either hyperpolarizing or depolarizing nature, which depends upon the charge flow: Anodal stimulation seems to depolarize neuronal firing, while cathodal stimulation seems to hyperpolarize neuronal firing (Utz, Dimova, Oppenlander, & Kerkhoff, 2010).

From the earlier explanation it can be assumed that tDCS influences the consolidation of memory processes. It has tenacious effects on neuronal firing in humans and influences long lasting effects in synaptic excitability, i.e. long-term potentiation (LTP) (Lang, et al., 2005). It has been affirmed that LTP and fear-conditioning share a common pathway for processing and have similar underlying mechanisms (Schafe, et al., 1999) stressing the importance of LTP in fear-conditioning (Schafe, et al., 1999). In addition, memory research affirms that the spindle activity generated during slow-wave-sleep (SWS) is important in sending information to the hippocampus, which acts as a buffer and stores the relevant information.

During the SWS spindle activity triggers the newly encoded information in the hippocampus leading to consolidation. This retrieval of hippocampal memory via spindle activity synchronizes with the neo-cortex and sends the newly stored information back to the neo-cortex which further triggers LTP processes (Born, Rasch, & Gais, 2006). The neural connection is strengthened and potentiates the consolidation processes with the help of spindle activity. Application of tDCS over the left DLPFC might facilitate the spindle activity, which has been suggested helping in the potentiation of consolidation processes (Marshall, et al., 2004).

Lin and colleagues (2003) showed that learning about having fear and safety (extinction) shares some common and uncommon mechanisms. They showed that both processes require protein synthesis (cAMP response element-binding protein), kinase (phosphatidylinositol 3-kinase), and NMDA receptors in the amygdala for the consolidation mechanism. They demonstrated long-term behavioural changes after fear and its extinction. In addition, NMDA supplies a ready explanation for the observation that learning about having fear or extinction triggers a Ca^{2+} ion influx in amygdala neurons. Increase in Ca^{2+} ion concentration promotes long-term synaptic depression (LTD) (Lin, Yeh, Lu, & Gean, 2003; Marshall, et al., 2004). This explains that extinction processes suppress Ca^{2+} ion concentrations followed by synaptic changes, which further lead to extinction of fear memory.

Our results are what would be expected with the application of tDCS over-left DLPFC in fear memory consolidation (FMC). Finding suggests that cathodal tDCS disrupts FMC via decreasing the neural firing. However, anodal stimulation, which might expect to increase firing, does not interfere with FMC. The present study suggests the role of the DLPFC in extinction. In turn negative effects of cathodal tDCS on fear memory might contribute to novel therapeutic approaches in the prevention and treatment of pathological memories, e.g. PTSD.

STUDY II: SINGLE BRIEF EXPOSURE MAKES AN OLD MEMORY SENSITIVE TO DISRUPTION AND LEADS TO RESTORATION WITH NEW INFORMATION

2.2.1. Introduction

It has been well documented in memory literature that memory consolidation is a time-dependent process. Initially trace of memory is labile in nature over time it attains a more stable form and is stored for future reference (Dudai, 2004). Earlier researchers believed that memory consolidation is a one-time event (Nader, et al., 2000). In recent years researchers targeted the consolidation memory and concluded that stored memory becomes labile after retrieval or reactivation (Przybyslawski & Sara, 1997). The reactivated labile memory is known as reconsolidation and is also a time (Monfils, et al., 2009; Schiller, et al., 2010) and protein-dependent process. (Schafe & LeDoux, 2000). Hence, any ineterfernce after the retrieval or reactivation of memory leads to amnesia and disrupts reconsolidaton (Tronson & Taylor, 2007).

Pharmacological treatment of reactivated memory has shown its potential; however, its use on humans is unpredictable (Schiller, et al., 2010). Another approach used in the treatment of anxiety or related disorders is exposure therapy. It offers a drug-free methodology in which an individual is exposed to a threat as long as his/her fear dissipates. In Pavlovian fear-conditioning humans or rodents are exposed to CS which dampens the CR. This repeated presentation of CS in absence of UCS is known as extinction process (Myers & Davis, 2006). Recently, Schiller and co-workers (2010) demonstrated how to prevent fear return by performing extinction training within the reconsolidation period. In this study, three groups underwent fear learning on day 1. A day later all participants were divided into three groups: in one group fear memory was reactivated via a reminder followed by a 10 minute break and then followed by extinction training, the second group also received the reminder, but the extinction training was performed after 6-hours from reactivation, and the third group did not receive any reminder. They observed that the group which received extinction training 10minutes after reactivation prevented the fear return, the group which received extinction training after 6-hours of reactivation did not prevent the fear return. Similarly the group that did not receive reactivation showed failure in preventing fear return.

The findings suggested the importance of reactivation followed by extinction training within the reconsolidation period. The study offered the first drug-free paradigm in the treatment of anxiety and related disorders. Using this study as a foundation, we took a similar paradigm and modifyed it a bit. Firstly by doubling the strength of reinforcement, i.e. 80% instead of 38% as presented in the earlier finding to investigate whether strong memories undergo consolidation or not. Secondly, by using an aversive sound as UCS instead of shock as reported earlier. We hypothesize that strong memory also undergoes reconsolidation and a reminder will prevent fear restoration.

2.2.2 Methods

2.2.2.1 Participants

143 healthy participants were recruited by advertisements to participate in the present study. Participants were eligible for inclusion if they met the following criteria: (i) age 18-31 years; (ii) German native; (iii) females taking contraceptives during the study period. Participants were excluded from the trial if they met the following criteria: (i) any neurological or psychiatric illnesses; (ii) pregnancy. Information about these criteria was obtained by questionnaires. After data analysis 34 participants were excluded due to artefacts (i.e. origin of response to stimuli before a baseline) and null responses of the conditioned responses (see below). Four participants did not volunteer to participate on Day-2. The demographical data of the remaining 105 participants is shown in Table 1. The study was in accordance with the declaration of Helsinki in their latest version from 2008 and has been approved by the Ethics Committee of the University of Würzburg. All participants gave written informed consent to participate in the study.

Table 4: Demographics of participants included in Study II

	Reminder	**No-reminder**
Nr. of Participants	47	58
Age	24.21 ± 3.17	23.84 ± 2.55
Female / Male	22 / 25	31 / 27

2.2.2.2 Stimulus

Blue and yellow colored squares were used as conditioned stimuli. Along with one woman's scream of code number 276 adapted from the International Affective Digital Sounds (IADS) for 2-sec of 102-db loudness, which served as unconditioned stimulus.

2.2.2.3 Self-report and Questionnaires Rating Scales

Nine questionnaires were considered in the present study in order to have a better understanding about the status of the participants (see table 6). Apart from these nine questionnaires, some self-report rating scales were also used, for example arousal & valence. Self-report rating scales were presented on the monitor and all participants were asked to rate (range 1 to 9). Both the arousal scale ranged from 1 to 9 (1 very aroused and 9 not at all aroused) as well as the valence scale (1 very positive and 9 very negative). To record their response the participants pressed assigned buttons on the keyboard. All participants were given German versions of total 9 questionnaires and were asked to fill these out before the experimental study.

The fear questionnaire (FQ) is widely used to measure phobias such as agoraphobia, social phobia, and blood-injury phobia. Issac and Mathews invented the FQ in 1979. It has a 0-8 point scale, which is divided into five-point values (0 = hardly at all, 1 = slightly troublesome, 2 = definitely troublesome, 3 = markedly troublesome, and 4 = very severely troublesome). It has a total of 20-items on the scale and the score range for all prior mentioned phobias varies from 0 to 40 (Marks, 1979)

The Positive Affect Negative Affect Scale (PANAS) is 20-item scale invested by Watson, Clark, and Tellengen (1988). It consists of five-point values (1 = very slightly or not at all, 2 = a little, 3 = moderately, 4 = quite a bit, and 5 = very much) and is used for the brief measurement of positive and negative affects (Watson, Clark, & Tellegen, 1988).

The Penn State Worry Questionnaire (PSWQ) is a scale used to measure the worry level in individuals. Mayer and colleagues invented this scale in 1990. It has 16-items that are rated on five-point values (1 = not at all typical to me, 2 = rarely typical of me, 3 = somewhat typical to me, 4 = often typical of me, and 5 = very typical of me). The total score range varies from 16 to 80.(Meyer , Miller, Metzger, & Borkovec, 1990).

