THE LONG-LASTING EFFECTS OF EXTINCTION DURING THE RECONSOLIDATION PROCESS OF FEAR MEMORY

EZGİ GÜR

MAY 2014

THE LONG-LASTING EFFECTS OF EXTINCTION DURING THE RECONSOLIDATION PROCESS OF FEAR MEMORY

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF SOCIAL SCIENCES
OF
IZMIR UNIVERSITY OF ECONOMICS

BY

EZGİ GÜR

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE
IN
THE GRADUATE SCHOOL OF SOCIAL SCIENCES

MAY 2014

Approval of the Graduate School of Social Sciences

Prof. Dr. Cengiz Erol

Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof. Dr. Hakan Çetinkaya

Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Assoc. Prof. Dr. Seda Dural

Supervisor

Examining Committee Members

Prof. Dr. Hakan Çetinkaya

Assoc. Prof. Dr. Seda Dural

Asst. Prof. Dr. Zübeyit Gün

ABSTRACT

THE LONG-LASTING EFFECTS OF EXTINCTION DURING THE RECONSOLIDATION PROCESS OF FEAR MEMORY

Gur, Ezgi

MS in Experimental Psychology, Graduate School of Social Sciences

Supervisor: Assoc. Prof. Seda Dural

May 2014, 122 pages

In the present study, we have examined the time dependent and long-term effects of extinction carried out during the reconsolidation process of fear memories. The reconsolidation update procedure developed by Schiller, et al. (2010) in a three-phase experiment (acquisition, extinction, and re-extinction of fear) was followed to see the time-dependent effects of the reconsolidation update on preventing fears; additionally, a one-year follow-up study was conducted to observe the long-term effects. Extinction training was given to the participants 10 minutes after the reminder (within the reconsolidation window), 6 hours after the reminder (outside of the reconsolidation window), and without reminder (standard extinction). The spontaneous recovery of fear reactions were tested 24 hours, 15 days or 3 months after the extinction. A 3 (Extinction: 10 minutes and 6 hours after the reminder and no reminder) x 3 (Re-extinction: 24 hours, 15 days, and 3 months after the extinction) between-groups design was used in the study. Skin conductance response of the participants was recorded as a measure of fear reactions in each phase. The results

revealed that when extinction training was given within the reconsolidation window, spontaneous recovery of the fear responses was significantly lower as compared to the extinction training outside of the reconsolidation window and standard extinction training independent of the re-extinction manipulation. However, in the one-year follow-up, long-term effects of extinction during the fear memory reconsolidation was not found to be significant.

Keywords: fear memory, reconsolidation process, fear conditioning, extinction, spontaneous recovery

ÖZET

KORKU BELLEĞİNİN YENİDEN BÜTÜNLEŞTİRME SÜRECİNDE UYGULANAN SÖNME İŞLEMİNİN UZUN SÜRELİ ETKİLERİ

Gur, Ezgi

Deneysel Psikoloji Yüksek Lisans, Sosyal Bilimler Enstitüsü

Tez Danışmanı: Doç. Dr. Seda Dural

Mayıs 2014, 122 sayfa

Bu tezde, korku belleğinin yeniden-bütünleştirme sürecine uygulanan sönme işleminin zamana bağlı ve uzun süreli etkileri incelenmiştir. Schiller ve arkadaşları (2010) tarafından geliştirilen ve üç aşamadan oluşan (edinim, sönme, yeniden sönme) yeniden-bütünleştirme güncelleme paradigması, söz konusu işlem yolunun korku tepkilerinin önlenmesindeki zamana bağlı etkilerini incelemek üzere kullanılmış, ayrıca uzun süreli etkilerin incelenmesi için sönme işleminden bir yıl sonra bir takip çalışması yürütülmüştür. Sönme işlemi katılımcılara hatırlatıcı sunumundan 10 dakika sonra (yeniden-bütünleştirme penceresi içinde), hatırlatıcı sunumundan 6 saat sonra (yeniden-bütünleştirme penceresi dışında) ve hatırlatıcı sunumu olmaksızın (standart sönme işlemi) uygulanmıştır. Korku tepkilerinin kendiliğinden geri gelmesi ise sönme işleminden 24 saat sonra, 15 gün sonra ve 3 ay sonra olmak üzere üç ayrı düzeyde manipüle edilmiştir. Çalışmada gruplar arası karşılaştırmaları gerçekleştirmek üzere 3 (Sönme: Hatırlatıcıdan 10 dakika ve 6 saat sonra ve hatırlatıcı sunumu olmaksızın) x 3 (Yeniden sönme: sönme işleminden 24 saat, 15

gün, 3 ay sonra) denekler arası desen kullanılmıştır. Katılımcıların deri iletkenliği tepkisi aracılığıyla ölçülen korku tepkileri her aşama için kaydedilmiştir. Çalışmanın sonuçları, yeniden sönme süreci dışında ve hatırlatıcı uyarıcı sunumu olmaksızın uygulanan sönme işlemlerine kıyasla, sönme işlemi yeniden bütünleştirme süreci içerisinde uygulandığında korku tepkilerinin anlamlı bir şekilde daha az geri geldiğini ve bu durumun yeniden-sönme manipülasyonundan bağımsız olduğunu göstermiştir. Fakat, uzun süreli etkilerin incelendiği bir yıl sonraki takip çalışmasında söz konusu etkiye rastlanmamıştır.

Anahtar Kelimeler: korku belleği, yeniden-bütünleştirme süreci, korku koşullaması, sönme, kendiliğinden geri gelme

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my advisor Assoc. Prof. Dr. Seda DURAL who has been a tremendous mentor for me. I cannot say thank you enough, without her supervision and support, this thesis would not have been possible.

I would also like to thank to Prof. Dr. Hakan ÇETİNKAYA, my former advisor, for his guidance, encouragement, and more importantly for giving me the opportunity to work with him and with many other great people in his laboratory.

I owe many thanks to my family, for being there all the time.

I cannot express how grateful I am to the friends I met along the way. Ayşegül, Emine, Akın, Ahmet, Banu, and Ezgi, for their endless support and patience throughout this jouney. I could not have been luckier.

Last but not least, I also want to thank to the Scientific and Technological Research Council of Turkey for the financial support granted through my graduate study that made this journey possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

After formation of a new memory was completed, memory was thought to be in a stabile state in which no change occurs according to the consolidation theories of memory, which has been widely accepted in the area for long time until the end of 90s. Previous studies of memory concluded that there were several conditions ending up with a change in memory but these changes were only possible just short after the initial learning, in other words, until consolidation process was complete (Nader & Hardt, 2009). This conclusion, derived from earlier studies (e.g. Flexner, Flexner, & Stellar, 1965; McGaugh, 1966), was offering a stabilization period for memory and confirming the consolidation theories of memory.

When Loftus and her colleagues observed the malleability of the human memory over "misinformation effect" in which misleading post-event information resulted in wrong recollection of the event, they suggested that during this process new information might be integrated to the original memory. On the other hand, others argued that this might be due to forgetting of the original event or source misattribution (Schiller & Phelps, 2011). Most probably, because of the prominent consolidation theory at the time and its lack of power to explain reconstruction of an old memory through integration of a new information, explanation of Loftus and her colleagues did not appreciated, as it should be.

However, more recent studies (Duvarci & Nader, 2004; Nader, Schafe, & Le Doux, 2000), rooted in studies from 70's (DeVietti, Conger, & Kirkpatrick, 1977;

Lewis, Bregman, & Mahan, 1972; Missanin, Miller, & Lewis, 1968), have challenged commonly accepted consolidation theory of memory. These studies suggested that consolidated memories, which were thought to be in a stable state after consolidation was complete, can turn into an active state under certain circumstances and requires another consolidation period, which is called as "reconsolidation", in order to persist. Existence of such state for the consolidated memory showed us that memory has a dynamic nature rather than being an inactive process of recalling from long-term memory, once memory was formed. This recent view of memory –its dynamic nature- massively changes the way we see and treat to the memory. Now, there is accumulating evidence that it is possible to interfere with consolidated memories by giving certain types of pharmacological or behavioral treatments following reactivation procedure when memory is in a labile state during reconsolidation process. Therefore, besides understanding the underlying basic mechanisms of memory processes, studies of reconsolidation may have important clinical implications. They might offer new possible directions for treatment of certain psychological disorders in a more effective way as compared to traditional methods currently used in the applied areas of psychology. These studies might serve to create persistent solutions to certain psychological problems such as post-traumatic stress disorder, obsessive-compulsive disorder, addiction, phobias… etc. Therefore, dynamic nature of the memory became one of the core concepts of many studies focusing on different memory systems and different levels of analysis (cellular, molecular, and behavioral) with different species, especially in the last decade.

In this thesis, main objective was to investigate the time dependent and long-

term effects of behavioral interference to the reconsolidation process for a fear memory acquired through differential Pavlovian paradigm by human subjects. In order to observe time-dependent effects, by scheduling different time points in addition to the 24 hours condition for re-extinction, we tested spontaneous recovery of fear 24 hours, 15 days and 3 months after the interference to the reconsolidation process by extinction training, because former studies employing the same paradigm examined the effect only 24 hours after the manipulation. Long-term effects were also examined with a one-year follow-up study as in Schiller et al. (2010), because there were no other studies conforming or disagreeing their findings supportive for the persistency of reconsolidation update paradigm. Prior stating the main hypotheses of the study, why traditional extinction approach is not sufficient to prevent return of fears, emergence of the reconsolidation phenomenon in the history, neural network - specifically within the amygdala- related to the fear conditioning, extinction, and their storage, re-emergence of the phenomenon in the scope of recent animal studies, and finally reconsolidation studies started to conduct with human subjects in the last couple of years will be introduced.

When and Why Does Extinction Fail?

Extinction is the traditional behavioral technique to reduce fear responses. Effect of extinction treatment alone was not found to be persistent since recovery of fear was observed following extinction (Dirikx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2004; Schiller, et al., 2008). Similarly, in treatment of anxiety and fear related disorders, extinction-based techniques are used predominantly and main problem with this behavioral techniques is that extinguished fears recover (Duvarci, & Nader, 2004; Field, 2006; Schiller et al., 2010). On the other hand, recent studies

of reconsolidation offer more promising results to extinguish fear memories (e.g. Schiller et al., 2010; Schiller, Kanen, LeDoux, Monfils, & Phelps, 2013; Soeter & Kindt, 2010). So why does extinction treatment fail to show its effect consistently in long-term?

Extinction occurs as a result of consistent unpairings of the conditioned stimulus (*CS*) that results in conditioned response (*CR*) with the unconditioned stimulus (*US*) that causes fear related responses (e.g. Bouton, 1988; Delgado, Olsson, & Phelps, 2006; Field, 2006; Kindt, Soeter, & Vervliet, 2009). Therefore, the *CS* that had a positive associative value initially, in terms of signaling the *US*, provides negative information about the occurrence of the *US* after extinction procedure took place (Field, 2006). However, as mentioned before, this technique does not provide a persistent solution since recovery of conditioned fear response was observed most of the time. Reason behind is that extinction does not erase or impair the previously formed association between the *CS* and the *US* but it creates a separate memory and inhibits the *CR* (Bouton, 2002; Rescorla & Heth, 1975). So, extinction memory express itself by inhibiting the acquisition memory. Eventually, there will be two distinct memories (acquisition and extinction memories) about the same stimulus (*CS*) and two possible actions when an organism comes across with the stimulus. Expression of one of these memories will be modulated by "contextual" and "temporal" factors. As a function of these factors, organism will express one of the existing memories in its behavior (Bouton, 2002).

There are certain known phenomena ends up with the recovery. Depending on contextual cues, it was observed that extinguished fear responses might recover, which is known as "renewal" effect (Bouton & Bolles, 1979). Passage of time since

the last presentation of the *CS* might also result in "spontaneous" recovery (Rescorla, 2004). Known as "reinstatement", the *US*-alone presentation was observed as another reason of response recovery (Dirikx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2004) or presentation of any other stimulus except the *CS*, and the *US* might induce the same effect. It is also worth to mention that even after full recovery of fear responses, memory for extinction found to be persistent (Quirk, 2002). Therefore, one might conclude that in certain circumstances mentioned above, it is not because acquisition memory expressed more, but existing extinction memory failed to play its inhibitory role on acquisition memory will result in recovery of fear responses. Thus, it is possible to say that there will always be an ongoing competition between acquisition and extinction memories and certain modulatory factors will determine the one to express.

Despite the substantial role of memory mechanisms in return of fear, not taking into account these related mechanisms as a major component on extinction-based techniques could be considered as the main reason behind the failure of previous attempts to extinguish fear. In a typical *CS-US* association, when *CS* is presented, this presentation evokes the mental representation of *US* and related response systems were activated (Lee, 2009). More clearly, mental representation of a traumatic experience is activated following the presentation of a conditioned fear stimulus. This mental representation triggers the behavioral defense mechanism and person demonstrates specific fear responses to the conditioned fear stimulus. As anticipated, these sequential set of events require a memory component. Pure extinction approach is lack of this memory component in extinguishing fears and so

original fear memories remains intact and as mentioned previously, this results in recovery of fear.

However, when reconsolidation process was targeted to disrupt the original fear memories, as different from the extinction treatment, it directly interferes with the original fear memory (Schiller et al., 2013). Therefore, rather than forming a new separate memory which will have an inhibitory effect depending on the context, as in the case of extinction, it appears that any procedure applied within the reconsolidation process would alter the original memory trace. Current studies of reconsolidation employ both invasive and non-invasive techniques for better understanding of this phenomenon (e.g. Duvarci & Nader, 2004; Kindt et al., 2009; Monfils, Cowansage, Klann, & LeDoux, 2009; Schiller et al., 2010). Their findings converged that when reactivated memory tries to return to its stable state to persist, pharmacological interference "blocks" the restabilization of the existing fear memory (e.g. Duvarci & Nader, 2004) or behavioral interference "updates" the existing memory by rewriting the association between *CS* and *US* as safe (e. g. Schiller et al., 2010) . Independent from the method used, applied treatment directly has an impact on the original memory trace, unlike extinction.

Towards Reconsolidation Theory

Back in the 1960's in one of a few laboratories working on retrograde amnesia it was revealed that it was possible to induce the amnesia on rats for a memory that had already been consolidated, by retrieving it before an amnesic treatment (Sara, 2000). This phenomenon was first demonstrated by Misanin, Miller and Lewis (1968) and was called as "cue-dependent amnesia". In this experiment, they employed a passive avoidance task in which rats were trained to drink from a drinking tube and

after this training; rats were given foot shock following a cue (*CS*) presentation. Foot shock was resulting in cessation of drinking response immediately and the presented cue was able to induce the same effect. A day later, they presented the cue alone as a reminder and electroconvulsive shock (*ECS*) was applied to the rats following the reminder presentation. Later on, when the cue was presented alone again, they observed that rats showed memory impairment –amnesia- evidently cue did not result in cessation of the drinking behavior. On a control condition, rats were also given the *ECS* without the reminder cue. However, they did not observe any memory impairment in these rats. So this was the first paper back in the history, providing a description for the reconsolidation (but, it was not called as "reconsolidation" until Spear coined the term in 1973) phenomenon and how this phenomenon should be studied.

Lewis (1969) defined the experimental procedure that should be followed to study cue-dependent amnesia. It consisted of three consecutive stages:

1. Presenting the reminder cue in order to reactivate the consolidated memory,
2. Interfering with reactivated memory by applying the proper treatment (e.g. *ECS*) following reactivation of the memory,
3. Testing for retention after the effect of treatment disappears.

In the scope of this experimental procedure, a significant difference observed in the retention of the original memory in the experimental group when compared to the control groups that no reminder cue was presented prior to the interference treatment or reminder was presented but no interference treatment was applied can be explained by an existing restabilization period for the reactivated memory and the treatment was effective to interfere with this restabilization process. For example, in

the study of Misanin et al. (1968), this treatment resulted in blockade of the original memory.

Lewis and his colleagues (see Lewis et al., 1972; Lewis & Bregman, 1973) continued their studies on the subject and they replicated their findings even for more complicated task like complex maze learning. Moreover, other studies using a similar protocol for consolidated memories, with an alternative pharmacological intervention method rather than *ECS* showed that it was also possible to enhance memories when interfered just after retrieval of the memory (see Gordon & Spear, 1973). As Spear (1973) stated, these studies were very important at that time because they had not only introduced a new experimental protocol to study memory but also provided crucial information about the nature of the memory. With appropriate stimuli (reminder cues), it was possible to create a state of memory which was like the state of memory just as after its initial formation in which possible to interfere until consolidation was complete.

Following their studies on cue-dependent amnesia, Lewis (1979) proposed a new theory of memory. This was one of the earliest theoretical attempts to explain both consolidation and reconsolidation processes. Because, findings were supporting the idea that even consolidated memories can be interfered by following a certain procedure. Consolidation theory of memory, commonly accepted at the time, was not able to explain this phenomenon. Because traditional consolidation approach to the memory was suggesting that the memory was in an active state only once during the formation and this was the only time interval, making interventions possible to the memory (Schiller & Phelps, 2011). On the other hand, Lewis (1979) proposed that memory can be in an active state, inactive state or in transition and retention turns

inactive memories into an active state and whatever the age of the memory this reactivation provides an interval to interfere with the original memory.

Rubin (1976) adapted the experimental procedure developed by Lewis and his colleagues to test reconsolidation hypothesis with human subjects that diagnosed with obsessive-compulsive disorder (*OCD*). This was one of the first attempts to use this procedure on human subjects and to make the clinical use of the phenomenon. In this study, since participants already had negative memory about an event because of their certain psychopathology, unlike the animal subjects, there was no need to create a new aversive memory. Therefore, study started with the presentation of a retrieval cue helping them to focus on their psychopathology. This reminder was thought as the equivalent of reminder cue in the animal studies and with this cue, Rubin expected maladaptive memories of the *OCD* patients to turn into a labile state, which was supposed to make intervention possible. Then, *ECS* administered to the participants following the retrieval procedure. Improvement in the *OCD* symptoms of these patients was observed as compared to the patients given *ECS* under anesthesia in another study. Therefore, result of the study was supporting the reconsolidation hypothesis derived from animal studies on human subjects as well.

On the other hand, there were other studies coming up with contradictory results (e.g. Dawson & McGaugh, 1969; Squire, Slater, & Chace, 1976). As Nader states (2003), there was no clear reason why these studies failed to replicate the findings supporting the reconsolidation hypothesis; however, the subject matter was very new in the area and maybe slight differences in the experimental procedure which might be crucial for the effect has been failed to notice.

Despite the fact that Lewis's theory about the dynamic nature of the memory was able to explain both the consolidation account of the memory and the other findings in the field that could not be explained by the consolidation account (Nader, 2013), and there was also accumulating support to this overarching hypothesis back in the time, studies testing the reconsolidation hypothesis of the memory remained silent after the 80s till the beginning of 2000. Reason for the stagnation is still unknown but as many researchers in the field suggested, re-emergence of the reconsolidation in the last decade can be linked to the recent advances in the field of neuroscience that allows for more detailed examination of the memory processes in different levels.

Certain studies of reconsolidation revitalized the reconsolidation account of the memory and gave inspiration for this thesis, will be referred in detail in the following sections. However, before moving to the more specific literature on the reconsolidation of the fear memories, I think it is important to underline certain important neural mechanisms related to fear conditioning and extinction for better understanding of the certain intervention to the reconsolidation process of fear memories. Especially neural network within the amygdala will be emphasized as an important brain site related to fear formation and its expression.

Fear & the Amygdala

Findings from different studies confirm that amygdala is the brain structure of interest when it comes to the understanding of fear and learning of fear. Large number of studies provide strong support that amygdala is the central site of the fear network in the brain (Öhman, 2009).

During fear conditioning, representation of *CS* is associated with somatosensory representation of aversive *US*. According to the proposed neural model, which can be seen in Figure 1, by Olsson & Phelps (2007), at first, representation of the *CS* from related sites of thalamus and cortex regarding stimulus modality, and representation of the *US* from somatosensory thalamus and primary and secondary somatosensory cortex sent to the lateral nucleus (*LA*) of amygdala. Same sites also projected to the hippocampal memory system (*HI*), anterior insula (*AI*), and anterior cingulate cortex (*ACC*). Besides projections from the thalamus and related sensory cortex, information related to the learning context and internal states of the organism delivered to the *LA* from the *HI*, *AI*, and *ACC* that carry secondary representations of *CS* and *US*.

Projections to the *LA* play an important role in conditioning of fear responses. Since sensory representation of the *CS* and the *US* converge here, the *LA* is proposed to be the site for learning. The central nucleus (*CE*), both directly and indirectly receives input from the *LA*. Indirect projections of the *LA* to the *CE* are done via basal nucleus (*B*) and intercalated cells (*ITC*). Moreover, the *B* directly sends information to the *ITC*, which provides an additional pathway to modification of responses provided by the *CE*. The *CE* controls the specific fear *CR*s by sending outputs to various regions of the brain (Phelps, 2009).

Therefore, any damage or inactivation of *CE* results in disruption of fear responses (Pare & Duvarci, 2012). Moreover, as the site that the *CS* and *US* converge, the *LA* keeps critical elements for conditioned fear responses; thus, when this site of the amygdala is damaged or inactivated, it results in disruption of the recall of

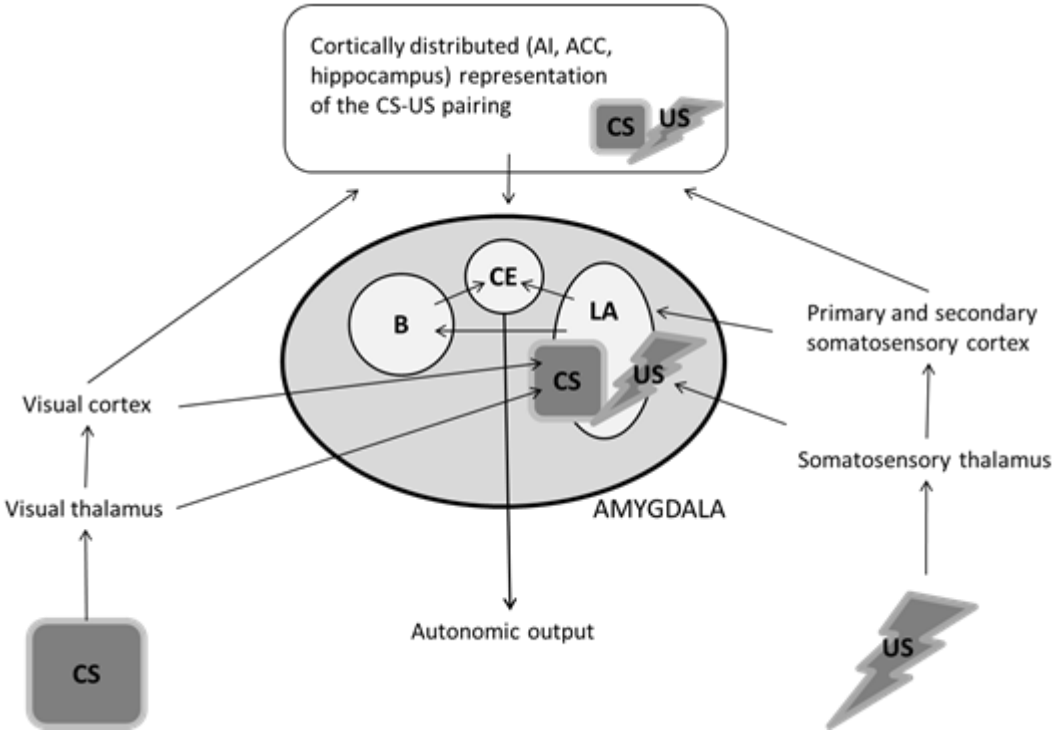

Figure 1. A neural model of fear learning in humans via Pavlovian fear conditioning (adapted from Olsson & Phelps, 2007).

fear responses triggered by the *CS* but does not affect the fear elicited by the *US* (Fanselow & LeDoux, 1999).