The Spielberg State-Trait Anxiety Inventory (STAI) is a widely used scale that measures the anxiety in individuals. Spielberger and colleagues invented it in 1983. It is a four-point scale (1= not at all, 2 = a little, 3 = somewhat, and 4 = very much so), and consists of two types: (i) state and (ii) trait (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983).

The Allgemeine Depressions Skala (ADSk) is a scale used to measure depression in individuals. Hautzinger and Bailer invented it in 1993; it is a four-point likert-scale (0 =rarely, 1 = sometimes, 2 = frequent, 3 = usually). This scale has two versions ADSl and ADSk. In the current study we used the ADSk version to filter out the depressives from the study (Hautzinger & Bailer, 1993; Meyer & Hautzinger, 2001).

The Behavioral Approach System / Behavioral Inhibition System scale (BISBAS) was invented in 1994 by Carver and White to measure the motivational system of individuals. This scale has 24-items that are rated on a four-point scale (1 = very true for me, 2 = somewhat true for me, 3 = somewhat false for me, and 4 = very false for me) (Carver & White, 1994).

The Anxiety Sensitivity Index-3 (ASI3) is designed for the measurement of anxiety disorders, which further assess three factors as follows: (i) physical, (ii) cognitive, and (iii) social aspects. Taylor and colleagues have invented it in 2007. It is a five-point scale (0 = very little to 4 = very much) and has a total of 18-items with the scores ranging from 0 to 72 (Taylor, et al., 2007).

2.2.2.4 Skin Conductance Response (SCR) Recording

To measure the physiological response, SCRs were recorded from the volar surfaces on medial phalanges of the participants' non-dominant palm (Dawson, et al., 2007; Michael E. Dawson, Anne M. Schell, Filion, & Berntson, 2007). SCRs were recorded by a V-Amp 16 (Brain Products GmbH, Gilching, Germany) 16-channel DC amplifier system using the BrainVision Recorder Software (V-Amp Edition 1.10, Brain Products GmbH, Gilching, Germany) at a sampling rate of 1000Hz. All data was filtered with a 50Hz notch filter while recording. SCRs were recorded by using two Ag/AgCl electrodes (diameter = 13 mm) filled with non-hydrating gel.

2.2.2.5 Procedure

The present study was conducted in three consecutive days, 20 hours to 26 hours apart. The study involved the five stages: habituation, acquisition, re-activation, extinction and re-extinction. The first session (day 1) consisted of the habituation and acquisition stages, in which participants learned the

association of conditioned (CS) and unconditioned stimuli (UCS). Blue or yellow colored squares (presented at a 16 degree visual angle) were presented to the participants in a randomized order on a monitor for 4 seconds (sec) with the inter-stimulus-interval (ISI) of 10-12 sec using Presentation® Version 13.0 software (Neurobehavioral Systems, Inc., Albany, Calif., USA).

Along with one of the colored squares, a women's scream adapted from the International Affective Digital Sounds (IADS) (Bradley, 1999) was presented for 2 sec (2 sec after CS+ onset), while the other square served as CS-. Sixteen CS+ and CS- were presented and an 80% reinforcement rate was used i.e. 80% CS+ trials were paired with UCS. On day 1, the experiment had two phases: (i) the habituation phase, in which 8 trial squares of each color were presented on the monitor to reach a stable response to the stimuli and (ii) the acquisition phase, during which 16 trials of squares of each color were presented. During the acquisition phase, one of the colored squares was paired with the scream (UCS).

During the second day (day 2), all participants first underwent re-activation (except the control group) followed by extinction training (16 CS+, 16 CS-). A re-activation single trial of CS+, served as a reminder trial and was presented before the extinction training and then followed by a break of 10 min in the experimental group. In contrast, the control group was never presented any reminder trial but the extinction training started after a 10 min break as well. During the extinction training, there was a repeated presentation of CS+ and CS- in absence of UCS. This generated new knowledge and helped participants learn implicitly that the threatening stimulus is safe. Decrease in the physiological response (SCR) was observed during the extinction training in contrast to during the acquisition phase. A day later (day 3), both groups control and experimental again underwent the extinction training (16 CS+, 16 CS-) in order to test the spontaneous recovery. All three days the headphone and the skin conductance electrodes were connected to all the participants. Physiological responses (SCR) were recorded from start until finish of the session on all three days (see figure 11).

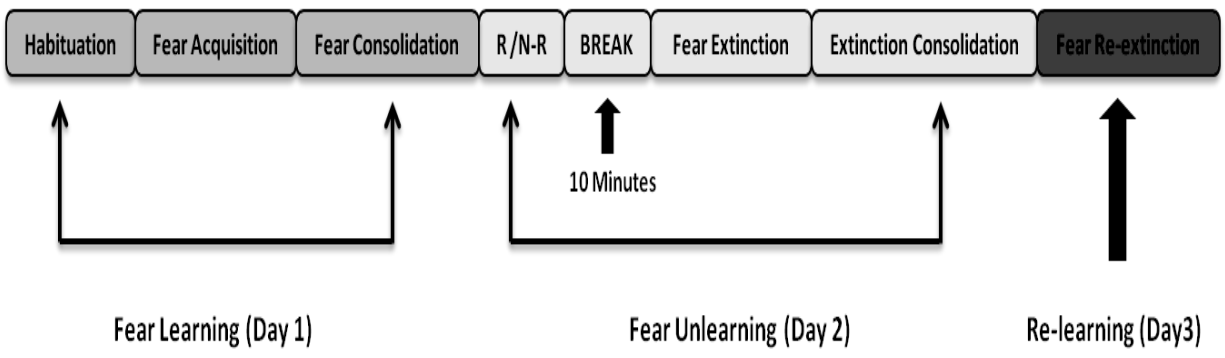


Figure 11: Schematic diagram depicting the experimental procedure

2.2.2.6 Statistical Analysis

Participants were excluded from the analysis when not showing conditioning (CS+ < CS- during acquisition) or having more than 50% artifacts (i.e. ≥ 8 trials in CS+ or CS-) in the raw data of SCR. Raw SCR scores were square root transformed to normalize distributions and were range-corrected. For the statistical analysis, the two trials of both CS+ and CS- were averaged across the total number of trials (i.e. 16 trials in each). Each single trial block is comprised of two-trials. Hence, in total we received 4-trail blocks during habituation and 8-trial blocks for each CS+ and CS- during acquisition, extinction, and re-extinction.

For the statistical analysis, the first 4-trial blocks (trials1-8) during acquisition, the last 2-trial blocks (trials 13-16) during extinction and the first 1-trial block (trials1-2) were taken into consideration. Two-way ANOVA was conducted to test the significant effect of the reminder between the two groups (reminder and non-reminder). To test the spontaneous recovery last 2-trail blocks of extinction and first 1-trail block were considered.

2.2.3 Results

2.2.3.1 Self-report Ratings

Table 5: Summary of t-test of mean (s.d.) differential (CS+ minus CS-) of self-report ratings during habituation, acquisition, extinction and re-extinction

	Arousal				**Valence**				
	Reminder (47)	**No-reminder (58)**	**t-value**	**P-value**	**Reminder (47)**	**No-reminder (58)**	**t-value**	**P-value**	**df**
Habituation	-0.60(2.51)	-0.43(2.03)	-0.37	0.72	0.62(2.82)	0.48(2.85)	0.24	0.81	103
Acquisition	-4.94(2.17)	-4.88(1.83)	-0.15	0.89	5.57(2.06)	5.53(2.09)	0.09	0.92	103
Extinction	-2.62(2.24)	-1.72(2.24)	-2.03	0.05	2.68(2.47)	1.77(2.53)	1.84	0.07	102
Re-extinction	-1.96(2.20)	-1.46(1.90)	-1.25	0.22	1.81(2.26)	1.47(2.13)	0.78	0.44	102

2.2.3.2 Questionnaire Ratings

Table 6: Summary of the t-test of the reported anxiety sensitivity, fear questionnaire, PSWQ, PANAS, ADSk, STAI, and BISBAS for the participants' selection in study II

	Groups [Mean (S.D.)]	t-value	P-value	df
FQ	Reminder [1.35 (0.73)] vs. No-reminder[1.22 (0.71)]	0.94	0.35	103
PANAS	Reminder [2.46 (0.34)] vs. No-reminder [2.38 (0.37)]	1.22	0.23	103
PSWQ	Reminder [2.34 (0.39)] vs. No-reminder [2.15 (0.43)]	2.31	0.02	103
STAI	Reminder [0.97 (0.47)] vs. No-reminder [0.84 (0.53)]	1.29	0.19	103
ADSk	Reminder [0.75 (0.25)] vs. No-reminder [0.73 (0.31)]	0.31	0.76	103
BISBAS	Reminder [1.98 (0.21)] vs. No-reminder [1.92 (0.22)]	1.29	0.20	103
ASI3	Reminder [0.97 (0.47)] vs. No-reminder [0.84 (0.53)]	1.29	0.20	103

2.2.3.3 Skin Conductance Response (SCR) Recording

Means and standard deviations for SCRs in response to the conditioned stimulus are shown in (see table 7). Conditioning was effective leading to a significant main effect of stimuli ($F [1, 104] = 203.86$; $p < 0.01$, time ($F [1, 104] = 210.31$; $p < 0.01$), and an interaction effect stimuli x time ($F [1, 104] = 305.17$; $p < 0.01$). A post-hoc t-test revealed that the SCRs in response to the CS+ were higher in comparison to the CS- ($t_{104} = 17.56$; $p < 0.01$), but only during acquisition, not during habitation. Only CS+ ($t_{104} = -18.42$; $p < 0.01$) but not CS- ($t_{104} = 0.84$; $p > 0.05$) increased from habitation to acquisition.

A Two-way ANOVA was designed to investigate whether two groups did not differ from each other during fear acquisition. We observed the significant main effect of stimuli ($F [1, 103] = 167.94$; $p < 0.01$), time ($F [2, 103] = 133.94$; $p < 0.01$), stimuli x group ($F [2, 103] = 4.52$; $p < 0.05$), stimuli x time ($F [2, 103] = 263.10$; $p < 0.01$), and an interaction effect stimuli x time x group ($F [2, 103] = 7.03$; $p < 0.01$). A post-hoc t-test was conducted to understand the interaction between stimuli x time x group, CS+ values differed significantly between the two groups during acquisition ($t_{103} = 2.30$; $p < 0.05$) but not during habituation and also CS- values did not change for both groups from the habituation to the acquisition phase.

The T-test revealed that CS+ values differed significantly between the groups during fear learning. Hence ANOVA (2 x 8 x 2) was designed to investigate the change in CS value over time between the two groups during the Acquisition phase. This showed the significant main effect of stimuli ($F [1, 103] = 280.90$; $p < 0.01$), time ($F [1, 103] = 17.79$; $p < 0.01$), and an interaction effect stimuli x group ($F [1, 103] = 7.53$; $p < 0.01$), no stimuli x time x group interaction was observed. We are unable to suggest

why the two groups differed during fear learning. The early phase did not show significant interaction however the sudden decline is observed in the no-reminder group perhaps this is the reason for stimuli x time x group significant interaction.

Three-way ANOVA was performed to evaluate the effect of the reminder on extinction in the groups from the acquisition phase to the extinction phase with the main effects of time (acquisition and extinction), stimuli (CS+ and CS-) and group (reminder and no-reminder). This showed the significant main effect of stimuli ($F [2, 103] = 238.92$; $p < 0.01$), a stimuli x time ($F [2, 103] = 109.33$; $p < 0.01$) and stimuli x group interaction ($F [2, 103] = 10.99$; $p < 0.01$) but no effect of group or interaction.

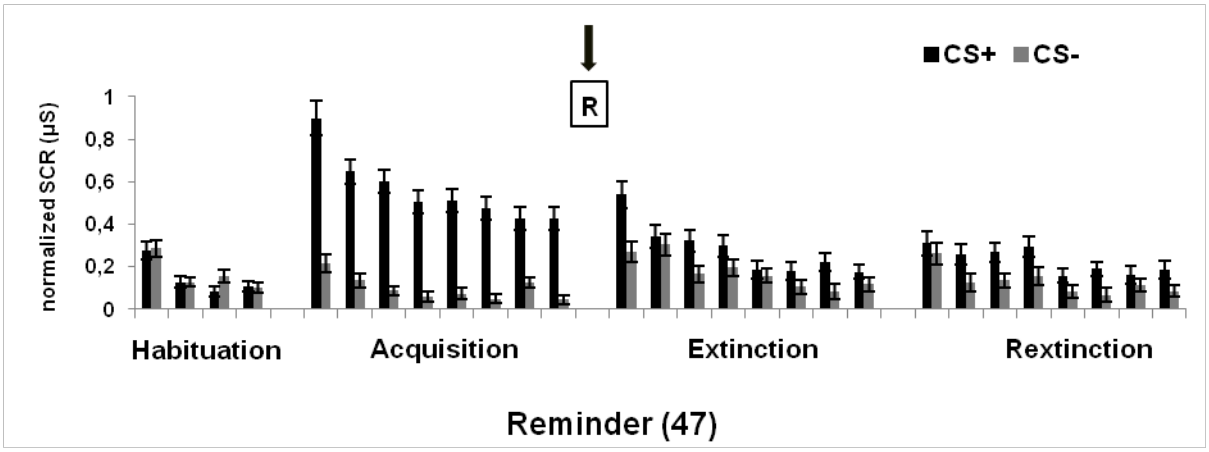
Table 7: Mean (S.D.) SCRs (CS+ and CS-) during habituation, acquisition, extinction and re-extinction for both groups (reminder and no-reminder) in Study II

	Reminder (47)		No-reminder (58)	
Mean SCR (S.D.)	**CS+**	**CS-**	**CS+**	**CS-**
Habituation	0.15 (0.15)	0.17 (0.13)	0.13 (0.12)	0.14 (0.13)
Acquisition	0.66 (0.36)	0.13 (0.11)	0.60 (0.20)	0.15 (0.12)
Extinction	0.21 (0.25)	0.10 (0.16)	0.16 (0.21)	0.12 (0.18)
Re-extinction	0.30 (0.33)	0.26 (0.36)	0.44 (0.43)	0.30 (0.32)

A post hoc t-test confirmed that participants had higher SCRs in response to the CS+ compared to the CS- during acquisition (trialblocks1-8; reminder: $t_{46} = 9.62$; $p < 0.01$; no-reminder: $t_{57} = 18.96$; $p < 0.01$). However, during extinction (trialblocks 7-8) the reminder group showed significant difference between the SCRs responses of CS+ and CS- ($t_{46} = 4.06$; $p < 0.01$), while the no-reminder group did not show significant difference between CS+ and CS- ($t_{57} = 1.46$; $p > 0.05$). Follow-up t-tests confirmed the reduction of differential response (CS+ vs. CS-) from the acquisition to the extinction phase in the reminder group ($t_{46} = 5.50$; $p < 0.01$) and the no-reminder group ($t_{57} = 6.65$; $p < 0.01$). There was no group difference for the differential values (CS+ vs. CS-) from the acquisition to the extinction phase (see figure 12).

Spontaneous recovery was assessed using a three-way ANOVA with main effects of time (extinction and re-extinction), stimuli (CS+ and CS-) and groups (reminder and no-reminder). This showed the significant main effect of stimuli ($F [2, 103] = 9.85$; $p < 0.01$), time ($F [2,103] = 38.08$; $p < 0.01$, and stimuli x time x group interaction ($F [2, 103] = 4.13$; $p < 0.05$). Follow-up t-tests were used to compare the reminder and no-reminder group with regard to the amount of attenuation between the extinction (day 2) and re-extinction (day 3) phase for the differential values between CS+ ($t_{103} = -2.38$; $p < 0.05$)

which differed significantly with lower values for the reminder group, while differential CS- (t_{103} = -0.253; p > 0.05) values did not differ significantly (see figure 13).