Studies investigating the neural mechanism underlying the extinction have drawn attention to two more significant neural structures in addition to the amygdala: prefrontal cortex and hippocampus. Interaction of these structures modulates the extinction learning and its expression (Quirk, Mueller, Kalivas, & Manji, 2008).

We already know that the *CE* is the center for expression of conditioned fear. So what does happen when extinction procedure took place to reduce fear responses? Via single-cell activity recordings of subnucleus of the *LA*, Repa and his colleagues (2001) observed that *CS* related activity in some population of cells reduced, while there was an increasing activity in other cell population during extinction training. Phelps (2009) proposed a working neural model for control of fear via extinction and cognitive regulation by combining previous work on both humans and animals (Figure 2). According to this model, when extinction memory is recalled, excitation of the *ITC* via ventromedial prefrontal cortex (*PFC*) inhibits the expression of fear via inhibiting communication between the *LA* and the *CE*, which results in reduced fear expression. Direct projections from the ventromedial *PFC* to the *LA* might also have a role on inhibition of the fear. Furthermore, projections to the ventromedial *PFC* from hippocampus mediate the contextual expression of extinction. Projections to the *B* from hippocampus might also serve for the same function, since the *B* modulates the *CE*. Finally, dorsolateral *PFC*, responsible for cognitive regulation of conditioned fear, inhibits the amygdala via the ventromedial *PFC*, so plays its role on expression of extinction.

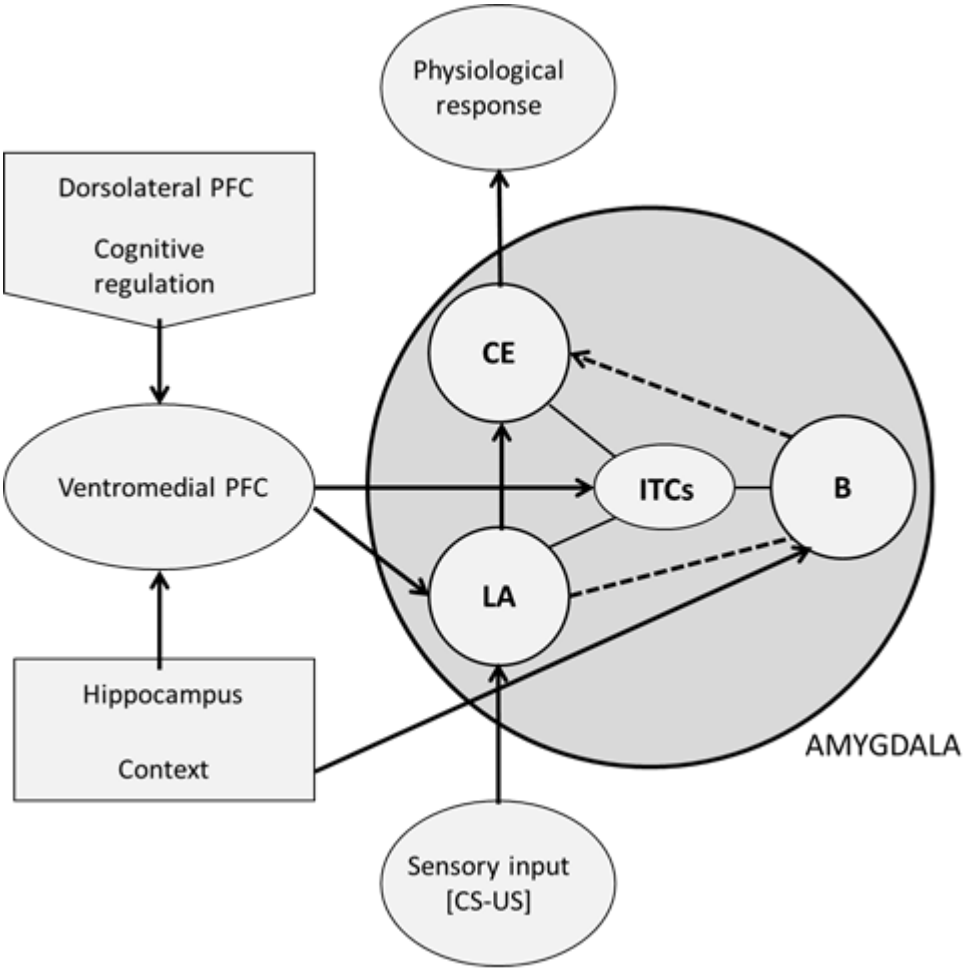

Figure 2. A neural model of for the control of conditioned behavior through extinction and cognitive regulation (adapted from Phelps, 2009).

Re-emergence of Reconsolidation & Extinguishing Fears

Nader, Schafe and LeDoux (2000) examined the reconsolidation process systematically by employing an auditory fear conditioning paradigm on rats and carried out a series of research. Several decades after the born of the reconsolidation concept, by providing a systematic and clear demonstration of the memory reconsolidation, this study renewed the interest in the subject and reconsolidation phenomenon started to be investigated by many scientists in the area.

First, they tested whether protein synthesis during reconsolidation is a requirement for reactivated fear memories to persist. It is now well known that consolidation of the auditory fear conditioning requires protein synthesis in the LA and infusion of protein synthesis inhibitors such as anisomycin to the LA, immediately after the fear conditioning, interferes with the memory consolidation and disrupts the long-term memory but not the short-term memory (Schafe & LeDoux, 2000). In addition, both the *LA* and the *B* (the *LBA* nuclei) of the amygdala is believed to be the site for memory storage in fear learning (Fanselow & LeDoux, 1999; Schafe, Doyére, & LeDoux, 2005). Therefore, in this study, the *LBA* was targeted and anisomycin was infused on this site in order to block protein synthesis.

In the first day, rats were given a single tone (*CS*) paired with a foot shock (*US*). Freezing behavior of rats given to the *CS* was used as the index of fear. Twenty four hours later, rats received a single presentation of *CS* as reminder (test 1) and this presentation was followed with anisomycin (high vs. low dose) or artificial cerebrospinal fluid (*ACSF*) infusion to the LBA bilaterally. Twenty four hours after the test 1, the rats were presented three *CS*s (test 2). For test 1, no difference was found between the rats infused anisomycin and *ACSF*. On the other hand, freezing

response to the *CS* in test 2 produced a dose-dependent decrease. High dose anisomycin group showed significantly lower response to the *CS* than low dose anisomycin and *ACSF* groups and the latter two groups were similar in terms of freezing behavior in test 2. In the control study, it was found that this response decreasing effect of high dose anisomycin revealed itself only when memory was reactivated; in other words, when the *CS* was presented prior to the anisomycin infusion. With these findings in mind, Nader and colleagues concluded that observed effect was not due to non-specific drug effect or amygdala damage by the drug since there were no histological evidence for the amygdala damage. Observed deficit in the original memory produced by the anisomycin infusion following the memory reactivation demonstrated that retrieval of the memory might turn it into a labile state open to the disruptions and protein synthesis was a requirement for these memories to persist.

Second study was designed to find out whether certain time window also exists to interfere with the original memory following the reactivation as in the case of consolidation; in other words, it was investigated if reconsolidation is a time-dependent process. Same experimental procedure was used but anisomycin infusion was delayed for 6 hours following the reactivation. No significant effect of anisomycin infusion has been found when it was given 6 hours later, in contrast to the anisomycin infusion immediately after retrieval; showing that after reactivation there is a certain time window that memory is open to the disruption after reactivation, and when window was closed, disruption was not possible.

Next, in the same series of experiments, Nader and colleagues investigated the effect of memory age in order to see if older memories were more resistant to the

reconsolidation. Again, same experimental paradigm was used. Only difference was the time of reactivation (presentation of the *CS*: test 1) after the initial learning. While memory for the original event reactivated 24 hours later in one group of rats as in the previous studies, other group waited for fourteen days for memory reactivation. Following reactivation, infusions and test procedures were the same for both groups. Results indicated that anisomycin infusion within the reconsolidation window resulted in less freezing behavior compared to the controls (*ACSF* infused group). Moreover, independent from memory age (one day old vs. fourteen days old memory), blockade of protein synthesis within reconsolidation window resulted in amnesia for the original fear memory. They concluded that even older memories can be disrupted when reactivated.

In the final experiment, they investigated the source of observed reconsolidation blockade, whether anisomycin inhibits the protein synthesis required for stabilization of the reactivated memory or induces nonspecific effects on amygdala making it dysfunctional during the process. If pharmacological manipulation directly acts on protein synthesis then short term memory (*STM*) for the original memory expected to remain intact while long term memory (*LTM*) was impaired. To the same paradigm, a new test stage was added four hours after the test 1 in which reactivation occurs. By doing so, they observed the *STM* for the original fear memory following anisomycin infusion and they referred it as the post reactivation *STM*. In other test phase, twenty for hour after the reactivation, similarly, post reactivation *LTM* was observed. As in the previous studies, results showed that post reactivation *LTM* was impaired only in the group infused anisomycin immediately after reactivation. Furthermore, for this group, intact post reactivation

STM but impaired post reactivation *LTM* was observed, which means that anisomycin shows its effect on fear behavior by acting directly on the protein synthesis mediating reconsolidation.

As mentioned previously, this study series of Nader and his colleagues (2000) drew considerable interest to the reconsolidation phenomenon and paved the way for new studies to understand this phenomenon in more detail. Milekic and Alberini (2002) proposed that older memories might not be sensitive to the disruptions by protein synthesis inhibitors following reactivation like the younger memories. To test this hypothesis, as different from Nader et al. (2000) they employed inhibitory avoidance (*IA*) task and latency to enter the shock chamber was used as the index of acquisition. They manipulated the time points that retention take place in order to reactivate the memory. Memory for the *IA* in different groups of rats was reactivated 2, 7, 14 or 28 days after the initial learning and following reactivation, half of each group received anisomycin injection and the other half received vehicle solution (saline solution). When all groups were tested two days later, rats injected anisomycin following reactivation 2 and 7 days after initial learning showed impairment in recall for the original memory as compared to rats injected saline solution. On the other hand, no impairment for the memory recall was observed in 14 and 28 days groups. Therefore, they concluded that older memories will be more resistant to the postreactivation interventions in contrast to recently acquired memories. This results were not consistent with the previous findings of Nader et al. (2000) showed that even 14 days-old memories could went through reconsolidation and interfered via protein synthesis inhibition. Researchers suggested that these might be due to different temporal requirements of protein synthesis for different

task used to create fear (auditory fear conditioning vs. inhibitory avoidance) and this aspect should be clarified by further investigation.

Findings from different studies were confirming the existence of a time-dependent memory process requiring protein synthesis following memory activation to keep original memory intact, which is reconsolidation. On the other hand, there were alternative explanations for these findings. One group argued that inhibition of protein synthesis might result in facilitated extinction (Fischer, Sananbenesi, Schrick, Spiess, & Radulovic, 2004) rather than reconsolidation blockade. Another group was also claiming that observed impairment was due to a retrieval failure not a restorage problem (Lattal & Abel, 2004). In order to rule these possibilities out Duvarci and Nader (2004) tested these hypotheses by using auditory fear conditioning. Firstly, they proposed that if facilitated extinction explanation was right, when extinguished memory was tested in a different context (renewal) following inhibition of protein synthesis, response recovery to the *CS* would be expected which is evident when the CR extinguished by extinction procedure (Bouton & Bolles, 1979). Response renewal was observed in the control group (received *ACSF*) but not in the anisomycin group when tested in a different context. This finding conformed the reconsolidation hypothesis.

Another study on the other hand, conducted by Pedreira and Maldonado (2003) on crabs showed that when duration of reminder cue presentation exceeds certain time period, it was serving for the extinction rather than reconsolidation. This was quite possible since the extinction involves presenting the *CS* alone. Then, what would happen when the *CS* was paired with the *US* in order to reactivate the memory? If facilitated extinction account was right, we would expect to see no memory

impairment due to intervention to the reconsolidation process, in case of presenting the *US* as a reminder. Duvarci and Nader (2004) also showed that when the *CS* paired with the *US* was used for the reactivation session, memory impairment was still evident in the rats. This finding was ruling out the facilitated extinction explanation and consistent with the reconsolidation.

Retrieval failure explanation for the findings was also refuted within the same study. Duvarci and Nader (2004) proposed that if there is a retrieval failure rather than re-storage problem, then certain procedures should overcome this retrieval problem and original memory should recover. It is already known that passage of time and reinstatement of the *US* results in return of the *CR* (Bouton, 2002), so they checked for any significant fear recovery by testing the memory 24 days after the manipulation and also by reinstating the *US*. Neither passage of time nor reinstatement of the *US* resulted in the recovery of fear responses for postreactivation anisomycin group as compared to the control group; confirming that blocking reconsolidation was resulting in a re-storage failure and the effect of the blockade was persistent in contrast to the extinction, which has a transient effect to reduce conditioned responding.

In another study, Debiec and LeDoux (2004) used propranolol, a beta adrenergic antagonist, to block protein synthesis instead of anisomycin on rats. By using auditory fear conditioning paradigm, they successfully showed that postreactivation propranolol injection resulted in impaired memory when tested 2, 9 and 16 days after manipulation as compared to saline injected rats. Moreover, they used reinstatement procedure (*US* exposure) to observe recovery of original memory, which was known to result in recovery given after memory extinction as mentioned

previously. Twenty four hours after the third test (on day 17), rats received a single *US* presentation, memory impairment was still evident in the experimental group as compared to the control group. Finally, they demonstrated that even two months-old memories went through reconsolidation, and observed impairment of the original memory via blockade of protein synthesis within reconsolidation process persist even after one month.

With the growing interest to the phenomenon, above mentioned and many other studies investigating the reconsolidation were successful at demonstrating the existence of such process and agreed that there was a time-dependent active state which opens consolidated memories to interruption. Regarding fear memories, it was possible to diminish fear responses by disrupting original fear memories during this certain time window via pharmacological manipulations. However, despite the fact that most of the studies were confirming reconsolidation, there were certain inconsistent findings. For example, while one study was showing that even older memories could underwent reconsolidation (e.g. Debiec and LeDoux, 2004), other study was concluding that when memory gets older it becomes resistant to the reactivation procedure so reconsolidation does not occur for old and strong memories (Milekic and Alberini, 2002). Actually, these inconsistencies did not mean that memory cannot undergo reconsolidation but pointed out the certain aspects of the memory or certain component of the employed method, and helped to specify boundary conditions that reconsolidation occurs. To illustrate, as well as the characteristics of the memory (such as memory age), characteristics of the reactivation session found to be an important parameter to induce reconsolidation process in different studies. Duration of the reminder to reactivate the memory

(Pedreira & Maldonado, 2003), environment that reactivation took place (Hupbach, Hardt, Gomez, & Nadel, 2008), predictability of the reminder cue (Morris, et al., 2006) were some of them (Nader, Hardt, Einarrson, & Finnie, 2013). Recently, it has also found that when presenting the *CS-US* pairing rather than the *CS*-only presentation as the reminder cue, temporal relationship between the *CS* and *US* association is an important property to induce reconsolidation. In order to reactivate the memory, detection of a temporal error between the *CS-US* association is necessary (Díaz-Mataix, Martinez, Schafe, LeDoux, & Doyère, 2013).

While animal studies using pharmacological reconsolidation blockade were offering great possibilities for further understanding of the memory reconsolidation in different levels of the analysis, and number of these studies were increasing day by day, studies of human reconsolidation was slow to emerge in the beginnings. As anticipated, pharmacological manipulation to the memory reconsolidation employed in animal research was impractical for the human research. Anisomycin was toxic in humans and also requirement for direct infusion of the pharmacological agent to the brain was an invasive way which cannot be considered as an option in the first place (Monfils et al., 2009; Phelps & Schiller, 2013). Therefore, this drug was not even an option to use in the human research. However, McGaugh (2000) found out that propranolol, a beta-adrenergic receptor blocker, was an alternative drug to the anisomycin to block protein synthesis in the amygdala during reconsolidation and it was amenable for humans as well as animals. Despite the fact that the dosage used in the animal research was much higher as compared to dosage administered in humans (Schiller & Phelps, 2011), this was one of the development pave the way for studying memory reconsolidation with humans.

Another contribution for the development of human research came from a study conducted by Monfils et al. (2009), it was offering a drug free behavioral procedure to interfere with reconsolidation process, allowing to diminish fear memories permanently as in the case of reconsolidation blockade by pharmacological intervention. In this study, Monfils and colleagues showed that by applying extinction treatment within the reconsolidation window, new information about the *CS* could be integrated to the original memory rather than forming an extinction memory. Such a behavioral method was much more safe and easy to use in humans.

In this paradigm, they conditioned rats to a tone signaling the shock in the first day and 24 hours later rats were presented a single *CS* as a reminder cue to reactivate the memory. As a control condition, one group of rats did not receive a reminder and directly went through extinction. Others were divided into four groups and while two of them underwent extinction within the reconsolidation window (10 minutes and 1 hour after reactivation), other two groups underwent extinction outside of the reconsolidation window (6 hours and 24 hours). When they tested all the groups 24 hours later to observe the consolidation of the extinction, all showed comparable levels of freezing behavior. One month after the initial test, they run another test for spontaneous recovery. There was significant increase for freezing behavior of the rats in control conditions (extinction without reminder and extinction outside of the reconsolidation window groups) as compared to the rats in other two conditions that took extinction training within the reconsolidation window. They were able to replicate the results when they employed renewal and reinstatement procedures, known to result in response recovery when only extinction training was given to

reduce conditioned responses. In addition, when they tried to recondition these rats, they found out that this manipulation might even retards the acquisition to the *CS* for the reactivated group prior to the extinction as compared to only extinction group and naive group of rats. These results were supportive for the idea that extinction during the reconsolidation rewrites the original memory by integrating the new information about the *CS*, which is "safe" in this case, and updates the original memory.

Reconsolidation of Fear Memories in Humans

After the re-emergence of reconsolidation studies, first human study to test the efficacy of the propranolol targeting the memory reconsolidation was conducted in a clinical population, with post-traumatic stress disorder (*PTSD*) patients (Brunet, et al., 2008). First, the patients were asked to write the event that caused their *PTSD* in order to reactivate the memory for the traumatic event. Following this procedure, half of the patients were given propranolol while the other half took the placebo pills. One week later, participants listened a recording of their traumatic event. In the meanwhile, heart rate (*HR*) and skin conductance responses (*SCR*) as autonomic system arousal measures were recorded. When these physiological measures were compared to the normative *PTSD* cutoffs, both measures were found to be below these levels for patients received propranolol treatment but above for placebo condition. This study was encouraging to continue investigating the effects of propranolol on disruption of fear memories through interfering with reconsolidation mechanisms.

In an attempt to investigate the effect of propranolol on fear memory reconsolidation, Kindt and her colleagues (2009) utilized the fear conditioning procedure for a better understanding of the basic conditions required for preventing

the return of fear in humans. First day, participants went through a discriminative fear conditioning protocol with fear relevant stimuli, in which two different spider pictures (*CS*s) were presented and one of the spider pictures was paired with the electrical stimulation (*US*) to the wrist during the acquisition phase. In the second day, in order to reactivate the fear memory, CS^{+}, the one paired with the electrical stimulation in the first day, was presented alone to the participants. One and a half hour prior to this reactivation session, either propranolol or placebo pill was administered to the participants orally. On the other hand, a third group of participants did not receive a reminder and only propranolol was given to them as the control condition. During the third day, all participants went through two-stage extinction training. In the first stage, both *CS*s were presented to the participants without the *US* and then unsignalled presentations of the *US* (reinstatement) were done. Reinstatement was followed by an additional extinction stage. During all stages, skin conductance response and startle response of the participants were recorded and *US* expectancy ratings were also collected as the indices of fear. According to the results, group received propranolol prior to the reactivation session showed substantial weakening of fear responses regarding startle response as compared to reactivated placebo and only propranolol groups. For skin conductance and the *US* expectancy data, effect of the manipulation was not observed.

Further inquiries were conducted with the same method by Soeter ve Kindt (2010) to replicate the previous finding and observe the long-term effect of the reconsolidation of fear memory blockade by propranolol in humans. Therefore, a one-month follow-up stage was added to the experimental procedure, in addition to the previously mentioned procedure (Kindt et al.,2009). For the follow-up, a

procedure similar to the third stage was applied. This study was succesful at replicating the previous results and more importantly, showed that effect of administrating propranolol prior to the reactivation was persistent over one month. It should have been noted that observed effect was evident only in startle response but not in other measures (skin conductance response and the *US* expectancy ratings).

While studies employing the reconsolidation blockade paradigm by using propranolol to erase fears permanently were successful at demonstrating this phenomenon to some extent, Schiller and her colleagues (2010) adapted the behavioral procedure proposed by Monfils et al. (2009) and turned this behavioral method, which is more safe and easy to use in humans, into a reconsolidation update paradigm to study with human. Colored squares were used as CS^+ and CS^- in a counterbalanced fashion and electrical stimulation from the wrist served as the US. As in the previous studies, in the first day, participants went through a differential Pavlovian fear conditioning procedure. Next day, participants were assigned to three different groups. These groups went through extinction procedure in three different condition. One group was presented a single CS^+ without the *US* as the reminder to reactivate the fear memory, formed in the first day, and after 10 minutes break, took the extinction treatment in which no *CS* was paired with the *US* (extinction 10 minutes after the reminder group). Other group went through the same procedure but waited for 6 hours after the reminder presentation (extinction 6 hours after the reminder group). Last group, on the other hand, underwent extinction without reactivation (extinction without reminder). For the third stage that took place 24 hours after extinction, all groups underwent an additional re-extinction procedure, in order to investigate the spontaneous recovery of the fear responses extinguished one

day before. During the all stages, skin conductance responses were collected from the participants and differential skin conductance response (CS^+ minus CS^-) was used as the index of fear.