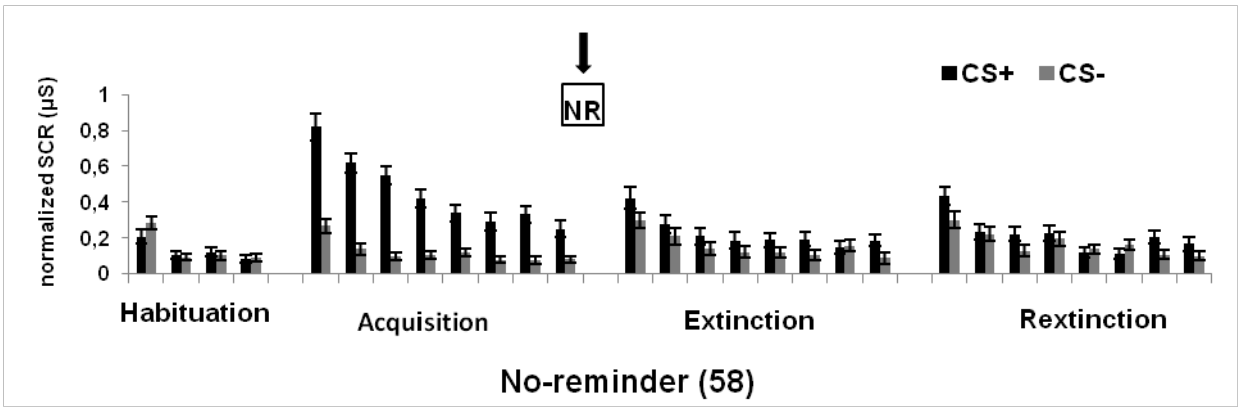


Figure 12: Mean range-corrected trialblocks (each trialblock is comprised of two-trials) plot for both reminder and no-reminder groups

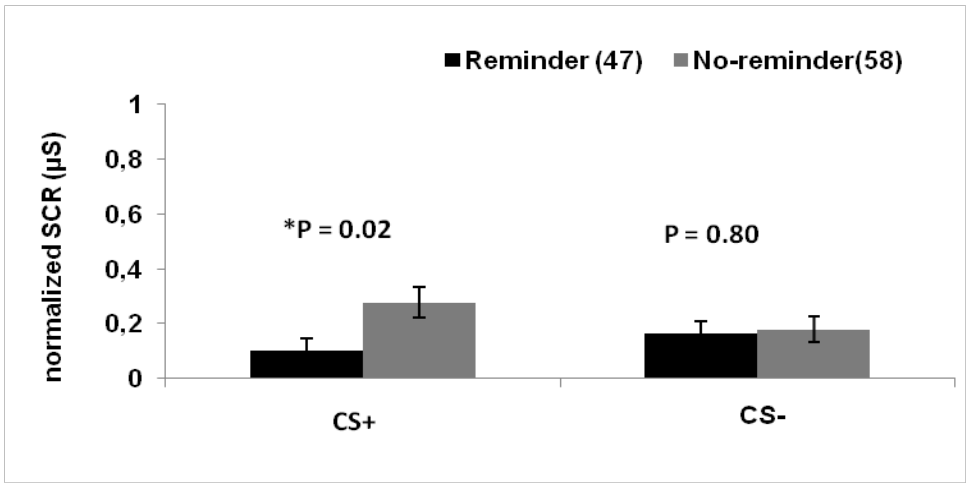


Figure 13: Plot shows differential SCRs mean CS+ (Re-extinction minus Extinction) and CS- (Re-extinction minus Extinction) for both groups. Note: n.s. denotes non-significant level and * denotes significant level at < 0.05

2.2.4 Discussion

Our hypothesis was to test whether a reminder (i.e., single CS) can make an old fear memory sensitive to restoration. Until now, the vast majority of studies have targeted the reconsolidation mechanisms (Agren, et al., 2012; Kindt, et al., 2009; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010). This makes it possible to study whether established memory becomes temporarily labile after its retrieval and becomes sensitive to disruption or modification (Agren, et al., 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010). After the retrieval or reactivation of a reinforced associative memory, the repetitive presentation of the conditioned stimuli without reinforcement results in a decrement in the conditioned response (CR), this is known as extinction (Myers & Davis, 2006).

Nevertheless, the fact remains that repetitive presentation of a non-reinforced conditioned stimulus decreases the CR. Results of the current study are similar to earlier findings (Agren, et al., 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010). When extinction training is followed by reactivation, it shows a decrement in CR as observed in the current study. Earlier studies have affirmed that a single brief exposure of non-reinforced conditioned stimulus (i.e., reminder) regulates the new learning (extinction or safety learning) (Agren, et al., 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010).

To investigate the proposed hypothesis we performed a simple auditory fear-conditioning paradigm and took SCRs and subjective rating scales as dependent measures. Different responses to the conditioned stimulus were seen for CS+ and CS-, which was recorded online and analysed offline. There was no SCRs response difference during the habituation phase, but a highly significant difference was observed between CS+ and CS- during the acquisition phase. This illustrates that participants learned about the association of CS-UCS. To investigate the spontaneous recovery we compared the SCRs response of the extinction process (Day 2) to the re-extinction process (Day 3). An arousal and valence property of the neutral stimulus changes after it becomes paired with an aversive stimulus. Hence, to measure the arousal and valence properties the 9-point Likert-scale was studied (see table 5).

The reminder trial triggered the CS-UCS associative learning in the individuals of the reminder group. This associative learning after a reminder may (Agren, et al., 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010) or may not (Chan, et al., 2010; Golkar, et al., 2012; Kindt & Soeter, 2011) be attenuated, followed by extinction. Extinction is a new form of learning which functions by changing the old associative learning (CS with UCS) with the new associative learning (CS without UCS) (Myers & Davis, 2006). This new learning has an uncertainty; and it may or may not suppress the old associative learning. It has been shown previously that the reminder of old associative learning leads to the

suppression of CRs and prevents fear recovery (Agren, et al., 2012; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010). In contrast, few studies have shown the reverse effects of reminder, i.e. augmentation of fear after successful extinction (Chan, et al., 2010; Golkar, et al., 2012; Kindt & Soeter, 2011). In the present study it has been shown that a reminder before the extinction resulted into an attenuation of fear and prevented fear recovery over time. This explains that the no-reminder group has good recall for CS+ stimulus on day 3, in contrast reminder group has poor recall for the CS+ after reminder of old associative learning followed by extinction and re-extinction.

Exposure therapy performed during the re-consolidation period can be effective in the suppression of fear and prevent fear recovery (Monfils, et al., 2009; Schiller, et al., 2010). Due to the lack of re-consolidation literature, and failure in targeting re-consolidation (Chan, et al., 2010; Golkar, et al., 2012; Kindt & Soeter, 2011), it becomes hard to explain the exact underlying mechanism for the attenuation or augmentation of fear return. Due to the equal success and failure rates in targeting re-consolidation. The questions: "What exactly is the re-consolidation phase and how does it work?" arise. In addition, the influence of a reminder on CS-UCS association during the extinction training is also an uncertainty. This raises a widely accepted idea by researchers, i.e. boundary conditions of memory (Tronson & Taylor, 2007).

Boundary conditions of memory are several factors, which play an important role in memory storage-reactivation-restoration. Factors such as memory strength, age of memory, and specificity of the CS are collectively known as boundary conditions of memory by researchers (Kwak, et al., 2012). It has been argued that strong memories are susceptible to disruption and do not undergo re-consolidation (Chan, et al., 2010; Golkar, et al., 2012; Kindt & Soeter, 2011). In the current study, we found that with 80% reinforcement level memory undergoes re-consolidation after retrieving fear compared to the no fear recall group. Results are similar to earlier findings with a high reinforcement CS-UCS association (Agren, et al., 2012). In addition, different brain faculties participate for processes other than the consolidation process after memory reactivation and its restoration (Agren, et al., 2012), suggesting that re-consolidation is a specific mechanism and a reason for disparity in earlier findings (Agren, et al., 2012; Kwak, et al., 2012).

Ample evidence affirms that amygdala is important in the fear formation. (Agren, et al., 2012) But when memory gets reactivated then amygdala only relays fear information irrespective to consolidation (Agren, et al., 2012; Diaz-Mataix, et al., 2011; Doyere, et al., 2007) Furthermore, duration of reactivation also plays a critical role in the reconsolidation of memory to make old memory sensitive to inhibition of protein synthesis (Kaang & Choi, 2011; Kaang, Lee, & Kim, 2009; Kwak, et al., 2012; Lee, Gardner, Butler, & Everitt, 2009). In our opinion we argue that the combined effect of both CS-UCS

association and the nature of the reminder might be effective in making strong memories undergo re-consolidation. Another reason for discrepancy in the results might also be due to the procedural differences in the previous findings such as usage of fear in relevant and irrelevant stimuli compared with geometrical figures. (Agren, et al., 2012; Chan, et al., 2010; Golkar, et al., 2012; Kindt & Soeter, 2011; Monfils, et al., 2009; Oyarzun, et al., 2012; Schiller, et al., 2010) In addition, different indexes were used to measure CR as an index for fear such as SCR and startle (Golkar, et al., 2012; Kindt & Soeter, 2011; Oyarzun, et al., 2012; Schiller, et al., 2010). In the present study we have used geometric figure as CS and SCR as a CR index.

Due to the equal success and failure rate in targeting re-consolidation, it would be hard to comment on which experimental conditions are needed to successfully target re-consolidation. From the current study, we suggest that it is possible to target re-consolidation and that strong memories also undergo re-consolidation. We also recommend that there is a need to conduct some more behavioral and translational research studies. And, we hope that future studies might provide a clear understanding about the reactivation of memory and its reconsolidation process under clinical set-up.

STUDY III: BDNF POLYMORPHISM ALTERS RECONSOLIDATION AND PREVENTS FEAR RECOVERY

2.3.1 Introduction

The current study investigated the effects of genetic polymorphism in the alteration of memory reconsolidation to prevent fear recovery. Recently, there has been a resurgence of interest in reactivation and performing extinction within the reconsolidation period (Agren, et al., 2012; Oyarzún, et al., 2012; Schiller, et al., 2010). This procedure has been effective in the prevention of fear return. Agren and colleagues (2012) demonstrated that the reactivation followed by extinction is modulated by genetic variants such as *5-HTT* and *COMT*. Animal and human studies have documented well that genetic factors are responsible for excessive fear and gave an understanding of the genetic basis of anxiety and related disorders (Gordon & Hen, 2004). Recent memory research has highlighted the importance of reconsolidation in the treatment of pathological memories. Reconsolidation occurs with the retrieval of consolidated memory and is important for the maintenance of memory (Tronson & Taylor, 2007). Another line of memory research has shown the role of LTP in learning and memory. It is not surprising that various genes have been considered to promote conditions which influence memory processes. So, far a number of animal and human studies have revealed that BDNF is involved in the LTP process (Monfils, et al., 2007). It also plays an important role in the maintenance of memory (Bekinschtein, et al., 2007; Bekinschtein, et al., 2008; Lee, Everitt, & Thomas, 2004). This developed our interest to investigate the role of the *BDNF* Val66Met polymorphism in the alteration of reconsolidation. The role of BDNF in fear processing has already been affirmed (Lonsdorf & Kalisch, 2011). We would like to hypothesize that the BDNF variation is also important for the reconsolidation process.

2.3.2 Methods

2.3.2.1 Participants

All 143 healthy participants (78 female, 65 male: ages 18-31 years) from Study II were considered for the investigation of the BDNF Val66Met polymorphism. After the data analysis (see below), 32 participants were excluded due to artefacts (i.e. origin of response to stimuli before a baseline) and regarded

as null-responses of the conditioned responses. Four participants refused to continue the study on the following experimental days. Sixteen participants either did not give blood or their samples were not genotyped correctly. The demo-graphical data of the remaining 91 participants is shown below in Table 8. The study was in accordance with the declaration of Helsinki in their latest version from 2008 and has been approved by the Ethics Committee of the University of Würzburg. All participants gave written informed consent to participate in the study and for the blood samples.

Table 8: Demographics of participants included in Study II

	Total	Val-Met & Met-Met Carrier (AG & GG)	Val-Val Carrier (GG)
Nr. of Participants	91	37	54
Age	24.15 ± 2.92	24.81± 3.45	23.70 ± 2.42
Female	42	18	24
Male	49	19	30
Reminder	41	14	27
No-reminder	50	23	27

2.3.2.2 Genotyping

DNA was extracted from buccal cells or EDTA blood using an advanced method (Mössner, et al., 2005; Sen, et al., 2003) Genotyping of the BDNF Val66Met polymorphism was performed with a 274-bp polymerase chain reaction (PCR) product containing the SNP rs6265. The forward and reverse primers and the PCR method for BDNF *Val66Met* are as follows: Forward primer: 5'-AAA GAA GCA AAC ATC CGA GGA CAA G and Reverse primer: 5'-ATT CCT CCA GCA GAA AGA GAA GAG G. PCR ran a total of 35 cycles. Denaturing at 95°C for 30 s, annealing at 55°C for 40 s, and extension at 72°C for 50 s were performed, followed by a final extension at 72°C for 5 min. After digestion, PCR gave the undigested product carrying A-variant and the digested product G-variant having three fragments of 57. 77, and 140 bp (Hunnerkopf, Strobel, Gutknecht, Brocke, & Lesch, 2007). The 123 participants were genotyped as follows: 7 participants were found to be homozygous for the *Met allele (Met66Met)*, 38 were *Val66Met* heterozygotes, and 78 were homozygous for the *Val allele (Val66Val)* (see table 9 a, b). Due to the low frequency of met66met compared to val66val and val66met, val66met and met66met (n = 45) were added together similar as in earlier works (Hajcak, et al., 2009; Lonsdorf, et al., 2010; Torrents-Rodas, et al., 2012).

2.3.2.3 Procedure

The experimental study was a part of Study II, hence there were no changes made in the experimental procedure. However, all participants were asked to give a blood sample on day 1, 2, 3 or 4 as convenient for them. For the blood sample all participants were paid 8€ as compensation and some who were afraid of blood were asked to give saliva. The blood samples were given for the DNA extraction and later the genotype data was correlated with the SCR data recording.

2.3.2.4 Statistical Analysis

This study was a part of Study II, hence there were no changes made in the statistical analysis. The blood samples were collected from all participants and were genotyped for this study. All participants, whose blood samples were genotyped and then gave the correct SCR response, were recruited for the study.

2.3.3 Results

2.3.3.1 Genotyping

The genotype frequencies were in Hardy-Weinberg equilibrium ($chi^2 = 0.66$, $df = 1$, n.s.): Val66Val = 78, Val66Met = 43, and Met66Met = 7.

2.3.3.2 Self-report Ratings

Table 9: Mean (S.D.) self-report (valence and arousal) ratings of CS+ and CS- during habituation, acquisition, extinction and re-extinction for both groups (reminder and no-reminder) in Study II

	Arousal				Valence				
	Reminder (47)	**No-reminder (58)**	**t-value**	**P-value**	**Reminder (47)**	**No-reminder (58)**	**t-value**	**P-value**	**df**
Habituation	-0.90 (2.37)	-0.38 (1.99)	-1.14	0.26	0.68 (2.96)	0.52 (2.84)	0.27	0.79	89
Acquisition	-4.85 (2.10)	-4.76 (1.84)	-0.23	0.82	5.49 (2.06)	5.48 (2.19)	0.02	0.98	89
Extinction	-2.63 (2.37)	-1.60 (2.30)	-2.10	0.04	2.71 (2.57)	1.74 (2.62)	1.77	0.80	89
Re-extinction	-2.02 (2.31)	-1.42 (1.98)	-1.34	0.19	1.83 (2.33)	1.46 (2.20)	0.78	0.44	89

2.3.3.3 Questionnaire Ratings

Table 10: Summary of t-test of the reported anxiety sensitivity, fear questionnaire, PSWQ, PANAS, ADSk, STAI, BISBAS for the all participants selection in study II between groups (reminder and no-reminder)

	Group		t-value	P-value	df
	Reminder (41)	No-reminder (50)			
	Mean (S.D.)	Mean (S.D.)			
FQ	1.34 (0.74)	1.23 (0.73)	0.66	0.51	89
PANAS	2.46 (0.35)	2.39 (0.37)	0.93	0.36	89
PSWQ	2.34 (0.38)	2.15 (0.45)	2.05	0.04	89
STAI	1.0 (0.48)	0.82 (0.54)	1.61	0.11	89
ADSk	0.75 (0.26)	0.73 (0.31)	0.51	0.61	89
BISBAS	1.98 (0.22)	1.90 (0.23)	1.79	0.08	89
ASI3	1.0 (0.48)	0.82 (0.54)	1.61	0.11	89