Results of this study revealed that fear recovery of the participants in which extinction procedure was applied within the reconsolidation window (extinction 10 minutes after the reminder) was significantly lower than the participants who directly underwent extinction (extinction without reminder) and who underwent extinction outside of the reconsolidation window (extinction 6 hours after the reminder). Moreover, with a one-year follow up study, they demonstrated that observed effect of the reconsolidation update paradigm was persistent when tested by reinstatement. Based on this result, Schiller and her colleagues (2010) suggested that by teaching to an organism that the *CS* is not paired with the aversive outcome anymore within the memory reconsolidation process, fear-related memory can be rewritten as safe, in other words, updated as safe and this results in that the aversive *CS* losts its previously acquired aversive properties.

In the same series of research, Schiller et al. (2010) also investigated how specific the effect of reconsolidation update. They suggested that in real life situations, a traumatic event might be associated with more than one cue and each of them might result in fear reactions. Therefore, they assessed the specificity of the reconsolidation update, by creating two *CS*s associated with the aversive outcome in the first stage and reactivated only one of them prior to the extinction intervention to the reconsolidation process in the second stage. They used only extinction 10 minutes after the reminder condition for this manipulation and reactivated one of the CS^+ and CS^-, while other CS^+ was not reactivated and participants went through

extinction. In the third day after the reinstatement procedure, fear reinstatement was found only to non-reactivated *CS* before the extinction. They concluded that extinction during the reconsolidation affects only reactivated memory trace but no other traces associated with the original event.

With the previous finding about the specificity of the reconsolidation intervention in mind, Soeter and Kindt (2011) approached to this issue in a different way. From the fact that fear generalization is the main characteristic of anxiety disorder, they proposed that disruption of the reconsolidation process should not only erase the fear reaction to the aversive stimulus associated by the *US* but also to the stimuli related to the same category with the original *CS*. In order to examine this hypothesis, they used reconsolidation blockade paradigm. An experimental procedure similar with the previous ones (Kindt et al., 2009; Soeter & Kindt, 2010) was followed. During the acquisition phase, two fear-relevant stimuli (spider and gun pictures) were paired with the electrical stimulation and one fear-irrelevant stimulus (mug picture) was presented alone. After propranolol administration in the second day, only spider picture was shown to the participants to reactivate the memory. On the third day, following the test sessions, all participants went through reacquisition training, additionally, in which spider and gun pictures paired with the electrical stimulation while the mug picture did not. In order to observe the generalization, for each stimulus a new generalization-stimulus (e.g. another spider picture for the conditioned spider picture) was presented to the participants. Consistent with their previous findings, reactivation group receiving the propranolol showed significant decrease in startle response but not in skin conductance and *US* expectancy ratings. Moreover, reacquisition to the reactivated spider picture was not as fast as

reacquisition to the gun picture when both paired with the electrical stimulation again. Despite the fact that fear reaction to the spider picture was diminished via reconsolidation blockade and forming the new association was slower as compared to the other conditioned stimulus (gun picture), it did not affect the reacquisition of the association. Results regarding the generalization of the fear reduction revealed that startle response to the generalization stimulus of the spider was lower than the startle response given to the other two generalization stimuli.

In the same series of studies, Soeter and Kindt (2011) also tested the behavioral approach proposed by Schiller et al. (2010). They used their own methodology and gave placebo instead of the propranolol to the participants. 10 minutes after the reactivation with a reminder, first extinction training took place. In the third day, although no recovery of startle response was observed for the reactivated *CS* at the beginning of the re-extinction session, after reinstatement procedure, effect of extinction within the reconsolidation window was not found to be persistent to diminish fear responses. Moreover, startle response recovery was comparable for all stimuli when reacquisition training was given. Finally, when generalization stimuli of all three stimuli were presented to the participants, startle response given to these stimuli revealed generalization of fear to all stimuli. Similar with the first experiment, no effect of behavioral manipulation was observed on skin conductance response and *US* expectancy ratings.

Another study using pharmacological manipulation examined the characteristics of the reminder session used for reactivation of the consolidated memory (Sevenster, Beckers, & Kindt, 2012). Based on the assumption that memory reactivation allows for integration of the new information to the original memory

trace, they hypothesized that retrieval does not necessarily induce reconsolidation, especially when there is nothing new to learn and outcome is fully predictable. In the first day of the study, differential fear conditioning paradigm was employed. 24 hours later, two groups of participants were given propranolol and the other group was given the placebo pills. Within the scope of reactivation session, the CS^{+} without the *US* was presented to the both groups given propranolol. However, one group received the reminder cue when electrical stimulation electrodes were attached to their wrists (propranolol group) while the other group received the reminder cue without electrical stimulation electrodes on their wrists (propranolol-no expectation group). Exactly same treatment was applied to the placebo group with propranolol group. When participants were tested on the next day, decrease in startle response to the aversive stimuli was observed only on propranolol group in which outcome of the reminder cue was not fully predictable as compared to the propranolol-no expectation group and placebo group. This finding revealed that pure retrieval of the fear related memory was not sufficient to induce reconsolidation process; outcome of the retrieval session should be unpredictable to some extend in order to open memory to the disruptions.

After the study of Schiller et al. (2010), first attempt by Soeter and Kindt (2011) to replicate this result using the behavioral intervention was unsuccessful to show that reconsolidation update paradigm could prevent return of fears permanently. Another attempt was done by Oyarzun and colleagues (2012), for fear conditioning geometrical figures were used as *CS*s and as different from Schiller's study, they used a loud noise as the *US* instead of the electrical stimulation. Skin conductance response was used as the fear index. They found out that fear responses of the

participants did not recover when memory reactivation was done via a single reminder *CS* prior to the extinction training, in other words, when fear responses were extinguished within the reconsolidation window. Therefore, finding of Schiller et al. (2010) was replicated for the first time with this study by using the same behavioral interference paradigm with a different aversive *US*.

On the other hand, another study series (Golkar, Bellander, Olsson, & Öhman, 2012) employed the reconsolidation update paradigm. Since the objects of the clinical fears are fear-relevant rather than being fear-irrelevant, they tested whether behavioral manipulation could prevent the return of fear both for fear-relevant (fearful male faces) and fear irrelevant stimuli (geometrical figures). Skin conductance response and startle response were recorded as the dependent measures. When recovery of fear was assessed with reinstatement procedure, fear recovery to fear-relevant and fear-irrelevant stimuli was observed consistently in both measures; therefore, they concluded that extinction treatment within the reconsolidation window is not sufficient to update fearful memories into the neutral ones independent from stimulus type.

Other branch of research used functional magnetic resonance imaging (*fMRI*) in order to examine the certain brain areas related with fear memory formation and extinction by using reconsolidation update paradigm and found supportive evidence for the effectiveness of behavioral approach to diminish fear memories. For example, Agren et al. (2012) showed that behavioral intervention to the reconsolidation process significantly weakened the fear memory trace formed in the amygdala and its coupling with other nodes of the fear network in the brain as compared to the extinction procedure without memory reactivation; therefore, attenuated memory

trace to recall and return of the fear. Effectiveness of extinguishing fears within the reconsolidation window was also supported by skin conductance response data collected during the experiment. Moreover, in a recent study carried out by Schiller, Kanen, LeDoux, Monfils, Phelps (2013), they proposed that giving extinction training within the reconsolidation process of fear memory would diminish the prefrontal cortex (*PFC*) involvement, which has an inhibitory influence over amygdala when standard extinction procedure was employed in order to extinguish fear responses. As in the second experiment of Schiller et al. (2010) two *CS*s was paired with the electrical stimulation while third one was not. In the second day, only one of the *CS*s, paired with the electrical stimulation (reminded *CS*) was presented to the participants and 10 minutes after the reactivation, extinction training was given to the participants. In terms of skin conductance response, fear responses were found to be extinguished for the reminded *CS* when reinstatement procedure was used to observe fear recovery. It was observed that there was an increasing activity in the *PFC* and its connections with the amygdala, after extinction procedure, during the presentations of non-reminded *CS* as compared to the reminded *CS*. In addition, amygdala activity to the presentation of non-reminded *CS* was higher than the amygdala activity to the presentation of the reminded *CS* after the extinction, confirming the observations of Agren et al. (2012).

One of the most recent studies, conducted by Kindt & Soeter (2013), tried to replicate the findings of Schiller and her colleagues (2010) with fear-relevant stimuli in order to see whether extinction procedure provided following reactivation allows for rewriting of the original fear association. As different from their previous study, testing the behavioral approach (Soeter & Kindt, 2011), this time no placebo pills

were given to the participants. Startle response, skin conductance response and the *US* expectancy ratings were collected as indices of the fear. In line with their previous study, any of these measures confirmed the finding that disrupting reconsolidation process with extinction training resulted in permanent erasure of fear memories via updating the old information, so they failed again to replicate Schiller et al.'s (2010) finding.

Keeping in mind that interest in fear memory reconsolidation regarding human studies has been recently developed, it is not surprising to come across certain contradictory findings. Given the heterogeneous findings from different series of studies in the human fear memory reconsolidation, especially the ones employing the behavioral intervention, current thesis aimed to examine the reconsolidation update paradigm and its long-term effects. When we proposed the study, there was only one study (Schiller et al., 2010) found evidence for both effectiveness and persistence of reconsolidation update paradigm, when tested 24 hours after the manipulation and one year after the manipulation. Answer to the question "what would be the result, if the effects of the behavioral intervention to the fear memories were investigated in a time dependent manner after the main manipulation took place rather than testing this effect only 24 hours later?" has remained unclear. Given that spontaneous recovery gradually shifted toward 100% with the passage of time (Quirk, 2002), in addition to test fear recovery 24 hours after the second stage, we tested for the spontaneous recovery of fear 15 days and 3 months after the second stage. Furthermore, a one-year follow-up employing reinstatement procedure was conducted. As well as replicating the Schiller et al.'s (2010) finding on reconsolidation update, by including different re-extinction conditions to test

spontaneous recovery, we wanted to find out whether spontaneous recovery scores differ between extinction groups depending on the testing time. Moreover, with one-year follow up, we intended to examine long-term effects of the behavioral intervention to the fear memory reconsolidation with a larger sample, considering the only study previously tested the long-term effects had a small number of participants.

Our first hypothesis, regarding extinction manipulation, was that when extinction treatment was given within the reconsolidation window (10 minutes after reminder), spontaneous recovery score of this group will be lower than the spontaneous recovery scores of the group took extinction treatment outside of the reconsolidation window (6 hours after reminder) and the group took extinction-only (no reminder). We also expected that latter two groups would have comparable levels of spontaneous recovery scores. In addition, by re-extinction manipulation, we aimed to observe if there would be an interaction between extinction and re-extinction variables. Therefore, if reconsolidation update paradigm is both effective and persistent, we expected that 10 minutes after reminder group tested 24 hours, 15 days and 3 months after extinction will differ from 6 hours after reminder and no reminder groups tested 24 hours, 15 days and 3 months after extinction for spontaneous recovery. Moreover, we expected no difference on spontaneous recovery scores within the 10 minutes after reminder group depending on the testing time (re-extinction condition); however, significant difference in spontaneous recovery scores was expected depending on the passage of the time on 6 hours after reminder and no reminder groups when tested. Finally, for the follow-up study, we expected the group extinction treatment was given within the reconsolidation window would display lower levels of spontaneous recovery score as compared to the groups extinction

treatment given outside of the reconsolidation window and without reactivation even after one year.

CHAPTER 2

Method

In this study, recovery of conditioned fear responses following the extinction inside and outside of the reconsolidation window were investigated with human subjects via creating physiological fear responses in laboratory conditions with arbitrary stimuli. *A memory reconsolidation update* paradigm developed by Schiller and her colleagues (2010) was employed to achieve this goal. This paradigm consists of a four-stage procedure including acquisition, extinction, re-extinction, and examination of long-term effects (reinstatement and extinction) stages. Therefore, during the experimental procedure;

1. the participants acquired fear through differential fear conditioning trials with arbitrary stimuli,
2. the acquired fear responses during the first stage of the procedure were extinguished in three different extinction conditions,
3. a re-extinction procedure including three different conditions was carried out to observe spontaneous recovery of fear responses,
4. a reinstatement and an additional extinction procedure was employed to examine the long-term effects of manipulation on recovery of fear responses.

Two independent variables included in the experimental design of the study were extinction that was manipulated in the second stage of the study, and re-extinction that was manipulated in the third stage of the study. The extinction variable was manipulated into three levels as extinction 10 minutes after reminder (10 minutes), 6 hours after reminder (6 hours), and without reminder (no reminder).

Re-extinction variable had also three levels including re-extinction 24 hours after extinction (24 hours), 15 days after extinction, and 3 months after extinction. Therefore, 3 (extinction group: 10 minutes after reminder, 6 hours after reminder, no reminder) x 3 (re-extinction group: 24 hours after extinction, 15 days after extinction, 3 months after extinction) between-groups design was used in the study. Skin conductance responses elicited by CS^+, CS^- and *US* used in each stage of the study was recorded as dependent variable. Thus, the levels of fear recovery were compared between the groups in terms of the levels of *SCR*s elicited by *CS*s.

Participants

Several elimination criteria were used in order to determine the eligibility of the participants to participate in the study. Some of these criteria were related to participants' current health status and prior experience with other fear related studies. These can be summarized as

- having any cardiovascular disease,
- having a history of any psychological/psychiatric disorder and not being on medication related to this condition,
- having prosthesis in any body parts,
- having any medical treatment using mild electrical stimulation such as physical therapy,
- taking part in a prior study related to fear and anxiety,
- having a score more than 15 at Beck Anxiety Inventory in the Participant Evaluation Form, higher scores means moderate and severe anxiety (Ulusoy, Şahin & Erkmen, 1998),

Anyone who met at least one of these criteria was not included in the study as participants. Specifically, 4% of the people who were volunteers to attend to the study were eliminated due to these issues. In addition, some further elimination criteria were set based on the performance of the participants. These were

- not attending the following stages of the study (dropout),
- not following the instructions properly,
- not meeting the criteria related to acquisition of fear (see preparation of data for analysis),
- not meeting the standard related to extinction of fear (see preparation of data for analysis).

Observing at least one of these was enough to exclude corresponding data from further analysis. Dropout caused data loss around 10%, participants who did not met the standards for acquisition and extinction of fear were around 30%. Additionally, a 6% of data were discarded due to technical problems and participant who did not follow the instructions properly during data collection.

To sum up, approximately 50 % of the scheduled participants were eliminated due to one of the aforementioned elimination criteria. Finally, valid data from 111 participants (41 male and 70 female) were obtained to use in statistical analysis. Ages of the participants were between 18 and 51, with a mean of 21.38 (SD = 5.07). Distribution of participants across experimental conditions can be seen from Figure 3.

In the fourth stage of the study, which was one year later from the main study, to examine long-term effects of experimental manipulation, 39 participants out of 111 were taken in. No one from the group that extinction training was given 6 hours after the reminder and tested 15 days later attended to the follow-up stage. Three of

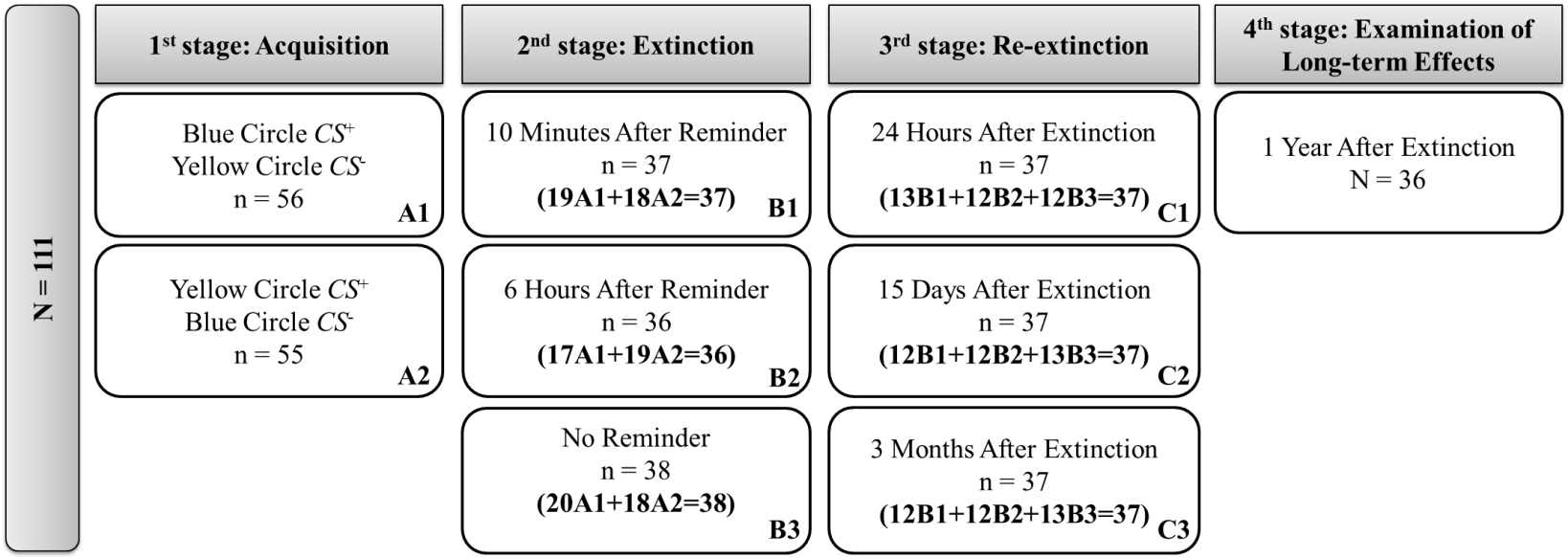

Figure 3. Experimental flow with conditions and distribution of participants across these experimental conditions

the participants were eliminated due technical problems (not receiving the electrical stimulation during reinstatement), therefore; valid data from 36 participants (14 male and 22 female) were used to examine long-term effects. Age of the participants were between 18 and 50, with a mean of 21.47 (*SD* = 5.47).

In order to create the study sample, convenience random sampling method was employed. Some of the participants were paid for their participation; some were given bonus credit for the Quantitative Methods in Psychology I class, if applicable.

Stimuli, Apparatus and Material

Stimuli. Yellow (R = 255, G = 255, B = 0) and blue (R = 0, G = 0, B = 255) circles (*d* = 375 pixels) are used as arbitrary *CS*s. These two stimuli were shown to evoke similar levels of skin conductance response in a pilot study carried out in our laboratory. Mean skin conductance response for all stimuli presented was 8.44 microsiemens (μS) in the pilot study. Yellow and blue circles that were most close to this value was selected to use in our study (*M* = 8.22 μS, *M* = 7.89 μS, respectively). Mild shock to the wrist was the *US*.

Participant Evaluation Form. This form was developed by academic staff of Psychology Laboratory in Izmir University of Economics previously for a study on fear learning and memory (Appendix A). It includes questions about both past and current physiological/ psychological well-being (e.g. Were you diagnosed with any phobic disorder? Are you on medication for any particular health problem?), and also questions to find out about previous experiences on any research participation (e. g. Did you participate in any other experiment in past 12 months?). Prior to the first

experimental session, an evaluation form was given to all recruited participants, in order to see whether they are able to satisfy the conditions of inclusion in the study.

Stimulus Presentation Programs. Presentation and randomization of stimuli was designed and controlled with Superlab^{TM} (Model: 4.5; Cedrus Corporation) which was run on a personal computer (AMD FX (TM)- 6100 six core processor, 3.30 GHz, 4 GB of RAM) and connected to a 20″ stimulus presentation monitor with a screen resolution of 1600*900 pixels, and refresh rate of 60 Hz. Table 1 represents 14 different stimulus presentation programs which were prepared for four different experimental sessions in accordance with behavioral paradigm and experimental conditions.

Each stimulus presentation program started with a five-minute habituation period. In the first two minutes, participants were expected to adapt environment that experiment will take place and certain instructions were given to make sure that participants understand the task and their duty during experiment, properly. During remaining three minutes, a countdown clock was presented showing time left to start the experiment and participants were asked to relax as much as possible. After five-minute habituation period, presentation of CS^{+} and CS^{-} were made, each lasted for 4000ms. As it is scheduled, electrical stimulation device- the STMISOLA (BIOPAC Systems, Inc.) was triggered by Superlab^{TM} 4.5 and presentation of *US* was made during the last 200ms of CS^{+} presentation. Inter-trial interval was 10s between the stimulus presentations.

In the first stage of the study (acquisition), all participants were subjected to a differential Pavlovian conditioning procedure with partial reinforcement in which blue and yellow circles were presented (Table 1). For the acquisition phase, two

Table 1. *Stimulus Presentation Programs*

Stage	Program Number	Condition
Acquisition	1	Blue circle CS^+
	2	Yellow circle CS^+
Extinction	3*	Blue circle reminder
	4*	Yellow circle reminder
	5	10 minutes after reminder (blue circle CS^+)
	6	10 minutes after reminder (yellow circle CS^+)
	7	6 hours after reminder (blue circle CS^+)
	8	6 hours after reminder (yellow circle CS^+)
	9	No reminder (blue circle CS^+)
	10	No reminder (yellow circle CS^+)
Re-extinction	9**	Blue circle CS^+
	10**	Yellow circle CS^+
Reinstatement	11	Blue circle: CS^+ first
	12	Yellow circle: CS^+ first
	13	Blue circle: CS^- first
	14	Yellow circle: CS^- first

*Reminder programs were used only for participants who underwent extinction 6 hours after reminder presentation.

**Stimulus presentation programs for re-extinction procedure were exactly same with stimulus presentation programs for extinction procedure that no reminder presented.

experimental programs were designed to counterbalance the presentations of blue and yellow circles as CS^+. Therefore, in the acquisition stage, half of the participants received electrical stimulation at the last 200ms of blue circle presentation, while the other half received electrical stimulation at the last 200ms of yellow circle presentation. Both programs consisted of 26 stimuli presentations in total. Sixteen CS^+ and 10 CS^- presentations were made. Six of 16 CS^+ presentations were terminated with electrical stimulation (CS^++*US* presentations). Moreover, in both programs, stimuli were presented in a pseudorandom order that equal numbers of CS^+, CS^- and CS^++*US* (5, 5, and 3, respectively) presentations in both first and second halves of the experimental programs took place. Hence, each half consisted of 13 stimulus presentations.

In accordance with extinction manipulation, eight experimental programs were designed for three extinction conditions: (1) Extinction 10 minutes after reminder, (2) Six hours after reminder, and (3) Without reminder. Through these programs, both blue and yellow circles were presented to the participants and none of these stimuli were paired with *US*. Presentation order was pseudorandom and similar numbers of CS^+ and CS^- were presented during the first and the second halves of the programs.