2.3.3.4 Skin Conductance Response (SCR) Recording

To investigate the role of the gene on fear acquisition along with group interaction, 4-way ANOVA revealed a significant interactive effect of stimuli ($F [4, 87] = 136.38$; $p < 0.01$), time ($F [4, 87] = 105.91$; $p < 0.01$), stimuli x time ($F [4, 87] = 193.91$; $p < 0.01$), stimuli x group ($F [4, 87] = 4.44$; $p < 0.05$), stimuli x gene ($F [4, 87] = 3.85$; $p < 0.05$), stimuli x time x group ($F [4, 87] = 6.22$; $p < 0.05$), but no significant interaction for stimuli x time x group x gene ($F [4, 87] = 237.38$; $p < 0.01$). Further, Two-way ANOVA was designed with the main effects: Time (habituation and acquisition), stimuli (CS+ and CS-) and group (reminder and no-reminder). This showed the significant main effect of stimuli ($F [2, 89] = 131.89$; $p < 0.01$), a stimuli x time ($F [2, 89] = 204.40$; $p < 0.01$) and stimuli x time x group interaction ($F [2, 89] = 5.61$; $p < 0.05$). A post-hoc t-test was conducted to understand the interaction between stimuli x time x group, CS+ values differed significantly between the two groups during acquisition ($t_{89} = 2.13$; $p < 0.05$) but not during habituation and also CS- values did not change for both groups from habituation to acquisition phase.

Prior analysis revealed that CS+ values differed significantly between the groups during fear learning. Hence ANOVA (2 x 8 x 2) was designed to investigate the difference in the CS value between the two groups during the Acquisition phase. This showed the significant main effect of stimuli ($F [2, 89] = 219.87$; $p < 0.01$), time ($F [2, 83] = 14.96$; $p < 0.01$), and an interaction effect stimuli x group ($F [2, 89] = 5.85$; $p < 0.05$), no stimuli x time x group interaction was observed. We are unable to suggest why two groups differed during fear learning in the early phase but did not show significant interaction,

however a sudden decline was observed in the no-reminder group at the end of fear learning, perhaps this could be the reason for stimuli x time x group significant interaction.

Table 11: Mean (S.D.) SCRs during habituation, acquisition, extinction and re-extinction for both groups (reminder and no-reminder)

	Reminder (41)		No-reminder (50)	
Mean SCR (S.D.)	**CS+**	**CS-**	**CS+**	**CS-**
Habituation	0.16 (0.15)	0.17 (0.13)	0.13 (0.13)	0.14 (0.13)
Acquisition	0.55 (0.33)	0.10 (0.08)	0.44 (0.15)	0.12 (0.09)
Extinction	0.21 (0.26)	0.09 (0.17)	0.15 (0.19)	0.11 (0.16)
Re-extinction	0.33 (0.34)	0.25 (0.37)	0.44 (0.44)	0.32 (0.33)

The effect of a reminder on extinction between the groups from the acquisition phase to the extinction phase was assessed using the three-way ANOVA main effects: Time (acquisition and extinction), stimuli (CS+ and CS-) and group (reminder and no-reminder). This showed the significant main effect of stimuli ($F [4, 87] = 196.68$; $p < 0.01$), a stimuli x time ($F [4, 87] = 78.06$; $p < 0.01$) and stimuli x group interaction ($F [4, 87] = 12.81$; $p < 0.01$) but no effect of the group or interaction. A post hoc t-test confirmed that participants had higher SCRs in responses to the CS+ compared to the CS- during acquisition (trialblocks1-8; reminder: $t_{40} = 8.41$; $p < 0.01$; no-reminder: $t_{49} = 18.36$; $p < 0.01$). The 3-Way ANOVA was designed to investigate the group (reminder vs. no-reminder) × BDNF (*Met vs. Val*) for range corrected Δ SCRs (CS+ minus CS-) during extinction and re-extinction. Results show the main effects of time x group ($F [1, 87] = 3.97$; $p < 0.05$) and time x group x BDNF ($F [1, 87] = 4.74$; $p < 0.05$) (see figure 14).

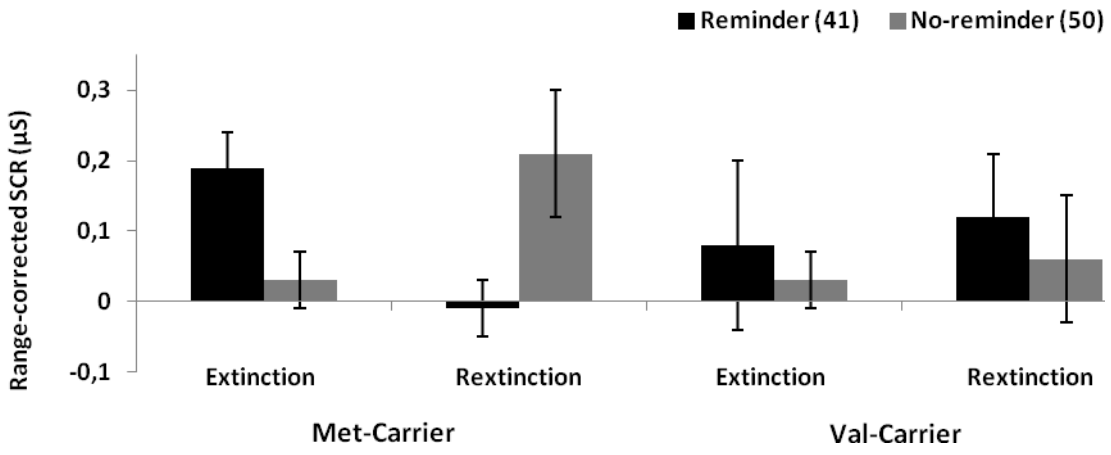


Figure 14: Plot shows differential SCRs means (CS+ minus CS-) during extinction and re-extinction for both groups and explicitly for Met (Met66Met and Val66Met) and Val/Val (Val66Val)

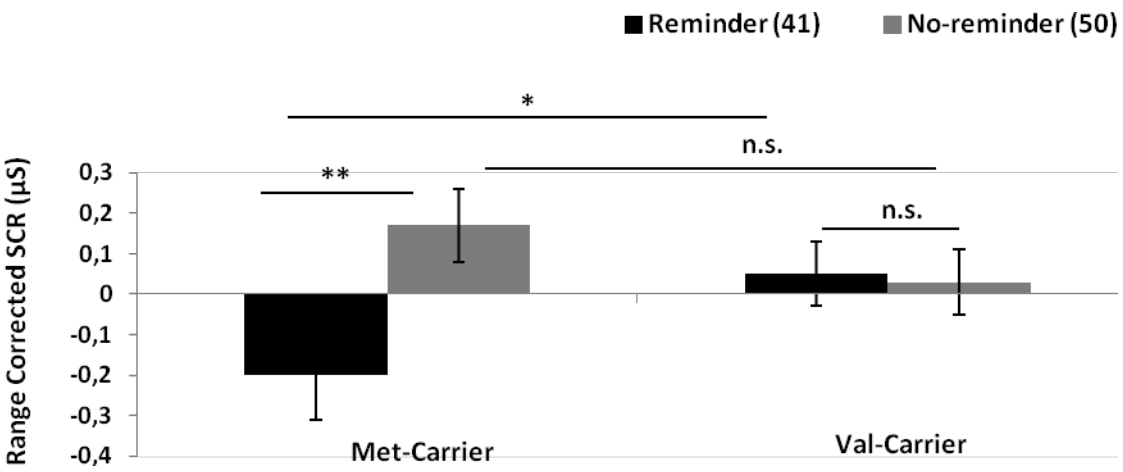


Figure 15: Plot shows differentials (CS+ minus CS-) SCRs mean (Re-extinction minus Extinction) for both groups and for Met (Met66Met and Val66Met) and Val/Val (Val66Val). Note: n.s. denotes non-significant level and asterisks denotes significant differences, *$p < 0.05$, **$p < 0.01$

For further clarity we performed another 2-way ANOVA with the main effects: Group (reminder vs. no-reminder) x gene (*Met vs. Val*)] for Δ SCRs (Day3 minus Day2). This showed the significant main effect of group (F [1, 87] = 3.97; p < 0.05) and group x gene (F [1, 87] = 4.74; p = 0.03) (see figure 15). Follow-up t-tests were used to compare the reminder and no-reminder group with regard to the amount of attenuation between Met vs. Val BDNF carriers for range corrected Δ SCRs (CS+ minus CS-) during extinction and re-extinction phase for the differential values between Met-carrier (t_{35} = -3.46; p < 0.001) which differed significantly with lower values for the reminder group, while differential Val-carrier (t_{52} = 0.13; p > 0.05) values did not differ significantly. Furthermore, Met vs. Val carrier differed significantly in the reminder group (t_{39} = -2.35; p < 0.01), while Met vs. Val carrier values did not differ significantly (t_{48} = 1.06; p > 0.05)

2.3.4 Discussion

In the present study, we did not find the effect of the *BDNF* gene variant on fear learning as shown previously (Lonsdorf, et al., 2010; Soliman, et al., 2010). In addition, we also did not observe a significant effect of the *BDNF* gene on extinction. However, we showed the significant effect of *BDNF* gene in the blockage of fear return via a targeting reconsolidation mechanism. Specifically, Met-carriers showed a significant effect compared to Val-carriers between and within the groups (Figure 15). Results suggest reminder modulated the *BDNF* Met carrier, i.e. fear memory return was prevented via reconsolidation in the reminder group. There was no significant effect observed in reconsolidation for the reminder in *BDNF* Val-carrier.