For extinction through 10 minutes after reminder condition, two stimulus presentation programs were designed. One was for the participants who received electrical stimulation with blue circle and the other was for the participants who received electrical stimulation with yellow circle during acquisition session. Both programs -consisted of 11 CS^+ and 11 CS^- presentations- started with a single CS^+ presentation as reminder. Following the reminder presentation, there was a 10-minute break in which participants did not leave the experimental room and watched

a short video about art of painting. After the break, extinction trials took place and participants in that condition were presented 10 CS^+ and 11 CS^- as one of the CS^+ was already presented as reminder. For extinction through 6 hours after reminder condition, four stimulus presentation programs were designed. Two of them were used for reminder presentation in which single CS^+ was presented without *US* while other two were used for extinction procedure. Latter two programs included 10 CS^+ and 11 CS^- presentations without *US* as in the 10 minutes after reminder condition. Finally, for extinction without reminder condition, two stimulus presentation programs including 11 CS^+ and 11 CS^- presentations without *US* were designed. These last two stimulus presentation programs were also used for the third stage of the study, namely re-extinction.

Four stimulus presentation programs were designed for the final stage, reinstatement & extinction procedure, which was one year later from the manipulation in order to investigate long-term effects. Two of the programs were used for participants who acquired blue circle as CS^+ during acquisition and the other two were used for remaining participants who acquired yellow circle as CS^+ to counterbalance the order of the stimulus presentations after the reinstatement of the *US*. All four programs started with four only-*US* presentations (reinstatement), and then 10 CS^+ and 10 CS^- presentations without *US* (extinction) were made.

Psychophysiological Stimulation and Assessment. Electrical stimulation was delivered through a bar electrode (Model: EL350; BIOPAC Systems, Inc.) attached with a plaster to the right inner wrist. Electrode site was cleaned with alcohol and a piece of electrode cream (Model: EC2; Grass Technologies) was applied to the electrode prior to replacement. A linear isolated stimulator (Model: STMISOLA;

BIOPAC Systems, Inc.) charged by a stabilized current was used to deliver electrocutaneous stimulation. All participants determined level of the electrical stimulation themselves with the assistance of experimenter at the beginning of the first session, starting from a mild level (around 20V) and gradually increasing the level within three trials, in maximum, until the shock is adjusted to a level which was interpreted as "uncomfortable but not painful". Maximum level was 60V, as in the previous studies using electrical stimulation with human participants (e.g. Schiller et al., 2010). During the test trials shock was delivered manually for 100ms; on the other hand, for the experimental sessions presented shocks were lasted in 200ms. Participants were informed about the issue when electrical stimulation level was adjusted.

Skin conductance response, which results from electrodermal activity (EDA), was assessed using disposable snap electrodes (Model: EL507; BIOPAC Systems, Inc.) that were designed for EDA studies and pre-gelled with isotonic gel. The electrodes were affixed to palm of the left hand, after cleaning the electrode sites with alcohol.

Data Acquisition System. Electrodermal activity during the experimental sessions were obtained via MP150WSW-G Data Acquisition System which was coupled with the Bionomadix Wireless Pulse and EDA Amplifier BN- PPGED via an Universal Interface Module UIM100C (BIOPAC Systems, Inc.). An isolated digital interface (Model: STP100C; BIOPAC Systems, Inc.) module was also used to connect MP system to the computer running stimulus presentation programs in order to isolate digital inputs and outputs to and from the MP system.

AcqKnowledge™ (Model: 4.2; BIOPAC Systems, Inc.) software was used for recording and offline analysis of the data. This software was run in another computer (Intel® Core™ i5- 2400CPU, 3.10 GHz, 4 GB of RAM) connected to a 21.5″ monitor, with a screen resolution of 1920*1080 pixels, and refresh rate of 60 Hz in a separate control room for real-time monitoring of the measurements, which was next to the experimental room that computer-controlled experimental task was administered (See Figure 4).

Procedure

Experimental sessions were carried out in two sound proof adjacent (experimental and control) rooms (Figure 4). In each room there was a computer connected to a monitor. Computer in the experimental room was used for stimulus presentations designed in Superlab™ 4.5. On the other hand, MP system was started in the control room and computer running AcqKnowledge™4.2 was used for recording the data and following the participants' responses during the sessions. In addition, all experimental sessions were recorded with a video camera to ensure that the participants were fulfilling their duty in accordance with the experimental terms and conditions. Ones who failed to follow instructions during the study were not called for the next stages of the study and their data were excluded from further analysis.

Before the first experimental session, volunteers were informed about the study and given a "Participant Information Form" (Appendix B) explaining the study and a "Consent Form" (Appendix C) to sign, stating they were aware of their rights as participants, their participation in the experiment was on a volunteer basis and we

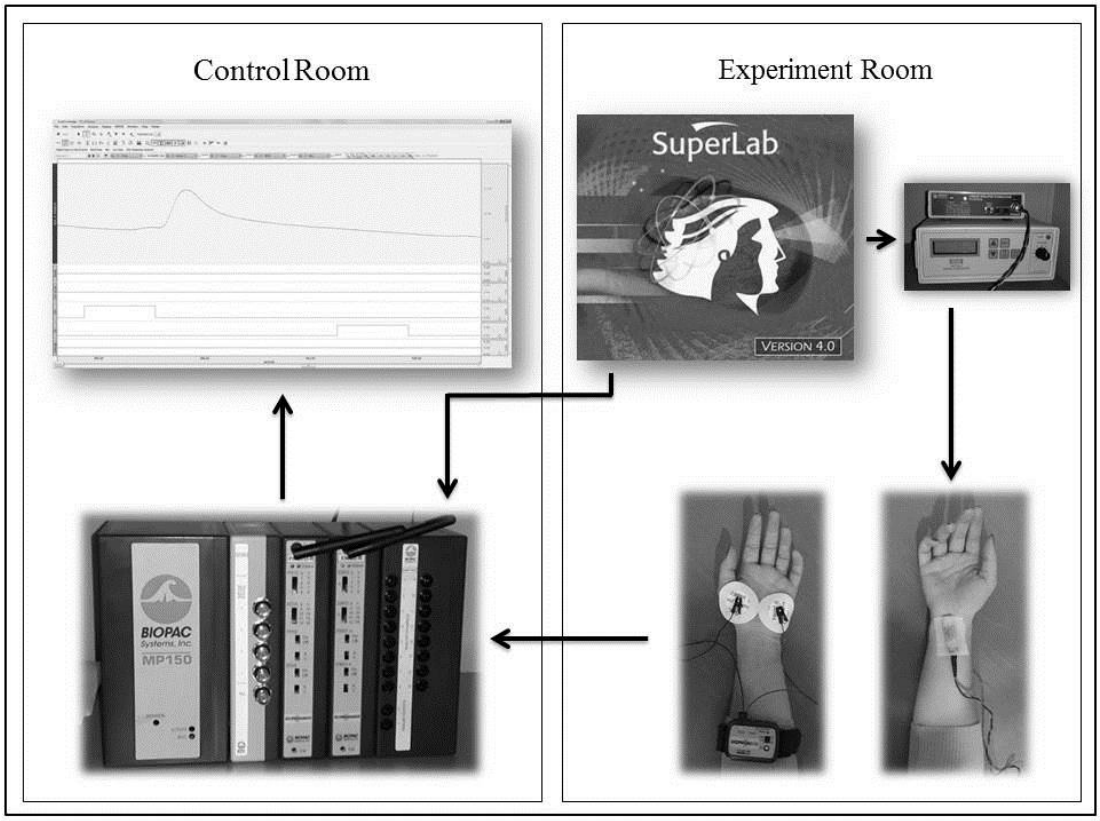

Figure 4. Integration of data acquisition system to experimental setup within experimental and control rooms. In the experimental room, a computer running Superlab software was used for stimulus presentations. Superlab also triggered the stimulator to deliver electrical stimulation to the participants' right wrist. In the control room, a computer running AcqKnowledge software recorded the stimulus presentation signals of Superlab and SCR data of the participants, which were acquired through MP system.

were allowed to use the acquired data for scientific purposes. Once they were agreed to continue to study, they filled out the "Participant Evaluation Form" (Appendix A). participant number in order to identify them through the experimental sessions. Also, these numbers were randomly assigned to experimental conditions previously; therefore, this helped experimenters to track the experimental conditions participant will be a part of, when arrived for next stages.

Afterwards, participants were taken to the experiment room. Before starting the stimulus presentation program, the stimulator was set to "ON" position and electrical stimulation electrode was attached to the participants' right inner wrist (Figure 5a). Level of the electrical stimulation was adjusted to the "uncomfortable but not painful" level (60V in maximum) as explained in the psychophysiological stimulation section. When participants decided to the level of the electrical stimulation, they were reminded that during the all experimental sessions this pre-determined level of electrical stimulation will be delivered to their right inner wrist, whenever required. So, adjustment of the electrical stimulation level was done only in the first day of the study and recorded to set the same level at the beginning of following sessions. It is important to note that even if there was no electrical stimulation during second (extinction) and third (re-extinction) stages, still electrical stimulation electrode was attached to the participants, electrical stimulator was set to "ON" position, and stimulation level was adjusted to the level that was previously decided by the participant. In order to measure electrodermal activity, disposable EDA electrodes were replaced to the palm of the left hand, specifically, to the thenar and hypothenar eminence (Figure 5b), before all experimental sessions. All electrode sites were cleaned with alcohol and waited until dry before attaching the electrodes.

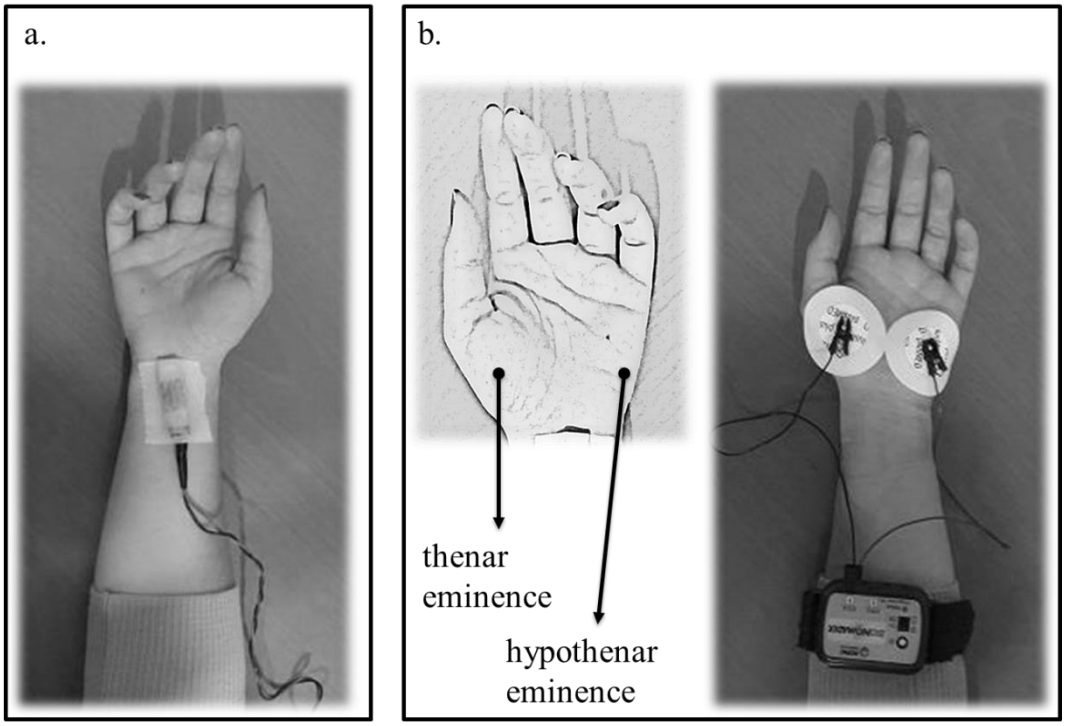

Figure 5. a) Attaching bar electrode to right inner wrist for electrical stimulation. b) Attaching electrodermal activity electrodes to thenar and hypothenar eminence of left hand.

Subjects were asked to sit still during the experiment and to use their right hand when they need to press a key, since electrodermal activity measurements are sensitive to the body movements and may cause motion artifacts. Participants were instructed to pay attention to the computer screen and try to understand the association between the circle on the screen and delivery of electrical stimulation. All experimental sessions began with a five-minute habituation period prior to the stimulus presentations so participants were expected to adapt to the experimental environment while electrodermal activity levels turn into baseline levels.

At the end of the experimental sessions, we asked participants whether they had received any electrical stimulation during the session to make sure that electrical stimulation was delivered properly during acquisition and reinstatement but not during extinction and re-extinction. Additionally, if they felt electrical stimulation, we asked what was on the screen while electrical stimulation delivered in order to see whether they paid attention to the task or not.

Reconsolidation update paradigm, as outlined by Schiller and her colleagues (2010) was followed as experimental procedure. This paradigm was formed by four consecutive stages:

1. Acquisition,
2. Extinction,
3. Re-extinction,
4. Examination of long-term effects (reinstatement & extinction).

Acquisition. In the first stage, participants underwent a differential Pavlovian fear conditioning procedure with partial reinforcement. *CS*s were blue and yellow

circles presented from computer screen and *US* was a mild shock given from the right inner wrist of participants (Figure 6). While one of the *CS*s was paired with the shock (CS^+) on a partial reinforcement schedule, other one was never paired with the shock (CS^-). Reinforcement rate was 38%. Partial reinforcement schedule was preferred for two reasons. First, we wanted to calculate acquisition, extinction and spontaneous recovery scores over *CR*s in the absence of *US* presentations. Second, when learning occurs with a partial reinforcement schedule, *CR* known to be more resistant to the extinction (Domjan, 2005). CS^+ and CS^- were counterbalanced across groups, so half of the participants had blue circle as CS^+ while the other half had yellow circle and vice versa for the CS^-. Fear conditioning paradigm included 26 trials in which 16 CS^+ and 10 CS^- presentations took place. Regarding the reinforcement schedule, 6 presentations of CS^+ co-terminated with the shock while remaining 10 CS^+ and 10 CS^- presentations were nonreinforced. Each *CS* was presented for 4000ms with 10s inter-trial intervals (*ITI*). *US* was delivered during the last 200ms of the CS^+ presentation in CS^++*US* trials. As explained previously on stimulus presentation programs section, presentation of the stimuli was done in a pseudorandom order. During acquisition procedure, skin conductance response of participants to given stimuli was collected. 24 hours after the acquisition stage, extinction procedure took place.

Extinction. The day after acquisition, the participants who had acquired fear successfully (will be explained in more detail in preparation of the data section), went through an extinction procedure in which all *CS*s were presented without *US*. Participants were split into three extinction conditions. Forming these groups we considered (a) whether or not participant will receive a reminder presentation before

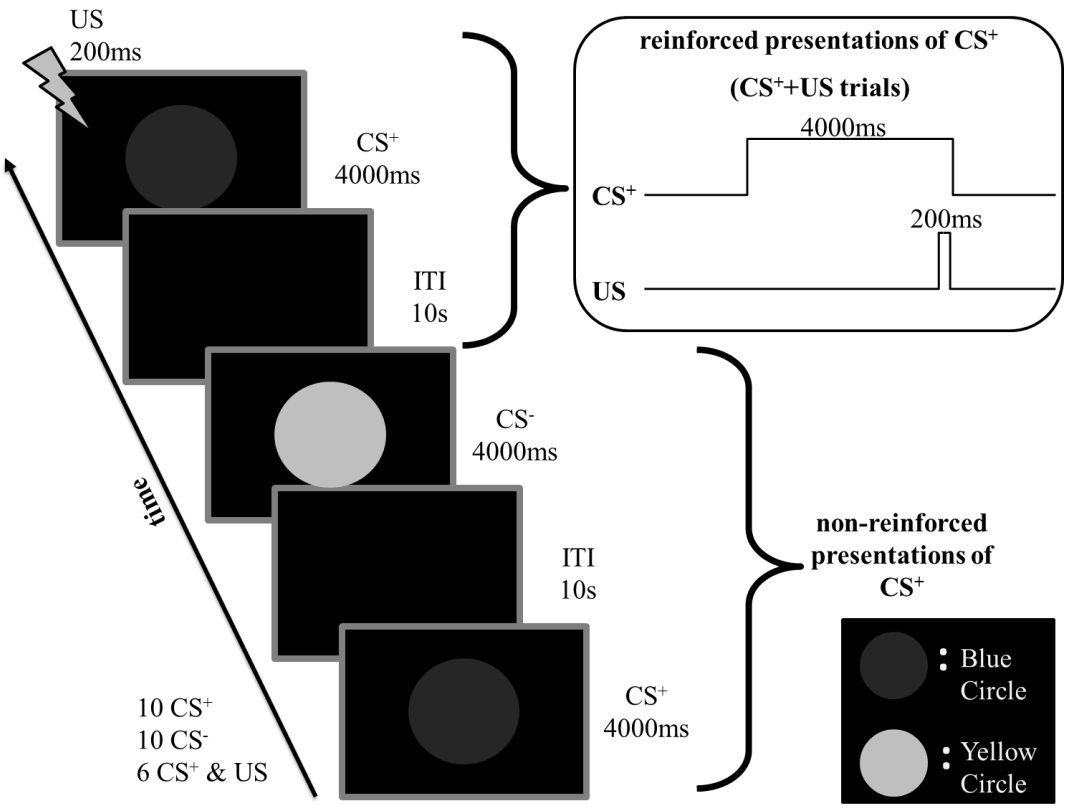

Figure 6. Experimental flow schema for acquisition stage.

the extinction, (b) if participant will receive a reminder, then when the extinction trials will begin. Consequently, extinction groups were:

- extinction 10 minutes after reminder,
- extinction 6 hours after reminder,
- extinction with no reminder.

At this stage, two groups (10 minutes and 6 hours after reminder conditions) received a single reminder prior to the extinction in order to reactivate the fear memory formed in the acquisition stage. Reminder was a single CS^+ presentation for 4000ms which was not paired with *US*. Therefore, once memory was reactivated, first group (10 minutes after reminder condition) had a ten-minute break in which they watched a pre-selected video so they did not have to leave the experimental room. Following the video, they went through extinction which was within the reconsolidation window. Second group (6 hours after reminder condition) had a six-hour break following reactivation and then went through extinction after reconsolidation window closed. On the other hand, latter group (no reminder condition) did not receive any reminder presentation before extinction and directly underwent extinction.

Extinction procedure included 22 trials, 11 CS^+ and 11 CS^- presentations without *US* were carried out in no reminder group, but 10 CS^+ and 11 CS^- presentations without *US* were performed in 10 minutes and 6 hours after reminder groups since they already had one CS^+ as reminder prior to the extinction. As a result, all groups received equal numbers of *CS*s in the second stage of the study. As in acquisition session, all *CS*s were presented for 4000ms with 10s inter-trial interval and skin conductance response of participants was collected during the extinction.

Re-extinction. Re-extinction was carried out in order to observe spontaneous recovery of fear responses which were extinguished through second stage, under three different extinction conditions. This stage had exactly same procedure with no reminder condition of extinction. All participants received 11 CS^+ and 11 CS^- presentations without *US*, lasted for 4000ms each, in random order with 10s inter-trial interval. Skin conductance response was collected from all participants.

Time point that participants went through re-extinction was manipulated in three levels for re-extinction variable. According to this manipulation one third of the each extinction group received re-extinction procedure in one of the three re-extinction conditions:

- 24 hours after extinction,
- 15 days after extinction,
- 3 months after extinction.

For instance, 1/3 of participants in extinction 10 minutes after reminder condition underwent re-extinction 24 hours after extinction, 1/3 of them underwent re-extinction 15 days after extinction and remaining 1/3 of them underwent re-extinction 3 months after extinction. Other two extinction groups (6 hours after reminder and no reminder) were split into three re-extinction groups in the same way (see Figure 3 in participants section).

Examination of Long-term Effects. Approximately one year after extinction, participants were invited to the laboratory, in order to observe long-term effects of behavioral manipulation done within reconsolidation. Thirty nine participants out of 111 agreed to join this follow-up. This stage included both reinstatement and extinction procedures. Firstly, in order to reinstate *US*, *US* was presented without any

CS for four times, each lasted for 200ms with 10s *ITI*. Right after *US* presentations, participants went through extinction in which 10 CS^+ and 10 CS^- were presented without *US*. As in all other trials, each *CS* lasted for 4000ms with 10s inter-trial interval. During the stage, skin conductance response was recorded.

Preparation of Skin Conductance Data for Analysis

As mentioned before, data acquisition was done via MP systems and recorded by AcqKnowledge^{TM}4.2. A recorded data sample can be seen at Figure 7. In this figure, first channel shows the electrodermal activity of participant during the experimental session, and following channels shows the time periods of stimulus delivery made by Superlab^{TM} 4.5, only *US*, CS^++*US*, CS^+, CS^-, and instructions, respectively.

Before the further analysis of the data, acquisition, extinction, re-extinction, and reinstatement scores of the participants were calculated. As it has been mentioned outset there were certain criteria related to acquisition and extinction to include the data of each participant for further analysis. Therefore, acquisition and extinction scores were calculated to make sure that participants had acquired the fear during acquisition and it was extinguished through extinction period. Since we intended to compare fear recovery scores of participants in different extinction and re-extinction conditions to see the effects of experimental manipulations, acquisition and extinction of fear were prerequisites for including participants' data in further analysis.

On the other hand, re-extinction and reinstatement scores were calculated to compute two distinct recovery scores. One was spontaneous recovery score showing

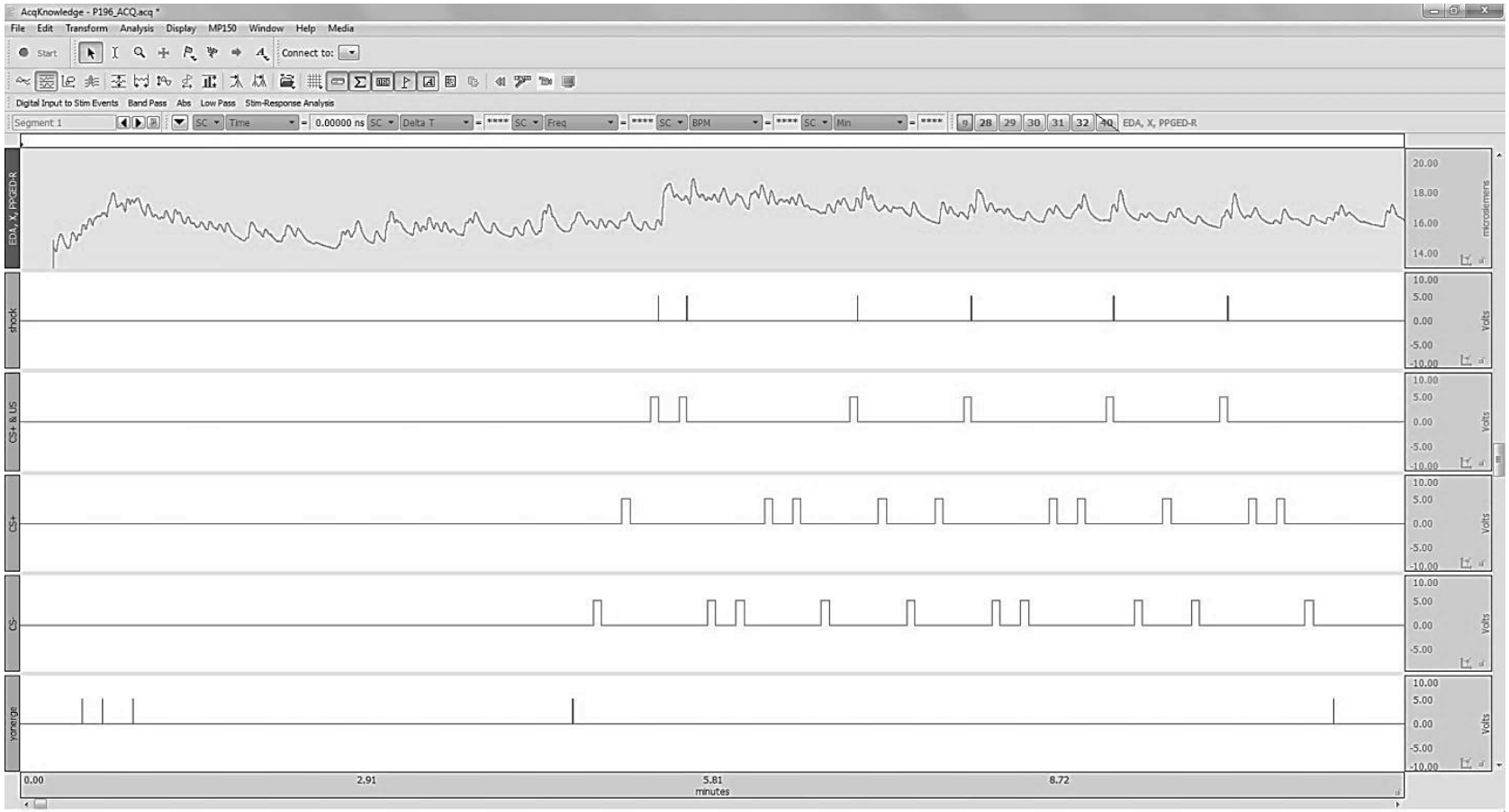

Figure 7. Data sample, recorded via AcqKnowledge^{TM}4.2.

how much fear recovered from the end of the extinction till the beginning of the re-extinction. The other one was recovery score, for long-term effects examination of experimental manipulations, showing how much fear recovered from the end of the re-extinction till the beginning of the extinction in the fourth stage, depending on reinstatement. During the statistical analysis main comparisons were done for these two scores.

In the following parts of this section, calculation of mentioned scores, which was adopted from Schiller and her colleagues (2010), will be explained. However, before moving to calculation of these scores, it is important to address the methodology used in measurement of skin conductance response elicited by experimental stimulations.

While measuring individual skin conductance responses to the specific stimulus, level of the response was determined as base to peak difference (amplitude, in microsiemens, μs) from the first response (waveform) in the 500ms to 50000ms time interval following the onset of stimulus (Figure 8). In order to consider a waveform as a response to the corresponding stimulus, base (starting point) of the waveform must be within this time window and must have an amplitude value greater than 0.02μs which was minimum skin conductance response criterion.

Calculation of Acquisition Score. Acquisition score was calculated for each participant from skin conductance responses given to all 6 CS^{+}+*US* (*US* trials) paired trials, as being of the last 5 trials of 10 CS^{+} presentations and last 5 trials of 10 CS^{-} presentations in acquisition stage. For each trial, amplitude of a response was calculated by subtracting peak microsiemens value from the base microsiemens value in the 500ms to 50000ms time interval following the onset of stimulus. Then,

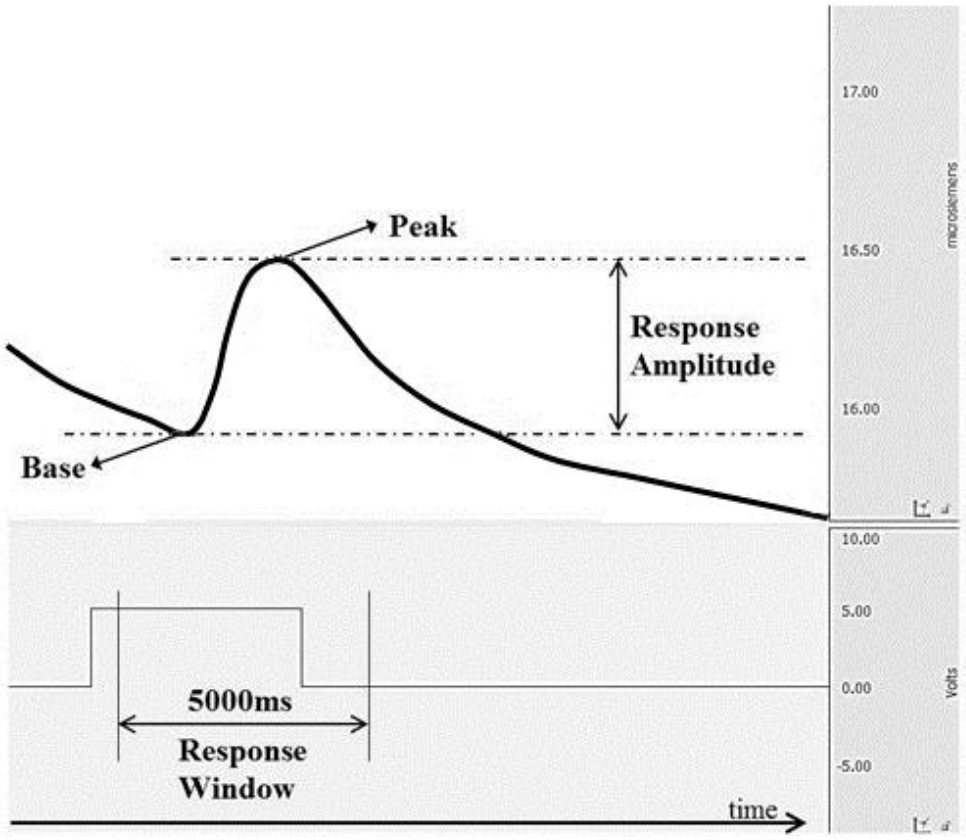

Figure 8. Measurement of skin conductance response given to a single stimulus

square root transformation was applied to normalize distribution for all 16 calculated values since it is suggested that in general, amplitude variable might have had a negatively skewed distribution (Boucsein, 2012). Transformed values of 6 *US* trials were averaged and each transformed value of the last 5 CS^+ and 5 CS^- were divided by this averaged value of *US*. Therefore, each single response given to the CS^+ and CS^- was scaled with participants' own unconditioned response. Difference scores were calculated between scaled CS^+ and CS^- responses and finally by averaging these difference scores, acquisition score was obtained (see Table 2 for an example). In order to decide whether acquisition took place or not, criterion proposed by Schiller et al. (2010) was used. According to this, participants who had acquisition scores "larger than 0.10" regarded as ones who acquired the fear and developed conditioned responses to CS^+. Anyone whose acquisition score was less than 0.10 was excluded from further analysis.

Calculation of Extinction Score. Extinction scores were calculated for each participant who acquired fear; in other words, had a 0.10 acquisition score at least. Extinction score was derived from skin conductance responses given to all 6 *US* trials in acquisition, last 5 trials of 11 CS^+ presentations and last 5 trials of 11 CS^- presentations in extinction stage. Firstly, amplitude of each response to the mentioned trials was obtained just like in the calculation of acquisition score. Then, for amplitude values of last 5 CS^+ and 5 CS^-, square root transformation was applied to normalize distribution. Transformed values of last 5 CS^+ and 5 CS^- were divided by average value of *US* which was gathered from acquisition calculations. Difference scores were calculated between scaled CS^+ and CS^- responses and finally by

Table 2. *Calculation of Acquisition Score*

CS^++US (Acquisition)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	
1	14.65607	11.50818	3.14789	1.77423	
2	14.46380	14.11590	0.34790	0.58983	
3	13.62915	11.28845	2.34070	1.52993	
4	13.07831	11.25030	1.82801	1.35204	
5	12.74872	11.36017	1.38855	1.17837	
6	13.70239	11.43494	2.26745	1.50581	
			Mean	**1.32170**	
CS^+ (Acquisition)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	Weighted CS^+ Value
6	11.43951	10.83374	0.60577	0.77831	0.58887
7	11.32660	10.67352	0.65308	0.80813	0.61143
8	12.36877	12.08343	0.28534	0.53417	0.40416
9	13.78784	13.09509	0.69275	0.83232	0.62973
10	12.78229	12.30469	0.47760	0.69109	0.52288
CS^- (Acquisition)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	Weighted CS^- Value
6	12.93182	12.63428	0.29754	0.54547	0.41270
7	12.01324	11.56616	0.44708	0.66864	0.50589
8	12.68463	12.42676	0.25787	0.50781	0.38421
9	11.22742	11.20148	0.02594	0.16106	0.12186
10	11.77673	11.47003	0.30670	0.55381	0.41901
					Difference Scores
					0.17617
					0.10554
					0.01995
					0.50787
					0.10387
				Acquisition Score	**0.18268**

averaging these differences, extinction score was obtained (see Table 3 for an example). Extinction criterion was having less than 0.10 extinction score to be able to say that extinction took place (Schiller et. al., 2010). Therefore, participants who had extinction scores "less than 0.10" regarded as the ones whose fear extinguished and they did not show conditioned responses to CS^+ anymore at the end of the extinction stage. Anyone who had extinction score larger than 0.10 was excluded from further analysis. In addition, difference score between last CS^+ and last CS^- was saved to use in calculation of spontaneous recovery score.

Calculation of Re-extinction Score. Re-extinction scores were calculated for participants with less than 0.10 extinction score. This score was derived from skin conductance responses given to all 6 *US* trials in acquisition, first trial of 11 CS^+ presentations and first trial of 11 CS^- presentations in re-extinction stage. Amplitudes of skin conductance response for first CS^+ and fisrt CS^- trials were found just like in the previous sessions by subtracting base from the peak value in certain time window that stimulus was presented. Then, square root transformation was applied to both *CS* values. Transformed values of the CS^+ and CS^- were divided by average value of *US* gathered from acquisition calculations. Difference between averaged CS^+ and CS^- responses was calculated and saved as re-extinction score for later use in order to compute spontaneous recovery score (see Table 4 for an example).

Additionally, the same computational procedure (square root transformation to response amplitude and dividing transformed values with average *US* response) was performed to find out the difference score between the last trial of 11 CS^+ presentations and the last trial of 11 CS^- presentations in re-extinction stage to use

Table 3. *Calculation of Extinction Score*

CS^++US (Acquisition)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	
1	14.65607	11.50818	3.14789	1.77423	
2	14.46380	14.11590	0.34790	0.58983	
3	13.62915	11.28845	2.34070	1.52993	
4	13.07831	11.25030	1.82801	1.35204	
5	12.74872	11.36017	1.38855	1.17837	
6	13.70239	11.43494	2.26745	1.50581	
			Mean	**1.32170**	
CS^+ (Extinction)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	Weighted CS^+ Value
7	10.27374	10.12421	0.14953	0.38669	0.29257
8	10.54230	9.95178	0.59052	0.76845	0.58141
9	10.64148	10.05096	0.59052	0.76845	0.58141
10	10.61859	10.22186	0.39673	0.62987	0.47656
11	10.86120	10.31799	0.54321	0.73703	0.55764
CS^- (Extinction)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	Weighted CS^- Value
7	0.00000	0.00000	0.00000	0.00000	0.00000
8	10.45685	9.94110	0.51575	0.71816	0.54336
9	10.16388	9.84192	0.32196	0.56742	0.42931
10	11.14349	10.02655	1.11694	1.05685	0.79962
11	11.29303	10.31189	0.98114	0.99053	0.74943
					Difference Scores
					0.29257
					0.03805
					0.15211
					-0.32306
					-0.19180
				Extinction Score	**-0.006425**

Table 4. *Calculation of Re-extinction Score*

CS^++US (Acquisition)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	
1	14.65607	11.50818	3.14789	1.77423	
2	14.46380	14.11590	0.34790	0.58983	
3	13.62915	11.28845	2.34070	1.52993	
4	13.07831	11.25030	1.82801	1.35204	
5	12.74872	11.36017	1.38855	1.17837	
6	13.70239	11.43494	2.26745	1.50581	
			Mean	**1.32170**	
CS^+ (Re-extinction)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	Weighted CS^+ Value
1	14.89715	13.82751	1.06964	1.03423	0.78250
11	0.14117	0.11251	0.02866	0.16929	0.12809
CS^- (Re-extinction)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	Weighted CS^- Value
1	15.48614	13.98926	1.49688	1.22347	0.92568
11	0.09671	0.05438	0.04233	0.20574	0.15566
					Difference Scores
				Re-extinction Score	**-0.14318**
					-0.02758

together with reinstatement score in order to calculate recovery score which was used in examination of long-term effects of the manipulation.

Calculation of Reinstatement Score. Reinstatement score was derived for each participant from skin conductance responses given to all 4 *US* trials during reinstatement, first trial of 10 CS^+ presentations and first trial of 10 CS^- presentations in following extinction procedure. For each one of those trials, calculation of response amplitude was done in the same way with previous sessions (base to peak difference). Then, square root transformation was applied to all 4 calculated *US* values. Transformed values of 4 *US* trials then were averaged, and transformed value of the first CS^+ and CS^- were divided by this averaged value of *US*. Finally, the difference between the averaged CS^+ and CS^- was recorded as reinstatement score (see Table 5 for an example). This score was later used to calculate recovery score.

Statistical Analysis

After all required scores were calculated, a preliminary examination of the data was performed; dependent measures were explored regarding distribution of the data. Consequently, extreme values were excluded from the analysis. In order to find extreme values, *z*-scores were used as suggested by Field (2009). According to this, all dependent measures were converted to *z*-score and then z-scores with absolute value greater than 3.29 were detected as extreme values and deleted from the data. In total, 3 extreme scores were deleted from extinction and re-extinction scores.

After data overview, mean differential skin conductance response of acquisition, extinction and re-extinction were compared in terms of conditioned stimuli used in the study to verify that these two stimuli did not differ. As can be remembered from previous sections, blue and yellow circles were used as CS^+ and

Table 5. *Calculation of Reinstatement Score*

CS^++US (Reinstatement)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	
1	8.43506	7.37762	1.05744	1.02832	
2	8.74023	7.85522	0.88501	0.94075	
3	9.40246	9.07287	0.32959	0.57410	
4	9.09729	8.70056	0.39673	0.62987	
			Mean	**0.79326**	
CS^+ (Reinstatement)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	Weighted CS^+ Value
1	7.51495	6.16607	1.34888	1.16141	1.46410
CS^- (Reinstatement)					
Presentation Order	Peak Value	Base Value	Response Amplitude	Square Root Transformation	Weighted CS^- Value
1	12.98065	11.10229	1.87836	1.37053	1.72773
					Difference Score
				Reinstatement Score	**-0.26362**

CS^- in a counterbalanced fashion. Therefore, by conducting independent *t*-test, mean acquisition score of participants who took blue circle as CS^+ were compared to mean acquisition score of participants who took yellow circle as CS^+. The same comparisons between two groups of participants were also made for extinction and re-extinction scores by independent *t*-tests.

Afterwards, procedural controls were done in order to see acquisition and extinction of fear response. Repeated measures of ANOVA allowed us to observe significant linear increase as acquisition trials proceed and significant linear decrease as extinction trials proceed in differential skin conductance response via linear trend analysis.

After stimulus control and procedural controls were completed, manipulation analysis were conducted via 3 (extinction group: 10 minutes after reminder, 6 hours after reminder and no reminder) x 3 (re-extinction group: 24 hours, 15 days and 3 months) factorial ANOVA. Therefore, effects of independent variables on mean differential skin conductance responses of acquisition, extinction, spontaneous recovery and recovery scores were examined. For any significant effect observed, planned contrasts (Helmert contrast) were used. Alpha level of 0.05 was used for all statistical comparisons.

CHAPTER 3

Results

Control and Procedural Analysis

In this section, first, the effect of conditioned stimuli on differential skin conductance response (derived via CS^+ minus CS^-) was examined in order to see whether there is a difference between using either blue circle or yellow circle as a CS^+ in different stages of the study. Then, a procedural control was performed by using differential skin conductance response in the first two stages of the study to make sure that employed differential fear learning paradigm worked throughout the stages, namely acquisition and extinction.

Stimulus Control. Mean differential skin conductance responses collected from participants during acquisition, extinction and re-extinction stages were compared regarding the stimulus used as CS^+, in order to see if using either blue or yellow circle as CS^+ makes any difference on differential skin conductance response during the different stages of the study.

The results revealed that mean differential skin conductance responses of acquisition (Figure 9), extinction (Figure 10), and re-extinction (Figure 11) stages were independent of CS^+ (blue or yellow circle) used. According to the independent *t*-test results, difference between mean differential skin conductance responses of participants who saw blue circle (M = .32, SE = .02) or yellow circle (M = .30, SE = .02) as a CS^+ during acquisition ($t_{(109)}$ = .84, p > .05), difference between mean differential skin conductance responses of participants whose CS^+ was blue circle (M = -.05, SE = .01) or yellow circle (M = -.02, SE = .01) during extinction ($t_{(109)}$ = 1.71,

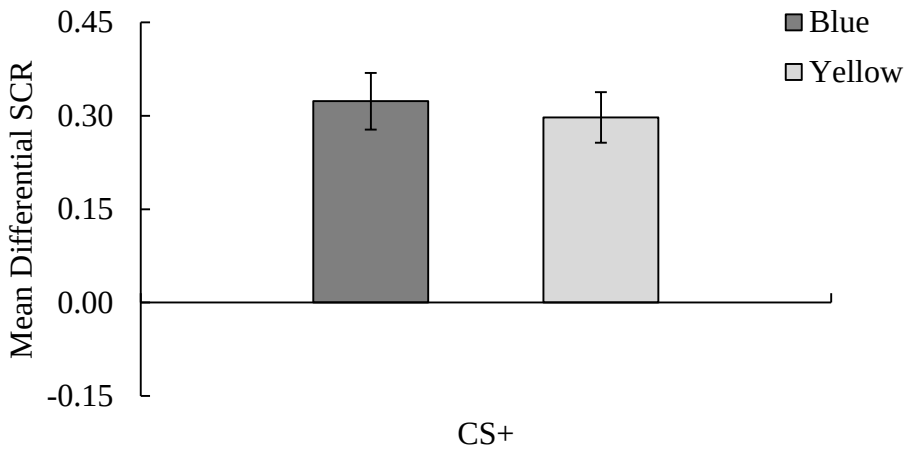

Figure 9. Mean differential skin conductance responses obtained in the acquisition phase as responses to blue and yellow circles that were used as CS^{+} (Error bars indicate 95% confidence intervals).

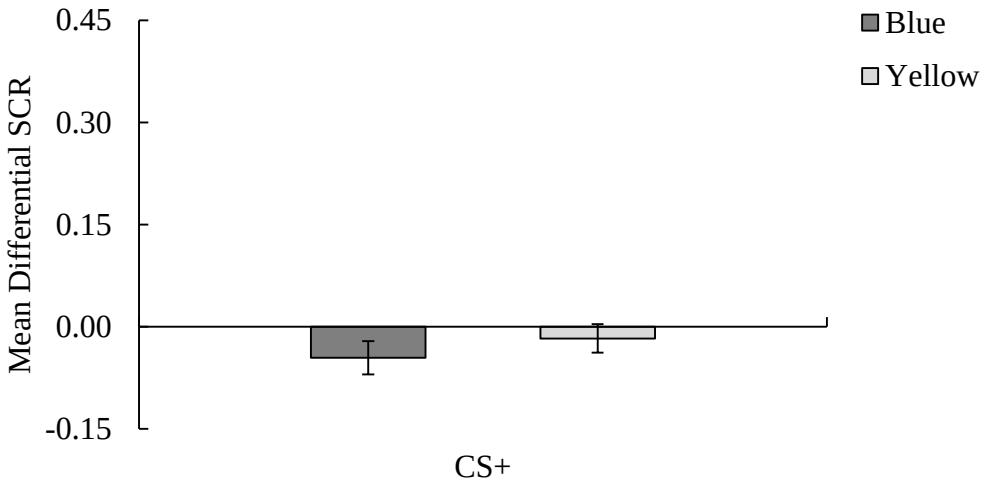

Figure 10. Mean differential skin conductance responses obtained in the extinction phase as responses to blue and yellow circles that were used as CS^{+} (Error bars indicate 95% confidence intervals).

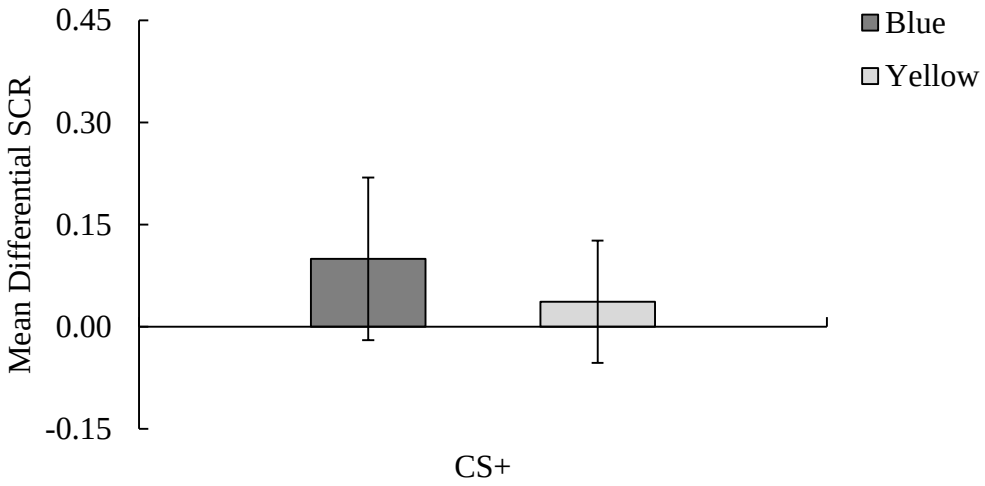

Figure 11. Mean differential skin conductance response obtained in the re-extinction phase as responses to blue and yellow circles that were used as CS^{+} (Error bars indicate 95% confidence intervals).

$p > .05$), and difference between mean differential skin conductance responses of participants whose CS^+ was blue circle ($M = .10$, $SE = .06$) or yellow circle ($M = .04$, $SE = .05$) during re-extinction ($t_{(109)} = .83$, $p > .05$) did not reach statistical significance.

Since using blue or yellow circle as CS^+ did not lead any bias in mean differential skin conductance responses during acquisition, extinction and re-extinction stages, type of CS^+ used was collapsed into one level as arbitrary stimulus and all participants treated same during following analysis in terms of acquired CS^+.