As it has been well documented in earlier findings, Met-carriers of *BDNF* have shown impaired fear extinction (Soliman, et al., 2010). The Met-carrier of *BDNF* is supposed to be the prime suspect for the anxiety or memory dysfunction (Hajcak, et al., 2009; Lonsdorf, et al., 2010). Solimon and colleagues (2010) designed a study to test the efficacy of the *BDNF* gene in extinction training in both human and animal models. In their study, they demonstrated that Met-carriers of both humans and animals failed to extinguish fear. They successfully showed the effects of *BDNF* on extinction: Met-carriers showed higher fear responses for both a threatening and neutral cue. This finding suggested that Met-carriers show dysfunction in neural circuitry; they also reported that the *BDNF* gene effect on fear acquisition was non-significant.

Our results did not replicate the previous findings of Lonsdorf and co-workers (2010). They showed the significant *BDNF* gene effect in fear acquisition using aversive differential fear-conditioning. Their finding suggested the dysfunctioning of the amygdala-dependent fear-conditioning in *BDNF* Met-carriers, also suggesting an important role of BDNF signaling in amygdala-dependent learning. Similarly, Hajack and colleagues (2009) reported the potentiation of a startle response for CS+ in *BDNF* No-met (i.e., Val-carriers) compared to Met-carriers. They demonstrated that Met-carriers show a specific deficit of fear learning. In our study, we did not observe such effect during fear acquisition and extinction. However, after fear retrieval followed by reconsolidation we found deficit behavior in Met-carriers in the reminder group compared to the no-reminder group. In addition, Met-carriers differed significantly from the Non-Met (Val)-carrier) in the reminder group. According to our knowledge, the reason for such discrepancy might be due to methodological differences. The current study used the geometric figures as a CS stimulus, which is similar to Torents-Rodez *et al*. (2012) while it is different from Lonsdorf *et al*. (2010), who used emotional stimuli as a CS. It is also noteworthy that the earlier evidence showed the genetic differences with the startle response not with the SCR (Hajcak, et al., 2009; Lonsdorf, et al., 2010). Results in the current study are similar to the earlier findings of Hijack (2009) & Lonsdorf (2010) with SCR.

Gene knockout studies have revealed the regulatory behavior of BDNF in synaptic plasticity (Kaplan, 2010). Liu and co-worker (2004) showed that a low-level of BDNF in the hippocampus leads to a learning deficit in animals. Poor learning was observed in contextual fear-conditioning suggesting that hippocampus-dependent learning requires normal levels of BDNF expression. In addition, contextual fear learning is amygdala and hippocampus dependent. Furthermore, it has been shown that animals with re-exposure of the fear context show decreased hippocampal BDNF mRNA expression (Rasmusson, Shi, & Duman, 2002). As Met-carrier dysfunctioning is reported in literature we hypothesize that re-exposure of the fear context reduces hippocampal BDNF mRNA expression, especially in Met-carriers, as observed in the current study.

Rasmusson and co-workers (2002) demonstrated in their study the down-regulation of BDNF mRNA in the dentate gyrus of hippocampus in rats. They argued that reduction of BDNF mRNA in dentate gyrus depends upon several factors such as: (i) intensity, (ii) duration, and (iii) type of stress exposure. They explained that exposure of stressful cues, i.e. UCS or cues associated with stressful situations regulates the level of BDNF mRNA down and reaches a normal level in 48-hours. The association between down-regulation of BDNF in dentate gyrus hints the influence of BDNF in the consolidation and maintenance of memory.

Another line of animal studies suggests that BDNF is required for the formation and maintenance of memory (Mahan & Ressler, 2012; Monfils, et al., 2007). BDNF up-regulation after 12 hours from learning is necessary for the memory and for its maintenance. This confirms that after memory reactivation BDNF is involved in the reconsolidation. It is required for the maintenance and formation of memory, but not for the consolidation of memory (Bekinschtein, et al., 2007). According to memory, consolidation hypothesis upon reactivation transforms stable memory into an unstable form. This unstable memory requires protein synthesis for formation as shown by earlier studies. In memory research, this protein-synthesis dependent memory process is known as reconsolidation. Reconsolidation is an update mechanism of memory processes (Dudai, 2004; Hardt, et al., 2010; Nader, et al., 2000; Sara, 2000). It is responsible for the maintenance of memory after reactivation. From genetic studies we receive an insight that similarly the *BDNF* polymorphism is involved in the maintenance of memory (Mahan & Ressler, 2012; Monfils, et al., 2007). Hence, we assume that reconsolidation and BDNF might share some similar cellular and molecular process and that their combined effect is responsible for the maintenance of memory after its reactivation.

To our knowledge, this is the first human study showing the importance of *BDNF polymorphism* in reconsolidation of fear memory. Due to the small sample size, the results are on the weak side. But this hints the role of *BDNF* in reconsolidation. This initial finding of reconsolidation and *BDNF* needs further attention in the future. Therefore we propose some more replication studies are needed to have a clear understanding about *BDNF* variants in reconsolidation of fear memory.

CHAPTER 3

BRIEF DISCUSSION AND CONCLUSION

The general aim of this thesis was to modulate the fear memory consolidation, reconsolidation, and extinction processes using various experimental methodologies such as behavioral, electrical stimulation, and genetics. Traditional pavlovian fear-conditioning has offered us a robust methodology to study anxiety and related disorders. Advancement in neuroscience has provided us with deep knowledge about the behavioral, cellular, and molecular mechanism of fear learning and its extinction. Over the past several decades several translational researches have documented the mechanisms involved in anxiety disorders from fear formation-to-consolidation-to-extinction-to-retrieval-to-reconsolidation. Neuroimaging data combined with animal studies have revealed the importance of the amygdala, hippocampus, and frontal cortex in the processes such as fear acquisition, extinction, and reconsolidation.

In fear-conditioning a neutral stimulus attains threatening a value and elicits a fear response via associative learning. If nothing has changed then this associative learning persists throughout life. In order to erase this associative fear learning trace pavlovian extinction has been performed. Pavlovian extinction is a process in which CS has been presented repeatedly in absence of UCS as a result fear response is attenuated. It follows the principle of the exposure-based method. However, the attenuation of fear response with extinction is not permanent and fear return has been observed, for e.g. renewal, reinstatement, and spontaneous recovery. Hence, a method is required which can block fear from returning. Pharmacological methods have shown some good results, but their effect on humans is unpredictable. Hence, a drug-free method is required for the treatment of anxiety or related disorders, i.e. pathological memories.

Study I describes the effects of weak electrical cranial stimulation on consolidation of fear memory. Early 1960s studies have highlighted the influence of ECS in modulation of memory processes. Recent research with tDCS suggested that it modulates memory processes and promotes the conditions required for consolidation. Earlier studies provide evidence that tDCS has either an excitatory or inhibitory effect depending upon the type of stimulation. It has been extensively shown that anodal tDCS

enhances learning and memory processes while cathodal tDCS has contrasting results. Although the exact mechanism is still unclear, it has been suggested that anodal tDCS depolarizes the cortical excitability and cathodal tDCS hyperpolarizes cortical excitability. To our knowledge it has now been shown for the first time that tDCS has potential to modulate non-declarative memory, i.e. fear associative memory. Results align with the earlier findings and show the inhibitory effect of cathodal tDCS on fear consolidation. We did not find the enhancing effect of anodal tDCS on fear consolidation (Asthana, et al., under review).