Procedural Control. Prior to the statistical manipulation check, procedural control analyses were conducted to control expected conditioned response patterns of participants as a result of acquisition and extinction trainings. In order to do this, regardless of the groups that participants were a part of, mean differential skin conductance responses derived from 10 CS^+ and 10 CS^- trials in acquisition (Figure 12) and 11 CS^+ and 11 CS^- trials in extinction (Figure 13) were computed.

By doing so, two basic questions related to employed procedure were aimed to answer:

1) Did participants acquire the fear during acquisition trials?
2) If fear is acquired, was it extinguished during extinction trials?

Figure 12 shows that skin conductance responses given to both CS^+ and CS^- are similar at the beginning of acquisition trials. As acquisition trials proceed CS^+ comes to elicit conditioned fear responses and the difference between the responses elicited by CS^+ and CS^- increases as a function of trial. On the other hand, as can be seen in Figure 13, within the last trials of extinction, observed difference between skin

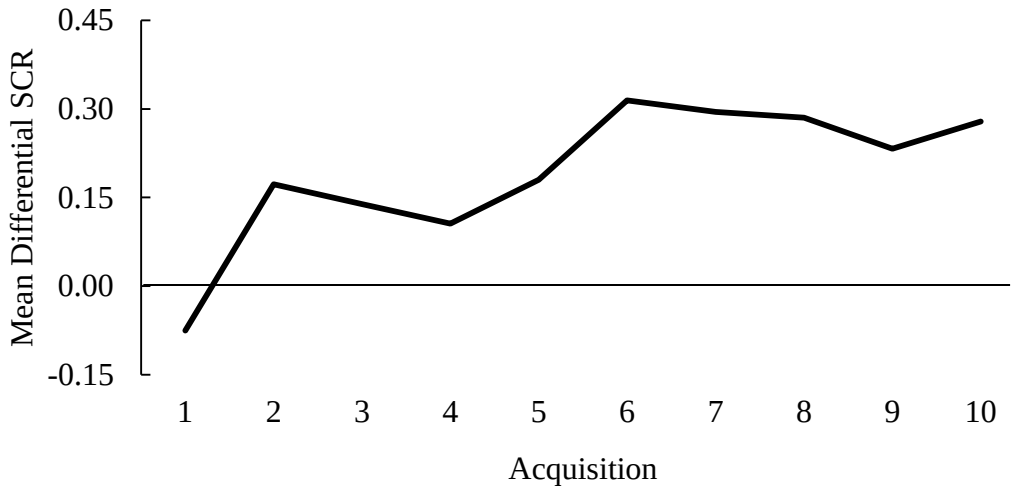

Figure 12. Mean differential skin conductance responses for acquisition trials.

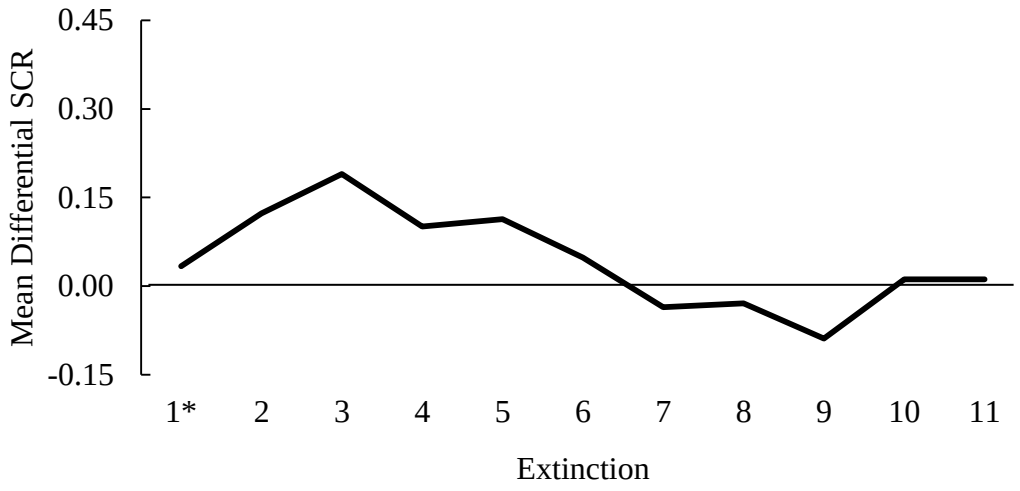

Figure 13. Mean differential skin conductance responses for extinction trials (* indicates reminder trial prior to the extinction in 10 minutes and 6 hours groups).

conductance responses given to CS^+ and CS^- disappears and returns to its initial level, in other words, conditioned fear response given to CS^+ extinguishes. Therefore, two separate linear trend analyses conducted to test statistical significance of observed linear increase during acquisition and linear decrease during extinction.

Trend analysis of acquisition data showed that observed linear increase in differential skin conductance response throughout acquisition trials was statistically significant, $F_{(1, 110)} = 72.98$, $p < .05$, partial $\eta^2 = .40$. The same analysis of extinction data revealed that linear decrease in differential skin conductance response throughout extinction trials was statistically significant as well, $F_{(1, 110)} = 25.02$, $p < .05$, partial $\eta^2 = .19$.

Manipulation Analysis

In the scope of manipulation analysis, effects of independent variables (extinction and re-extinction manipulations) on acquisition, extinction and spontaneous recovery scores were examined.

Acquisition. Mean acquisition scores of participants according to their extinction and re-extinction groups can be seen from Figure 14 and Figure 15, respectively. For acquisition phase, it was expected that mean differential skin conductance response (acquisition score) of participants in three different extinction groups and in three different re-extinction groups should be similar since two main manipulations did not take place yet. Likewise, for extinction and re-extinction interaction, mean acquisition scores (Figure 16) were expected to be similar. Otherwise, it would create a bias in the main results of study from the beginning of the experiment. Therefore, in order to see whether there were any effects of independent variables on acquisition score, derived from the first stage of the study,

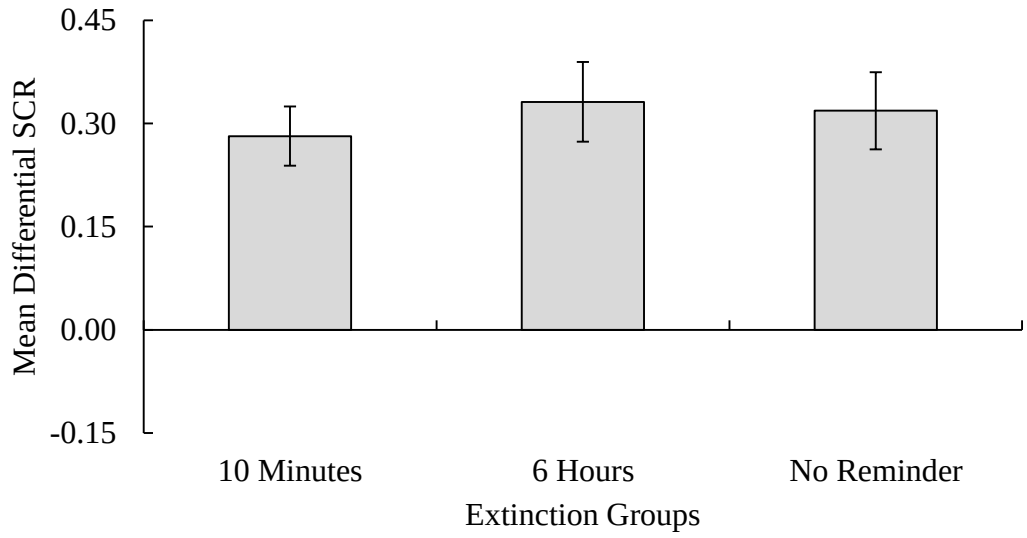

Figure 14. Mean differential skin conductance response for acquisition score with respect to extinction conditions (Error bars indicate 95% confidence intervals).

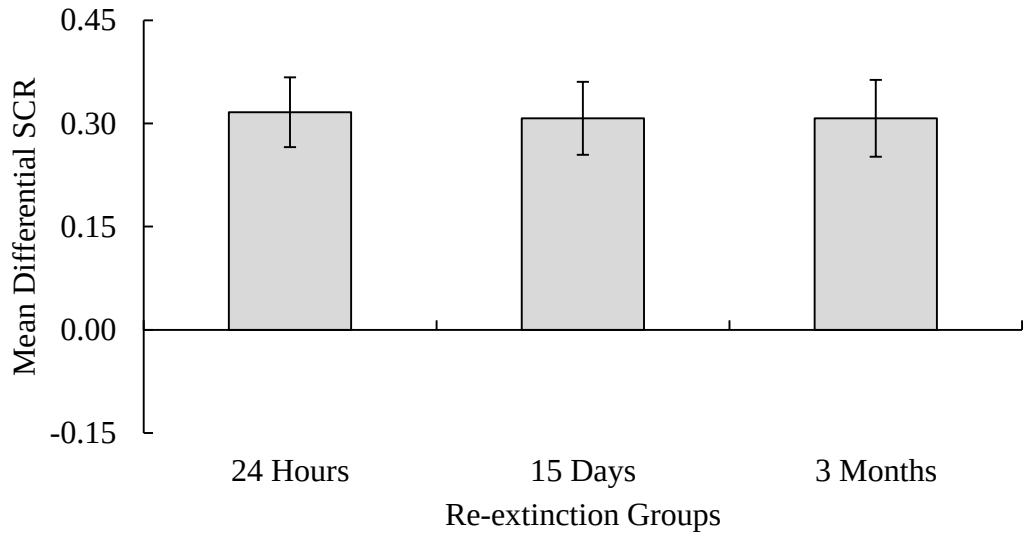

Figure 15. Mean differential skin conductance response for acquisition score with respect to re-extinction conditions (Error bars indicate 95% confidence intervals).

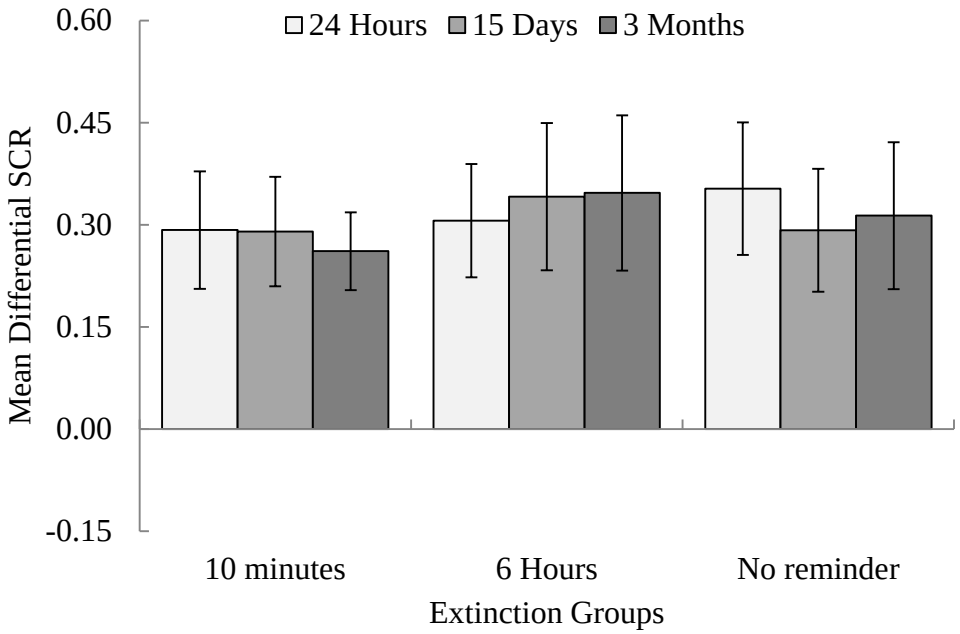

Figure 16. Mean differential skin conductance response for acquisition score with respect to extinction depending on re-extinction (Error bars indicate 95% confidence intervals).

3 (extinction group: 10 minutes after reminder, 6 hours after reminder and no reminder) x 3 (re-extinction group: 24 hours, 15 days and 3 months) factorial ANOVA was conducted.

Results indicated that there were nonsignificant main effect of extinction manipulation on mean differential skin conductance response for acquisition phase, $F_{(2, 102)} = .90$, $p > .05$. Extinction 10 minutes after reminder group ($M = .28$, $SE = .02$), extinction 6 hours after reminder group ($M = .33$, $SE = .03$), and extinction with no reminder group ($M = .32$, $SE = .03$) did not differ from each other in terms of acquisition scores regardless of re-extinction manipulation. Similarly, main effect of re-extinction manipulation on mean differential skin conductance response for acquisition phase was not significant, $F_{(2, 102)} = .04$, $p > .05$. Independent of extinction manipulation, acquisition scores of participants in 24 hours after extinction ($M = .32$, $SE = .03$), 15 days after extinction ($M = .31$, $SE = .03$), and 3 months after extinction ($M = .31$, $SE = .03$) groups were similar. Moreover, extinction*re-extinction interaction effect on acquisition score was not found to be significant, $F_{(4, 102)} = .36$, $p > .05$. Acquisition scores of participants in different extinction conditions did not change depending on re-extinction conditions. Therefore, acquisition score of participants in 10 minutes after reminder condition were similar for participants who underwent re-extinction 24 hours after extinction ($M = .29$, $SE = .04$), 15 days after extinction ($M = .29$, $SE = .04$), and 3 months after extinction ($M = .26$, $SE = .03$). Acquisition score of participants in 6 hours after reminder condition were also similar for participants who underwent re-extinction 24 hours after extinction ($M = .31$, $SE = .04$), 15 days after extinction ($M = .34$, $SE = .06$), and 3 months after extinction ($M = .35$, $SE = .06$). Finally, in no reminder condition, acquisition scores of

participants who underwent re-extinction 24 hours after extinction (*M* = .35, *SE* = .05), 15 days after extinction (*M* = .29, *SE* = .05), and 3 months after extinction (*M* = .31, *SE*= .06) were similar like in other two re-extinction conditions. Thus, just as we expected, acquisition scores were similar for all conditions.

Extinction. Regarding different extinction (Figure 17) and re-extinction (Figure 18) conditions, mean extinction scores of participants were expected to be similar since the effect of extinction manipulation done in this stage should display itself in spontaneous recovery scores. For the same reasons, no effect of interaction between extinction and re-extinction groups were expected on extinction scores (Figure 19). Therefore, in order to eliminate any bias, effects of independent variables on mean differential skin conductance response (extinction score), derived from the extinction phase of the study, was examined via 3 (extinction group: 10 minutes after reminder, 6 hours after reminder and no reminder) x 3 (re-extinction group: 24 hours, 15 days and 3 months) factorial ANOVA.

According to the results, main effect of extinction manipulation on mean differential skin conductance response for extinction phase was not statistically significant, $F_{(2, 102)} = 2.17$, $p > .05$. Extinction 10 minutes after reminder group (*M* = -.06, *SE* = .02), extinction 6 hours after reminder group (*M* = -.01, *SE* = .01), and extinction with no reminder group (*M* = -.03, *SE* = .01) did not differ from each other in terms of extinction scores regardless of re-extinction manipulation. Main effect of re-extinction manipulation on mean differential skin conductance response for extinction phase was not statistically significant, $F_{(2, 102)} = .00$, $p > .05$. Extinction scores of participants in 24 hours after extinction (*M* = -.03, *SE* = .02), 15 days after extinction (*M* = -.03, *SE* = .01), and 3 months after extinction (*M* = -.03, *SE* = .02)

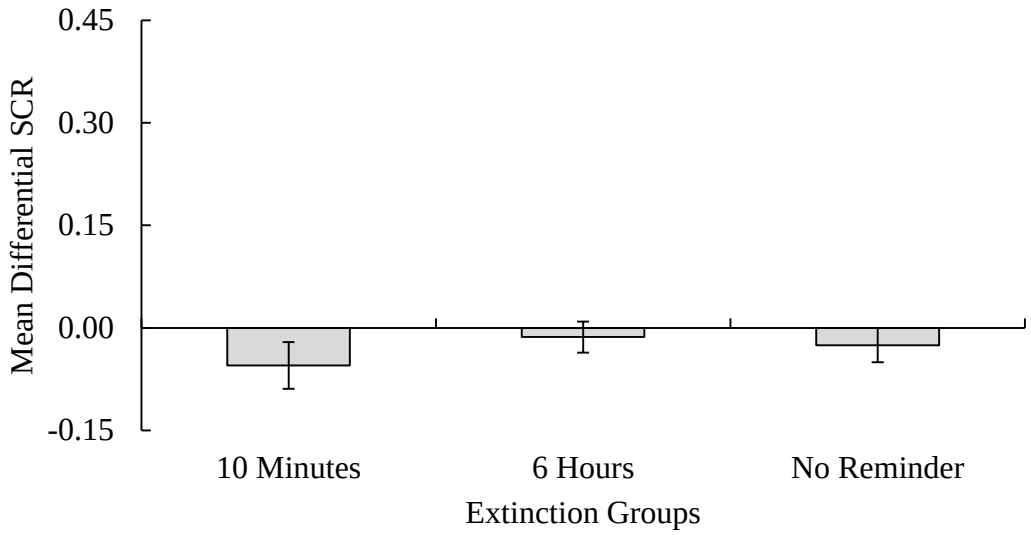

Figure 17. Mean differential skin conductance response for extinction score with respect to extinction conditions (Error bars indicate 95% confidence intervals).

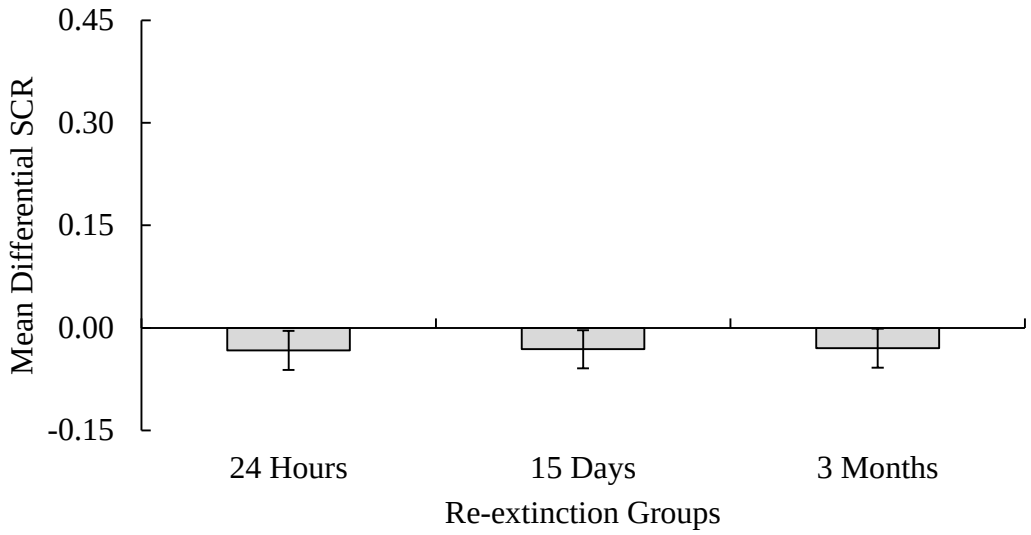

Figure 18. Mean differential skin conductance response for extinction score with respect to re-extinction conditions (Error bars indicate 95% confidence intervals).

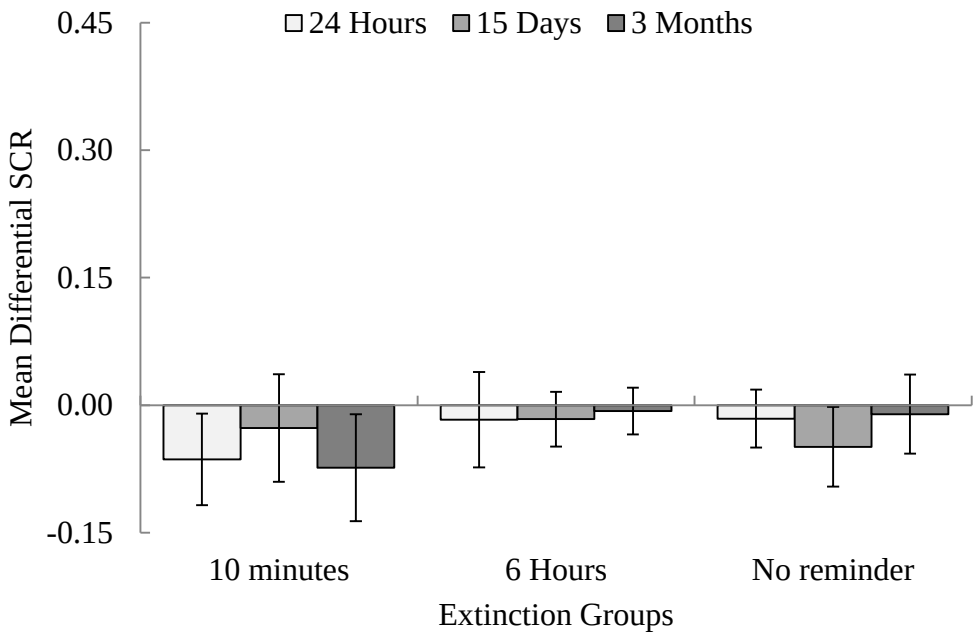

Figure 19. Mean differential skin conductance response for extinction score with respect to extinction conditions (Error bars indicate 95% confidence intervals).

groups were similar independent of their extinction condition. There was also a nonsignificant interaction between extinction and re-extinction, $F_{(4, 102)} = .87$, $p > .05$. Extinction scores obtained in 10 minutes after reminder condition were similar to those who underwent re-extinction 24 hours after extinction ($M = -.06$, $SE = .03$), 15 days after extinction ($M = -.03$, $SE = .03$), and 3 months after extinction ($M = -.07$, $SE = .03$). Extinction score of participants in 6 hours after reminder condition were also similar to the scores of participants who underwent re-extinction 24 hours after extinction ($M = -.02$, $SE = .03$), 15 days after extinction ($M = -.02$, $SE = .02$), and 3 months after extinction ($M = -.01$, $SE = .01$). In no reminder condition, extinction scores of participants who underwent re-extinction 24 hours after extinction ($M = -.02$, $SE = .02$), 15 days after extinction ($M = -.05$, $SE = .02$), and 3 months after extinction ($M = -.01$, $SE = .02$) were similar, as well. To conclude, examination of extinction phase showed that extinction scores of participants did not differ by the condition they were assigned.

Spontaneous Recovery. Conditioned responses, acquired through first stage of the study and extinguished during second stage, were examined in terms of spontaneous recovery. Spontaneous recovery scores were calculated by subtracting the last difference score in extinction from re-extinction score (first difference score in re-extinction). To clarify, difference between differential skin conductance response derived from the first trial of re-extinction and the last trial of extinction was used as an index of spontaneous recovery of fear. Figure 20 and Figure 21 represent mean differential skin conductance response of spontaneous recovery scores of participants which were calculated both for extinction and re-extinction variables, respectively. Mean differential skin conductance responses for

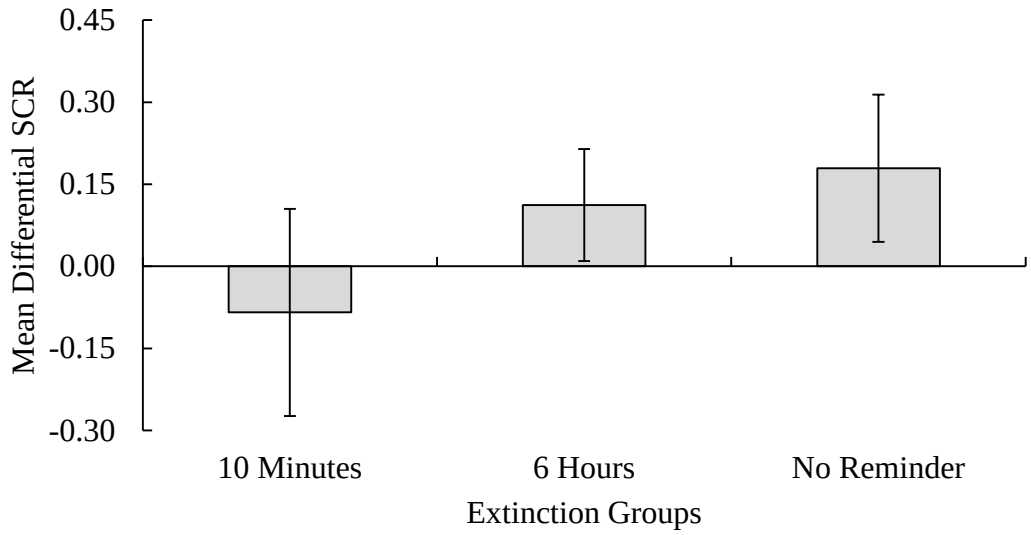

Figure 20. Mean differential skin conductance response for spontaneous recovery scores with respect to extinction conditions (Error bars indicate 95% confidence intervals)

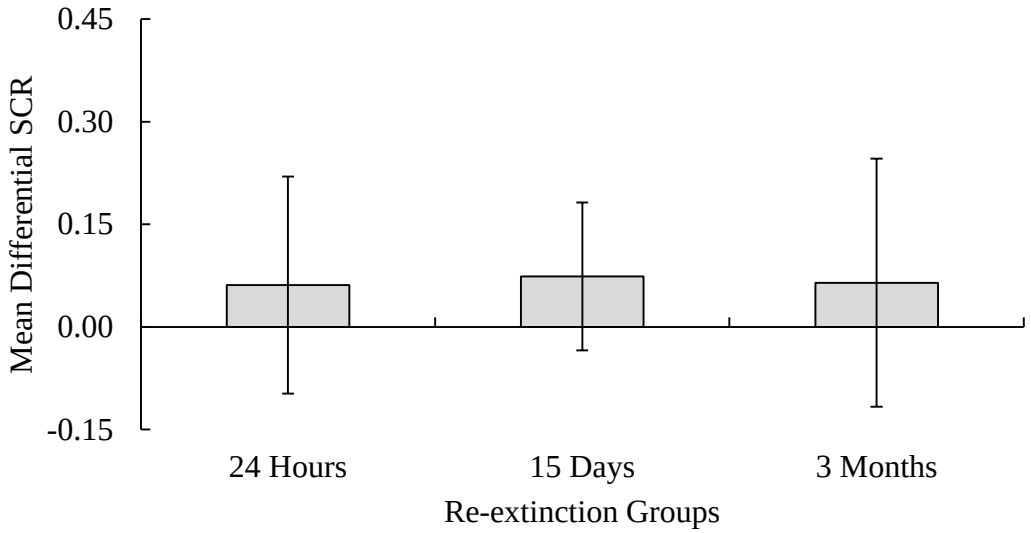

Figure 21. Mean differential skin conductance response for spontaneous recovery scores with respect to re-extinction conditions (Error bars indicate 95% confidence intervals)

spontaneous recovery scores also can be seen in Figure 22 for extinction*re-extinction interaction.

For extinction manipulation, it was expected that mean spontaneous recovery scores of participants underwent extinction 10 minutes after reminder (group that went through extinction within the reconsolidation window) would be less than mean spontaneous recovery scores of participants underwent extinction 6 hours after reminder (group that went through extinction outside of the reconsolidation window) and participants underwent extinction with no reminder (group that went through extinction without reminder manipulation). Moreover, while mean spontaneous recovery scores of these latter extinction groups (6 hours after reminder and no reminder) were expected to be differentiated from 10 minutes after reminder group, no difference between the mean spontaneous recovery scores of these two control groups were expected. We also wanted to examine the effects of re-extinction manipulation on spontaneous recovery scores. Effects of independent variables on spontaneous recovery scores were assessed with 3 (extinction group: 10 minutes after reminder, 6 hours after reminder and no reminder) x 3 (re-extinction group: 24 hours, 15 days and 3 months) factorial ANOVA.

Results revealed that main effect of extinction was statistically significant independent of re-extinction manipulation, $F_{(2, 99)} = 3.27$, $p < .05$, partial $\eta^2 = .06$. Then, in order to examine the source of significant difference found in the analysis, pairwise comparisonsamong the extinction conditions were conducted, since there were specific hypothesis to test. Helmert contrast procedure was employed to make two different contrasts. For the first contrast, 10 minutes after reminder group was compared to other two extinction groups (6 hours after reminder and no reminder).

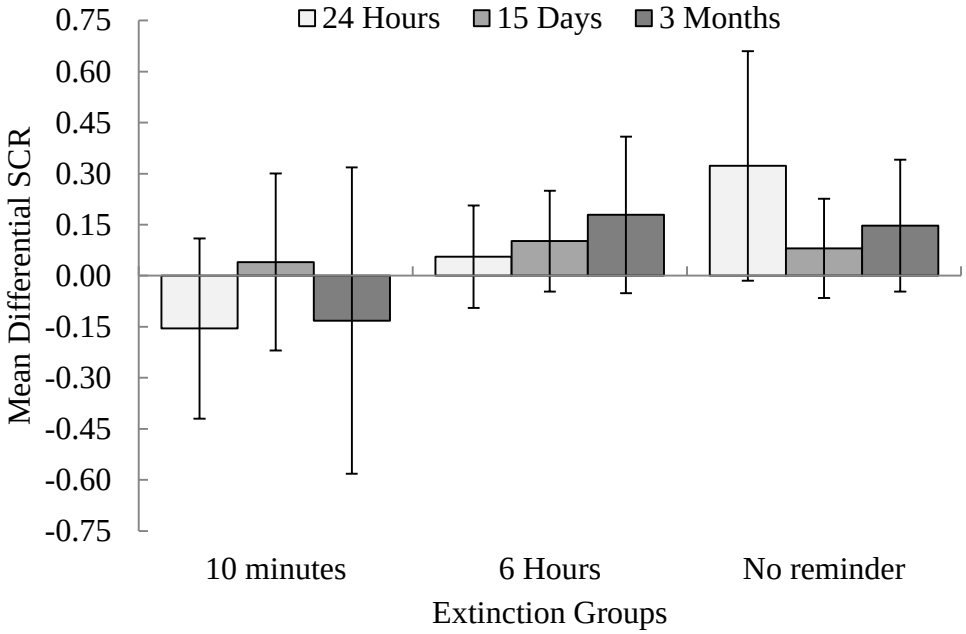

Figure 22. Mean differential skin conductance response for spontaneous recovery scores with respect to extinction depending on re-extinction (Error bars indicate 95% confidence intervals).

So, it was tested whether the mean spontaneous recovery score of 10 minutes after reminder group differentiate from combined mean spontaneous recovery score of 6 hours after reminder and no reminder groups. For the second contrast, 6 hours after reminder group was compared to no reminder group in terms of mean spontaneous recovery scores. Results of contrast analysis showed that participants went through extinction10 minutes after reminder (M = -.08, SE = .10) had significantly lower mean spontaneous recovery scores compared to participants went through extinction procedure 6 hours after reminder and with no reminder combined (M = .15, SE = .04), $t_{(105)}$ = 2.47, p < .05, r = .06. However, having extinction treatment 6 hours after reminder (M = .11, SE = .05) or without receiving a reminder (M = -.18, SE = .07) did not create a significant difference on the mean spontaneous recovery scores of these two conditions, $t_{(105)}$ = .65, p > .05. As consistent with our hypotheses, extinction manipulation affected recovery of fear in terms of skin conductance response, since extinction taking place within reconsolidation window (10 minutes after reminder) resulted in lower spontaneous recovery scores as compared to extinction outside of the reconsolidation window (6 hours after reminder) and extinction without manipulation (no reminder). Moreover, results showed that two control groups (6 hours after reminder and no reminder) did not differ from each other as expected.

Main effect of re-extinction on spontaneous recovery score was not significant, $F_{(2, 99)}$ = .01, p > .05. Spontaneous recovery scores of participants in 24 hours after extinction (M = .06, SE = .08), 15 days after extinction (M = .07, SE = .06), and 3 months after extinction (M = .07, SE = .09) groups were similar regardless of extinction condition they took part in. Testing groups for spontaneous recovery in

different time points did not affect the results, independent of extinction manipulation. Extinction*re-extinction interaction effect on spontaneous recovery scores was not significant, as well, $F_{(4, 99)} = .87$, $p > .05$. Spontaneous recovery scores in 10 minutes after reminder condition were similar to the scores obtained from the participants who underwent re-extinction 24 hours after extinction (*M* = -.16, *SE* = .14), 15 days after extinction (*M* = .04, *SE* = .13), and 3 months after extinction (*M* = -.13, *SE* = .23). Extinction scores in 6 hours after reminder condition were also similar to the scores obtained from the participants who underwent re-extinction 24 hours after extinction (*M* = .06, *SE* = .08), 15 days after extinction (*M* = .10, *SE* = .08), and 3 months after extinction (*M* = .18, *SE* = .11). In no reminder condition, extinction scores of participants who underwent re-extinction 24 hours after extinction (*M* = .32, *SE* = .17), 15 days after extinction (*M* = .08, *SE* = .07), and 3 months after extinction (*M* = .15, *SE* = .10) were similar, too. This means that for extinction groups, when re-extinction treatment took place did not affect the spontaneous recovery scores of participants.

Long-term Effects. Mean differential skin conductance response, attained through the difference between first differential score from reinstatement stage and last differential score from re-extinction stage used as an index of fear recovery. This difference corresponds to recovery of conditioned fear response in long-term due to reinstatement procedure. Mean differential skin conductance response for recovery scores of participants are represented in Figure 23 and Figure 24, regarding extinction and re-extinction variables. In addition, Figure 25 shows mean differential skin conductance response for recovery scores in respect to extinction*re-extinction interaction.

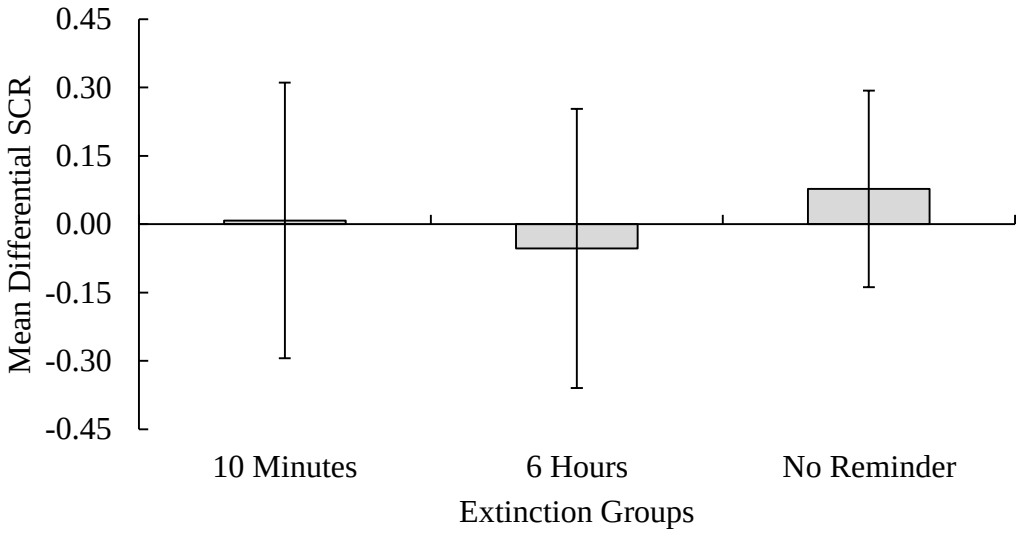

Figure 23. Mean differential skin conductance response for recovery scores in long-term with respect to extinction conditions (Error bars indicate 95% confidence intervals).

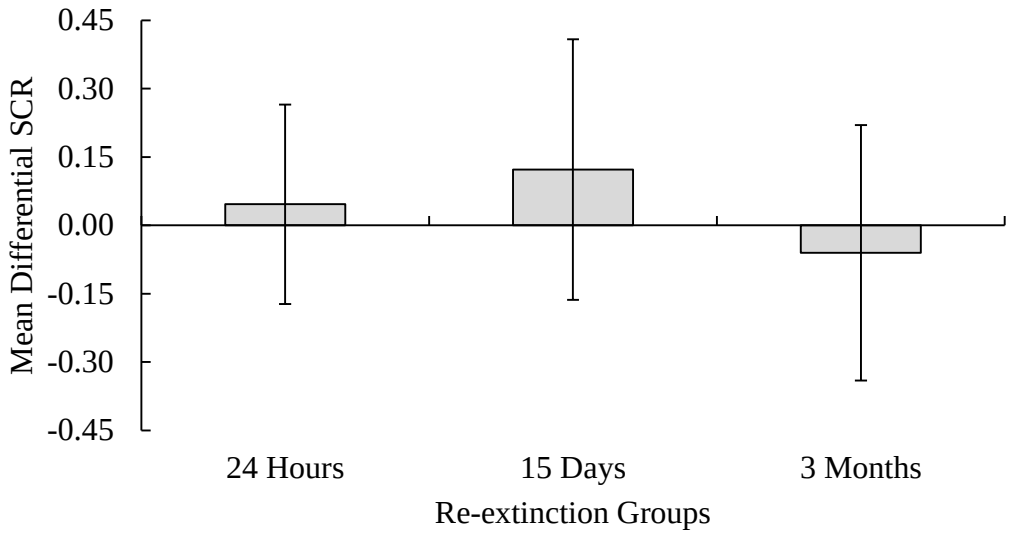

Figure 24. Mean differential skin conductance response for recovery scores in long-term with respect to re-extinction conditions (Error bars indicate 95% confidence intervals).

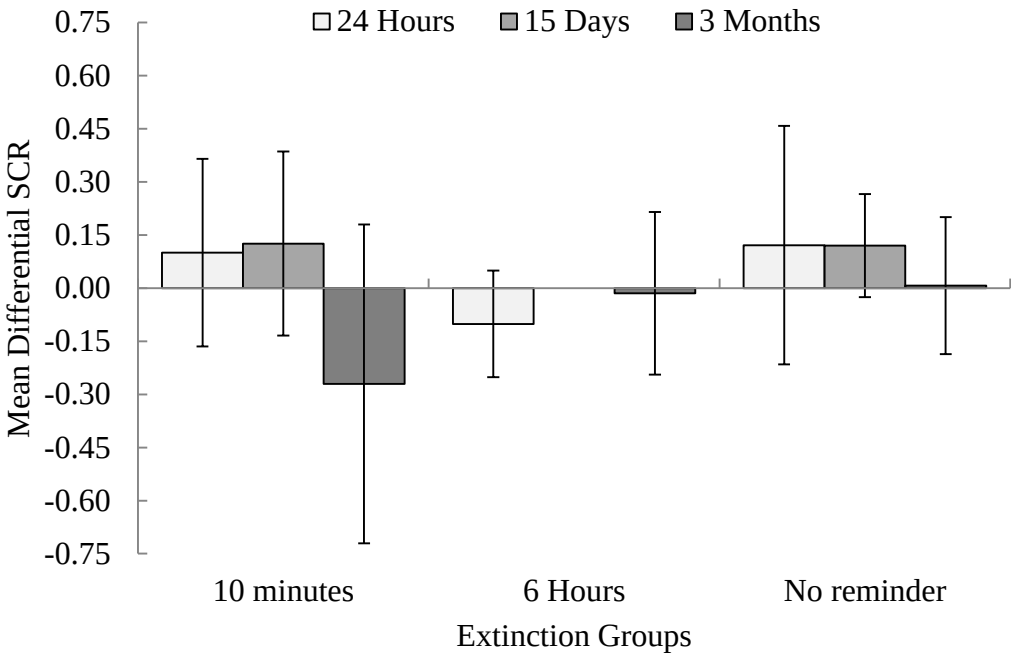

Figure 25. Mean differential skin conductance response for recovery scores in long-term with respect to re-extinction depending on extinction (Error bars indicate 95% confidence intervals).

Note: No one from the group that extinction training was given 6 hours after the reminder and tested 15 days later attended to the follow-up stage.

At this stage statistical analysis were conducted to observe if effect of extinction manipulation persists as Schiller and her colleagues (2010) found in their study, when recovery of fear responses were investigated one year after the main manipulation. 3 (extinction group: 10 minutes after reminder, 6 hours after reminder and no reminder) x 3 (re-extinction group: 24 hours, 15 days and 3 months) factorial ANOVA was conducted in order to examine the long-term effects of extinction and re-extinction manipulations on fear recovery scores of participants.

Results indicated that main effect of extinction manipulation on mean differential skin conductance responses were not significant regardless of re-extinction manipulation, when fear recovery was tested one year after the extinction manipulation, $F_{(2, 28)} = .17$, $p > .05$. Mean differential skin conductance response for fear recovery in long-term was similar to the scores of participants in 10 minutes after reminder ($M = .01$, $SE = .15$), 6 hours after reminder ($M = -.05$, $SE = .16$), and no reminder ($M = .08$, $SE = .11$) conditions of extinction.

Independent from the extinction manipulation, main effect of re-extinction manipulation on recovery of fear responses in long-term was also not significant, as expected, $F_{(2, 28)} = .43$, $p > .05$). Regarding of fear recovery in long-term, mean differential skin conductance responses of 24 hours after extinction ($M = .05$, $SE = .11$), 15 days after extinction ($M = .12$, $SE = .15$), and 3 months after extinction ($M = -.06$, $SE = .14$) groups did not differentiate from each other. Furthermore, interaction effect between extinction and re-extinction on fear recovery in long-term did not reach the significance level, $F_{(3, 28)} = .31$, $p > .05$. In long term, recovery scores of participants in 10 minutes after reminder condition did not change depending on when they receive re-extinction; 24 hours after extinction ($M = .10$, SE

= .23), 15 days after extinction (*M* = .13, *SE* = .18), and 3 months after extinction (*M* = -.27, *SE* = .46). Similarly, for participants in 6 hours after reminder condition, recovery scores did not differ from each other depending on being in the 24 hours after extinction (*M* = -.10, *SE* = .30), and 3 months after extinction (*M* = -.02, *SE* = .18) groups. There was also no significant difference between the recovery scores of participants who received extinction without any manipulation (no reminder condition) when re-extinction took place 24 hours after extinction (*M* = .12, *SE* = .07), 15 days after extinction (*M* = .12, *SE* = .24), and 3 months after extinction (*M* = .01, *SE* = .23). To sum up, there was no effect of any manipulation on fear recovery scores collected one year after the manipulation.

CHAPTER 4

Discussion

Regarding the aforementioned studies testing the fear memory reconsolidation on humans via both invasive and non-invasive methods, there is accumulating evidence implying that by reactivating consolidated memories, these memories might turn into a state susceptible to the interference. Although, not all the studies achieved to observe the phenomenon, or inconsistencies found between different measures of the fear responses, considering the limited time period human studies of reconsolidation evolved over the last couple of years, results are very promising in terms of developing new treatment techniques to overcome certain psychological disorders associated with fear and anxiety.

Running theme of the current thesis has been to investigate the efficacy and persistency of the extinction training within the reconsolidation process of fear memories acquired through Pavlovian fear conditioning on updating of fear memories with the "safe" information in order to diminish fear responses. To do so, participants, conditioned to the colored circles paired with the electrical stimulation in the first day of the study, were subjected to the extinction training inside (10 minutes after reminder) or outside (6 hours after reminder or without reminder) of the reconsolidation window, spontaneous recovery of participants' fear responses were tested in different time points, and a one-year follow-up study was also conducted.

In the first place, we analyzed whether independent variables in the research design have an effect on acquisition scores derived from the first stage that

acquisition training took place. Given that manipulations regarding extinction and re-extinction groups have not been done yet, we have already expected not to observe such an effect of independent variables on the acquisition scores. Otherwise, any difference found between extinction or re-extinction groups for acquisition scores would reflect a bias towards a certain group at the beginning of the study. Consistent with our expectancies, we confirmed that mean differential skin conductance responses for acquisition scores were comparable among extinction and re-extinction groups.

Similarly, in order to eliminate any bias that might be resulted from the different extinction scores of participants in different extinction and re-extinction groups, we tested the effects of independent variables on extinction scores derived from the extinction phase of the study. Considering that re-extinction manipulation has not taken place yet, such difference between extinction scores would create a bias towards a certain re-extinction group in the results. Again, no significant effect of extinction or re-extinction manipulations found on the mean differential skin conductance responses for extinction scores as expected.

Concerning the examination of the spontaneous recovery of the fear responses, acquired through the first stage and extinguished during the second stage of the study that was measured by skin conductance response, we found out that group receiving extinction training within the reconsolidation window (extinction 10 minutes after reminder) significantly differed from other two groups (extinction 6 hours after reminder and without reminder). Spontaneous recovery score of extinction 10 minutes after reminder group was lower than the other two groups and these latter two groups showed similar levels of the spontaneous recovery. However, testing for

spontaneous recovery in different time points (re-extinction groups: 24 hours, 15 days, 3 months) per se did not reveal any significant effect. Moreover, interaction of extinction and re-extinction manipulations on spontaneous recovery scores was not significant.

When the results of extinction manipulation that took place in the second stage of the study were taken into consideration, as consistent with our hypothesis, we found out that extinction manipulation affected the recovery of fear responses, when measured by skin conductance response. Our result implies that given extinction treatment within the reconsolidation window prevents the return of fear. Therefore, we were able to replicate the findings of Schiller et al. (2010) previously showed that behavioral intervention to the fear memory reconsolidation is effective to update fear memories as safe. Parallel with their findings, we observed that extinction intervention to a consolidated fear memory within the reconsolidation process after the reactivation occurs, attenuated the fear responses as compared to the extinction outside of the reconsolidation window or traditional extinction approach, independent from when the test for spontaneous recovery took place. In this respect, we provided additional supporting evidence for the dynamic nature of the memory and the effectiveness of behavioral intervention to the fear memory reconsolidation in humans.