Study II was the replication with some modification of Schiller *et al.* (2010) published in Nature. The idea was to test the reconsolidation process in an auditory fear-conditioning paradigm. It has been well documented that memory upon retrieval becomes labile and is susceptible to disruption or modification. Recently, Schiller and colleagues (2010) demonstrated that retrieval followed by extinction training performed within reconsolidation period (i.e. 10 minutes after memory retrieval) prevents fear from returning. We also observed the similar effect in the auditory conditioning paradigm. Our results were similar to earlier findings and observed the attenuation of fear memory. With the findings of the first study we suggest that reactivation of memory followed by extinction training within the reconsolidation period shows a potential in the treatment of pathological memories. However, due to some failure studies in targeting reconsolidation it is important to have some more replication before one plans to implement this new idea in a clinical set-up(Asthana, et al., under preperation).

Study III reports association of BDNF genetic polymorphism on reactivation and reconsolidation of fear memory in an auditory fear-conditioning paradigm. Recently, Agren and co-workers (2012) demonstrated that genetic polymorphism plays a role in the retrieval and reconsolidation of fear memory in humans. Like earlier studies we did not observe any significant allele difference, for e.g. *Met vs. Val* BDNF carriers during acquisition phase. In addition, we also did not find the allelic differences during extinction. However, we found the significant effect of BDNF *Met-carriers* in reconsolidation. 4-way ANOVA revealed a significant difference for the BDNF genes (*Met vs. Val*) and groups (Reminder vs. No-reminder). The study reports an important role of BDNF in reconsolidation of memory (Asthana, et al., under preperation).

Results from **Study I** conclude that the tDCS effect on non-declarative memory is a boost in the declarative memory. This thesis reported the inhibitory effect of cathodal tDCS on fear memory consolidation. However, it is important to consider that the current finding reveals that the cathodal tDCS over the left DLPFC influences fear memory consolidation. This suggests an importance of the left DLPFC in fear consolidation. We know that the frontal cortex has a dense connection with the hippocampus and amygdala, which is important for fear expression and suppression. However, earlier tDCS studies have

shown involvement of other brain regions in the memory consolidation process. It would be hard to comment what will happen if the right DLPFC is stimulated. In addition, one may also wonder whether tDCS influenced the consolidation or the associative learning process, i.e. CS-UCS value. Some more replication are needed to fully understand the effects of tDCS over the left DLPFC in fear consolidation, furthermore different brain regions should also be considered in the future.

Study II concludes the efficacy of a reminder in the prevention of fear return. It offers a way in the treatment of pathological memories; it now is a well-established fact that information provided within the reconsolidation window gets integrated into an old memory or even erases it. However, there are some discrepancies in recent years regarding the reconsolidation. Some studies failed to target reconsolidation as a result some crucial questions have been raised: "Whether memory really undergoes reconsolidation upon retrieval? Does all memory undergo reconsolidation? Does post-retrieval extinction induce new learning and not reactivate memory?" Reasons for such discrepancies might be methodological differences. In addition, boundary conditions of memory are also a deciding factor whether memory will undergo reconsolidation after post-retrieval or not. Boundary conditions of memory discuss the nature of memory, age of memory, and strength of memory. So far, the research studies have provided an insight into molecular and cellular mechanism of memory after retrieval. Moreover, this understanding will help us reveal why some memories undergo reconsolidation while others do not. Findings of this thesis might help and support the understanding of the reminder effect in the prevention of fear return.

Study III concludes the role of genetic variant BDNF in retrieval followed by reconsolidation, furthermore suggesting that the met-carrier prevents fear return in the reminder group compared to the no-reminder group. Results described in the thesis report are the second study showing the allelic differences in memory reconsolidation. Not many studies have been reported so far observing the effects of genetic variants in memory reconsolidation. We have gained depth in our understanding about reconsolidation of memory at cellular and molecular level. Nevertheless, in the future some more studies are required in order to understand why only a few individuals are able to overcome the fear and why only few individuals develop anxiety or related disorders? The significant finding of BDNF in reconsolidation suggests its modulatory and its potential effect in the treatment of pathological memories. However, due to the low number of participants in this report some more replication targeting reconsolidation is needed for clarity.

In Closing,

Firstly, the work provided in this thesis supplies evidence for memory modulation of fear consolidation using tDCS, suggesting the future application of tDCS in the treatment of pathological memories. Secondly, it has been demonstrated successfully in the thesis report that the reactivation followed by extinction training within the reconsolidation period blocks fear return. Reconsolidation processes offer an effective drug-free paradigm in the treatment of anxiety and related disorders. The only concern now is whether similar findings are achieved within the clinical population. Finally, the work included in this thesis provides evidence for BDNF *Val66Met* polymorphisms. We provide a possible explanation for the association of BDNF with the reconsolidation process. This finding will lay the foundation for the investigation of genetic variants and future studies.

CHAPTER 4

PERSPECTIVE

Pavlovian conditioning is a simple learning about the CS-UCS association. Decades of research using conditioning paradigm have given a deep insight about the neurobiological pathways of fear, fear learning and extinction. It has helped researchers to develop an effective paradigm in the treatment of anxiety and related disorders. In the recent past, researchers have tried to use several methodologies such as behavioral, electrical or magnetic stimulation, and pharmacological methods to modulate fear learning. Moreover, several treatment methodologies have been implemented to cure fear learning and to prevent its relapse.

Despite our deep knowledge about anxiety disorders, we are still not able to answer why some individuals acquire PTSD and others do not. Why some are more conditioned than others? Why some are good in fear acquisition or in extinction learning? Studies on fear-conditioning have tried to answer these questions over the past one century. In the recent past, fear-conditioning studies have moved from consolidation to extinction and reconsolidation processes. Studies targeting reconsolidation have broadened our understanding about the retrieval and maintenance of memory processes. Reconsolidation studies have also demonstrated a drug-free paradigm under laboratory conditions in the treatment of anxiety disorders. Future studies should be focused to understand memory reactivation and reconsolidation under normal conditions and further its application on patients. Such studies will broaden our perspective and might offer us an effective methodology in the treatment of anxiety disorders.

In recent years, electrical or magnetic stimulation studies have gained much attention because they offer a drug free safe paradigm. It has been reported that mostly psychiatric disorders are due to the dysfunctioning of the neural circuit. In addition, electrical or magnetic stimulation has shown its potential in influencing the neural circuitry; it might enhance or inhibit cortical excitability of brain regions depending upon experimental factors. In the near future, it would be important if electrical or magnetic stimulation studies would be given preference in the treatment of anxiety disorders. Investigation of consolidation, extinction, and reconsolidation processes with electrical or magnetic stimula-

tion will enhance our understanding in the treatment of anxiety disorders. Electrical or magnetic stimulation studies might also help us understand why some individuals are more prone to anxiety disorders compared to others. Stimulation studies show great potential for future research in the field of anxiety disorders.

Furthermore, advances in the genetic studies have helped us understand gene-gene interaction and gene-environment interaction in psychiatric disorders especially in anxiety disorders. Genetic studies provide evidence for the individualistic differences; and specific mechanisms involved behind anxiety disorders. It is helping researchers from the past several decades to develop an effective treatment methodology for anxiety disorders. Future studies need to investigate why some individuals are sensitive to develop PTSD in comparison to others? In addition, translational studies in this area are required because such studies will help researchers understand the connection between associative learning, neural network dysfunctioning, and individualistic differences.

Finally, findings of this thesis should be stretched into the domain of neuroimaging methods. Such studies may provide a clear picture about the understanding of associative learning and stimulation; associative learning and genes; associative learning, neural-network and genes.

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AFFIDAVIT / EIDESSTATTLICHE ERKLÄRUNG

Here I declare that my doctoral thesis entitled "***Associative learning – Genetic modulation of extinction and reconsolidation and the effects of transcranial Direct Current Stimulation (tDCS) / Assoziatives Lernen - Genetische Modulation der Auslöschung und Rückverfestigung und die Auswirkungen der transkraniellen Gleichstromstimulation (tDCS)***" has been written independently with no other sources and aids than quoted.

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