On the other hand, re-extinction manipulation in the third stage found to have no effect on spontaneous recovery scores. Independent from what kind of extinction training was employed, this analysis showed us that there is no difference between the spontaneous recovery of fear responses when tested 24 hours, 15 day or 3 months later from the extinction training. One might expected to find an increasing trend on

spontaneous recovery scores of participants when the time interval to test spontaneous recovery get longer from 24 hours to 3 months, given that extinguished responses may recover by the passage of time (Rescorla, 2004). However, we did not observed such an effect of time on response recovery. On the contrary, spontaneous recovery scores of the participants in the different re-extinction conditions were very similar to each other as previously reported, which might be due to the extinction group 10 minutes after reminder divided among all three re-extinction groups, reducing the mean spontaneous recovery scores in each of these groups.

With respect to interaction between extinction and re-extinction manipulation no significant difference was found. However, when we further look through the data, spontaneous recovery scores of participants in extinction 10 minutes after reminder group was still lower than the extinction 6 hours after reminder and extinction without reminder groups when they tested 24 hours, 15 days and 3 months after the extinction manipulation. When spontaneous recovery scores were tested 24 hours after the extinction manipulation, as in Schiller et al. (2010), participants in the extinction without reminder group showed the highest spontaneous recovery, and participants in the extinction 10 minutes after reminder group did not even show recovery of fear. Furthermore, highest spontaneous recovery score observed was in the extinction without reminder group. Despite the fact that difference observed between different extinction and re-extinction groups did not reach the statistical significance, our data showed a pattern that can be interpreted as consistent with the reconsolidation hypothesis. Moreover, we could not observe an increasing trend on spontaneous recovery scores when these scores for 24 hours, 15 days and 3 months were compared regarding extinction 10 minutes after reminder group. If we did

observe, it could be concluded as behavioral intervention to the reconsolidation process failed to prevent fear responses with the passage of time and as a function of time extinguished fears recovered like in the traditional extinction training, and reconsolidation update paradigm did not have a persistence effect on extinguishing fear responses.

At this point, it is important to note that we took verbal statements from the participants in the end of the each session by asking the stimulus paired with the electrical stimulation in the first day of the study and recorded their answers. At the end of the second and third stages, we confirmed that all the participants successfully recalled the *CS* paired with the *US* correctly as well as confirming that mean differential skin conductance responses of participants calculated for acquisition and extinction stages met our inclusion criteria. Therefore, we can conclude that explicit knowledge of the *CS-US* contingency was intact in the participants after both of our experimental manipulations. It is known that explicit knowledge of the *CS-US* contingency rely on the hippocampus (Bechara, et al., 1995). On the other hand, succesful demonstrations of the behavioral intervention to the reconsolidation process of fear memory revealed itself as reduced skin conductance response and reduced activity in the amygdala to the presentations of the *CS* in the recent studies (Agren et al., 2012; Schiller et al., 2013). Given that explicit knowledge of the *CS-US* contingency might also result in increased levels of skin conductance response (Phelps, et al., 2001), comparable levels of spontaneous recovery of the fear responses observed in our interaction analysis might be due to the fact that when reconsolidation update paradigm is used for fear memories, behavioral intervention targets only fear association stored in the amygdala and leaves explicit knowledge of

the *CS-US* contingency stored in the hippocampus intact, which in turn results in expression of extinguished fear on skin conductace response.

These in mind and as previous studies suggested (see Lee, 2009; Soeter and Kindt, 2010) skin conductance response might be sensitive to the cognitive influences. Consequently, this measure might be affected from cognitive/verbal (subjective experience) response systems and might not be able to provide the best option to use as the fear index for human subjects considering the complex cognitive processes going on. When animal studies were reviewed, we came across behavioral measures as the fear indeces (e.g. freezing, avoidance to enter the certain part of the box) most commonly. Considering the emotion component in the fear memories, it is important to remember that emotion theory defines emotions like fear in three different response systems: subjective experience, physiological activity and behavioral impulses (Beckers, Krypotos, Boddez, Effting, & Kindt, 2013). All three components might be considered as equally important; however, Frijda (1986) argued that behavioral tendencies should be considered as the core component of the emotions given that the ultimate function of the emotion is to direct behavior. Keeping in mind that emotional disorders are quite related with behavioral dysfunctions, as consistent with Frijda's argument, tendency towards an avoidance behavior is one of the criteria to diagnose many anxiety disorders (American Psychiatric Association, 2000).

Therefore, while translating animal research to the human subjects, it might be useful to mimic the behavioral measures used in animal studies for the human research as well as employing the certain procedural aspects of these animal studies. A simple avoidance task can be used for this purpose. According to the two-factor

theory of avoidance, learning of avoidance from an aversive stimulus should require a Pavlovian component in which fear is conditioned to a *CS*. Only after this, may avoidance learning be possible. However, the subject can successfully avoid from an aversive *US* by responding to *CS* instrumentally whenever it is in effect. Interestingly, each successful avoidance response may be considered as a step towards an obvious extinction of fear responses but it strengthened the fear response (Maia, 2010). For example, one way might be teaching a simple key pressing behavior in response to a certain picture appearing on the screen with frequent intervals. An auditory stimulus is added to the environment after they acquire this behavior via reflecting it in their performance successfully (without making a mistake for certain times of trial). They are asked to stop key pressing following the sound even if the picture appears on the screen and if they press the key after the sound, they are informed that there will be a punishment (e.g. mild electrical stimulation from the wrist). With this task, the sound will acquire aversive properties that will result in avoidance from key pressing behavior, which can be used as the index of fear. A day later, this sound can be used as the reminder cue to reactive the memory formed a day ago and same task is given to the subjects but this time without punishment even in the case of key pressing following the sound 10 minutes or 6 hours after the reminder or without reminder. In the test phase, same task (without punishment) is given and percentage of the avoidance from key pressing during the task is used as the dependent measure of the study. Then, comparisons are done over this measure between groups. However, while using behavioral indices of fear on such tasks, one should not ignore the fact that learning is not evident in the behavioral performance all the time (Domjan, 2005), so other measures such as skin conductance response, startle response, US expectancy ratings should not be ignored but the complex nature of these response

systems should be understood in more details to make more sound conclusions. For future research, this might suggest that in order to develop more effective behavioral intervention techniques for prevention of the fear memories, multiple systems of the fear memory network affecting the expression of fear response systems should be taken into consideration.

In the one-year follow-up study, we failed to observe any significant effect of extinction and re-extinction manipulations or their interactions on fear recovery scores when *US* was reinstated prior to the test. At first glance, one might say that effect of the extinction manipulation did not persist when tested one year later, which is contradictory with Schiller et al.'s (2010) study, showed that extinguishing fear responses within the reconsolidation window prevent the return of fear even after one year following the extinction manipulation. However, detailed examination of our long-term data revealed that there was no significant fear recovery in terms of skin conductance response of the participants in any of the groups.

For more clear understanding of this finding, we turned back to the personal statements of the participants to the question "Which was the picture paired with the electrical stimulation in the first day of the study?" asked at the end of the one year follow-up stage. We realized that approximately 20% of the participants from different conditions attended to the follow-up study could not come up with the correct answer or was not even able to recall the stimulus paired with the *US* a year before. This situation reveals that for some participants, there was not even explicit knowledge of the *CS-US* contingency when asked one year after the main study. Given that no recovery was observed in any of the conditions, one explanation might be related to the arbitrary stimuli (blue and yellow circles) used in our experimental

procedure because arbitrary stimuli can be evaluated as low in ecological validity to associate with an aversive outcome. Although using arbitrary stimulus is very beneficial for the development of laboratory models of acquired fear, it does not provide us the opportunity to test our inborn tendencies related to the fear, which was explained as preparedness or the existence of a fear module (Öhman & Mineka, 2001; Seligman, 1971). It is true that many objects may elicit fear under certain circumstances; however, intense fears are more related with objects and situations that are fear-relevant. Mineka and Öhman (2002) claimed that the fear module enable us to associate fear-relevant stimuli more easily than fear-irrelevant stimuli with an aversive outcome. Such that, even when there is no awareness of the *CS-US* association, before the conscious collection of information related to the *CS*, if *CS* is fear-relevant, then *CS* activates the fear module automatically. On the other hand, fear conditioning to the fear-irrelevant *CS* requires a conscious collection of this association and information related to this stimulus. Therefore, it is easier to develop fear responses to a fear-relevant stimulus rather than a fear-irrelevant stimulus. Regarding extinction, in an opposite manner with acquisition, association built up with a fear-relevant stimulus is harder to extinguish than extinguishing the association formed with fear-irrelevant stimulus (Mineka & Öhman, 2002).

In our case, most probably due to the lack of ecological validity of our fear-irrelevant *CS*, formed *CS-US* association might have been weakened easily by the extinction trainings and passage of time. Given that fear-relevant stimulus is more commonly associated with objects and situations related to the survival as in the anxiety disorders, and more resistant to the extinction as different from fear-irrelevant stimulus; this might explain why we did not observe any spontaneous

recovery in none of our groups. So, it might be more appropriate to use fear-relevant stimulus in fear memory reconsolidation studies to make more clear investigation of the subject. On the other hand, one study using fear-relevant stimulus failed to find the effect of reconsolidation update paradigm to update fear memory, even in the short-term when fear indices were startle and skin conductance responses (Golkar, et al., 2012), conflicts with Schiller et al.'s suggestion that return of fears can be prevented by behavioral interference to the reconsolidation process. This innate tendency to develop fear for threatening stimulus might have a significant role on the reconsolidation interference, which should be further investigated within the boundaries of reconsolidation studies.

Additionally, supporting results presented for the long-term effects of reconsolidation update paradigm by Schiller et al. (2010) should be approached cautiously. If the calculation of the fear recovery score over the skin conductance response is examined closely, it is seen that they used the difference between the mean value of first four responses from the extinction following reinstatement and mean value of the last two responses from the re-extinction. Moreover, they omitted the first response following the reinstatement by considering it as the orienting response, which might be the most important response that will tell us about the influence of reinstatement procedure on fear recovery. Also further examination of the calculation of the spontaneous recovery scores 24 hours after the extinction manipulation showed that they used the difference between the first response from the re-extinction and the last response from the extinction, which can be considered more appropriate as a recovery measure and we expected to see a similar calculation procedure for the long-term effect analysis as well. Therefore, reported findings by

Schiller et al. (2010) should not have been reliably concluded as the persistent effect of reconsolidation update paradigm; in my opinion, more detailed examination of the data is required to make this inference. With all these in mind, the long-term effect of this paradigm is still an area waiting for further investigation.

Another point worth to mention is that during our data collection process, we had a subject loss around 30% because these participants did not meet the acquisition or extinction criteria when these scores were calculated through *SCR*. On the other hand, Schiller et al.'s (2010) study reported a subject loss around 10% due to the same reason. The difference observed between the subject loss rates of these two studies is quite interesting given the fact that same methodology was followed in our study. Therefore, this issue should not be underestimated and reason behind this remarkable difference should be further investigated.

Taken all together, our results are supportive for the effectiveness of the reconsolidation update paradigm to some extent but persistency of the observed effect is still questionable. Given the fact that there are several papers studying with clinical populations (e.g. *PTSD* patients) by employing pharmacological interference to the reconsolidation process and successful at demonstrating the effectiveness of this paradigm even up to three months (see Poundja, Sanche, Tremblay ve Brunet, 2012), it is highly important to deepen our knowledge on both reconsolidation update and blockade paradigms. Although fear memory reconsolidation research to prevent return of fear in humans with behavioral interference is still in its infancy period and there is no conducted research on translation of these studies to clinical populations, better understanding of this phenomenon together with the pharmacological techniques might serve to develop better treatment options for certain psychological

problems such as *PTSD*, *OCD*, addiction and phobias. Furthermore, as well as providing new directions for treatments of these disorders, same techniques might contribute to the protective and preventative interventions. Clearly, further inquiries are needed to understand the basic mechanisms underlying the fear memory reconsolidation update process in order to develop more precise interference techniques suitable for the specific properties of the human fear memory.

References

Agren, T., Engman, J., Frick, A., Björkstrand, J., Larsson, E. M., Furmark, T., & Fredrikson, M. (2012). Disruption of reconsolidation erases a fear memory trace in the human amygdala. *Science, 337*, 1150-1552.

American Psychiatric Association (2000). *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR.* Washington DC.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science, 269*, 1115-1118.

Beckers, T., Krypotos, A. M., Boddez, Y., Effting, M., & Kindt, M. (2013). What's wrong with fear conditioning? *Biological Psychology, 92*, 90-96.

Bouton, M. E. (1988). Context and ambiguity in the extinction of emotional learning: Implications for exposure therapy. *Behavior Research and Therapy, 26*(2), 137-149.

Bouton, M. E. (2002). Context, ambiguity, and unlearning: Sources of Relapse after behavioral extinction. *Biological Psychiatry, 52*, 976-986.

Bouton, M. E., & Bolles, R. C. (1979). Contextual control of the extinction of conditioned fear. *Learning and Memory, 10*, 445-466.

Brunet, A., Orr, S. P., Tremblay, J., Robertson, K., Nader, K., & Pitman, R. K. (2008). Effect of post-retrieval propranolol on psychophysiologic

responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *Journal of Psychiatric Research*, 503-506.

Dawson, R. G., & McGaugh, J. L. (1969). Electroconvulsive shock effects on a reactivated memory trace: further examination. *Science, 166*, 525-527.

Debiec, J., & LeDoux, J. E. (2004). Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience, 129*, 267-272.

Delgado, M. R., Olsson, A., & Phelps, E. A. (2006). Extending animal models of fear conditioning to humans. *Biological Psychology, 73*, 39-48.

DeVietti, T. L., Conger, C. L., & Kirkpatrick, B. R. (1977). Comparison of the enhancement gradients of retention obtained with stimulation of the mesencephalic reticular formation after training or memory. *Physiology and Behavior, 19*, 549-554.

Díaz-Mataix, L., Martinez, R. C., Schafe, G. E., LeDoux, J. E., & Doyère, V. (2013). Detection of a temporal error triggers reconsolidation of amygdala-dependent memories. *Current Biology*, *23*(6), 467-472.

Dirikx, T., Hermans, D., Vansteenwegen, D., Baeyens, F., & Eelen, P. (2004). Reinstatement of extinguished conditioned responses and negative stimulus valence as a pathway to return of fear in humans. *Learning and Memory, 11*, 549-554. doi:10.1101/lm.78004

Domjan, M. (2005). *The Esssentials of Conditioning and Learning* (Third edition ed.). Canada: Thomson Wadsworth.

Duvarci, S., & Nader, K. (2004). Characterization of fear memory reconsolidation. *the Journal of Neuroscience, 24*(42), 9269-9275.

Fanselow, C. S., & LeDoux, J. E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron, 23*(2), 229-232.

Field, A. P. (2006). Is conditioning a useful framework for understanding the development and treatment of phobias. *Clinical Psychology Review, 26*, 857-875.

Fischer, A., Sananbenesi, F., Schrick, C., Spiess, J., & Radulovic, J. (2004). Distinct roles of hippocampal de novo protein synthesis and actin rearrangement in extinction of contexual fear. *The Journal of Neuroscience, 24*(8), 1962-1966. doi:10.1523/JNEUROSCI.5112-03.2004

Flexner, L. B., Flexner, J. B., & Stellar, E. (1965). Memory and cerebral protein sytnhesis in mice as affected by graded amounts of puromycin. *Experimental Neurology, 13*(3), 264-272.

Frijda, N. H. (1986). *The Emotions.* Cambridge: Cambridge University Press.

Golkar, A., Bellander, M., Olsson, A., & Öhman, A. (2012). Are fear memories erasable? –Reconsolidation of learned fear with fear-relevant and fear-irrelevant stimuli. *Frontiers in Behavioral Neuroscience, 6*, 1-10.

Gordon, W. C., & Spear, N. E. (1973). The effects of strychnine on recently acquired and reactivated passive avoidance memories. *Physiology and Behavior, 10*, 1071-1075.

Hupbach, A., Hardt, O., Gomez, R., & Nadel, L. (2008). The dynamics of memory: context-dependent updating. *Learning and Memory, 15*(8), 574-579.

Kindt, M., & Soeter, M. (2013). Reconsolidation in a human fear conditioning study: a test of extinction as updating mechanism. *Biological Psychology, 92*, 43-50.

Kindt, M., Soeter, M., & Vervliet, B. (2009). Beyond extinction: Erasing human fear responses and preventing the return of fear. *Nature Neuroscience, 12*(3), 256-258.

Lattal, K. M., & Abel, T. (2004). Behavioral impairments caused by injections of the protein synthesis inhibitor anisomycin after contextual retrieval reverse with time. *Proceedings of the National Academy of Sciences of the United States of America, 101*(13), 4667-4672.

Lee, J. L. (2009). Reconsoldiation: maintaining memory relevance. *Trends in Neuroscience, 32*, 413-420.

Lewis, D. J. (1969). Sources of experimental amnesia. *Psychologicaş Review, 76*, 461-472.

Lewis, D. J., & Bregman, N. J. (1973). The source of cues for cue-dependent amnesia. *Journal of Comparative and Physiological Psychology, 85*, 421-426.

Lewis, D., Bregman, N. J., & Mahan, J. (1972). Cue-dependent amnesia in rats. *Physiological Psychology, 81*, 243-247.

Maia, T. V. (2010). Two-factor theory, the actor–critic model, and conditioned avoidance. *Learning and Behavior, 38*(1), 50-67. doi:10.3758/LB.38.1.50

McGaugh, J. L. (1966). Time dependent processes in memory storage. *Science, 153,* 1351-1358.

Milekic, M. H., & Alberini, C. (2002). Temporally graded requirement for protein synthesis following memory reactivation. *Neuron, 36,* 521-525.

Mineka, S., & Öhman, A. (2002). Phobias and preparedness: the selective, automatic, and encapsulated nature of fear. *Biological Psychiatry, 52*(10), 927-937.

Missanin, J. R., Miller, R. R., & Lewis, D. J. (1968). Retrograde amnesia produced by electroconvulsive shock after reactivation of consolidated memory trace. *Science, 160,* 554-555.

Monfils, H. M., Cowansage, K. K., Klann, E., & LeDoux, J. E. (2009, May). Extinction-reconsolidation boundaries: Key to persistent attenuation of fear memories. *Science, 324,* 951-955.

Morris, R. G., Inglis, J., Ainge, J. A., Olverman, H. J., Tulloch, J., Dudai, Y., & Kelly, P. A. (2006). Memory reconsolidation: sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. *Neuron, 50*(3), 479-489.

Nader, K. (2003). Memory traces unbound. *Trends in Neuroscience, 26*(2).

Nader, K. (2013). The discovery of memory reconsolidation. In C. M. Alberini (Ed.), *Memory reconsolidation* (pp. 1-13). United States of America: Academic Press.

Nader, K., & Hardt, O. (2009, March). A single standart for memory: the case for reconsolidation. *Nature, 10*, 224-234.

Nader, K., Hardt, O., Einarrson, E. Ö., & Finnie, P. S. (2013). The Dynamic Nature of the Memory. In C. M. Alberini (Ed.), *Memory Reconsolidation* (pp. 15-41). United States of America: Academic Press.

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000, August). Fear memories require protein synthesis in amygdala for reconsolidation after retrieval. *Nature, 406*, 722-726.

Olsson, A., & Phelps, E. A. (2007). Social learning of fear. *Nature Neuroscience, 10*(9), 1095-1102.

Oyarzun, J. P., Lopez-Barroso, D., Fuentemilla, L., Cucurell, D., Pedraza, C., Rodriguez-Fornells, A., & Diego-Balaguer, R. (2012). Updating fearful memories with extinction training during reconsolidation: A human study using auditory aversive stimuli. *Plos One, 7*. doi:10.1371/journal.pone.0038849

Öhman, A. (2009). Human Fear Conditioning and the Amygdala. In P. J. Whalen, & E. A. Phelps (Eds.), *The Human Amygdala* (pp. 118-154). New York: The Guildford Press.

Öhman, A., & Mineka, S. (2001). Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. *Psychological Review, 108*(3), 483-522.

Pare, D., & Duvarci, S. (2012). Amygdala microcircuits mediating fear expressions and extinction. *Current Opinion in Neurobiology, 22*, 717-723.

Pedreira, M. E., & Maldonado, H. (2003). Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron, 38*, 863-869.

Phelps, E. A. (2009). The Human Amygdala and Control of Fear. In P. J. Whalen, & E. A. Phelps (Eds.), *The Human Amygdala* (pp. 204-219). New York: The Guilford Press.

Phelps, E. A., & Schiller, D. (2013). Reconsolidation in humans. In C. M. Alberini (Ed.), *Memory Reconsolidation* (pp. 185-211). United States of America: Academic Press.

Phelps, E. A., O'Connor, K. J., Gatenby, J. C., Grillon, C., Gore, J. C., & Davis, M. (2001). Activation of the left amygdala to a cognitive representation of fear. *Nature Neuroscience, 4*, 437-441.

Poundja, J., Sanche, S., Tremblay, J., & Brunet, A. (2012). Trauma activation under the influence of propranolol: an examination of clinical predictors. *European Journal of Pychotraumatology*(3). doi:10.3402/ejpt.v3i0.15470

Quirk, G. J. (2002). Memory for extinction of conditioned fear is long-lasting and persist following spontaneous recovery. *Learning and Memory, 9*, 402-407.

Quirk, G. J., Mueller, D., Kalivas, P. W., & Manji, H. K. (2008, January). Neural Mechanisms Of Extinction Learning and Retrieval. *Neuropsychopharmacology, 33*(1), 56-72. doi:10.1038/sj.npp.1301555

Repa, J. C., Muller, J., Apergis, J., Desrochers, T. M., Zhou, Y., & LeDoux, J. E. (2001). Two different lateral amygdala cell populations contribute to the initiation and storage of memory. *Nature Neuroscience, 4*, 724-731.

Rescorla, R. A. (2004). Spontaneous Recovery. *Learning and Memory, 11*, 501-509.

Rescorla, R. A., & Heth, C. D. (1975). Reinstatement of fear to an extinguished conditioned stimulus. *Journal of Experimental Psychology, 1*, 88-96.

Rubin, R. D. (1976). Clinical use of retrograde amnesia produced by electroconvulsive shock: A conditioning hypothesis. *Canadian Psychiatric Association Journal, 21*, 87-90.

Sara, S. J. (2000). Retrieval and reconsolidation: toward a neurobiology of remembering. *Learning and Memory, 7*, 73-84. doi:10.1101/lm.7.2.73

Schafe, G. E., & LeDoux, J. E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *The Journal of Neuroscience, 20*, RC96.

Schafe, G. E., Doyére, V., & LeDoux, J. E. (2005). Tracking the fear engram: the lateral amygdala is an essentila locus of fear memory storage. *The Journal of Neuroscience, 25*(43), 10010-10015.

Schiller, D., & Phelps, E. A. (2011). Does reconsolidation occur in humans. *Frontiers in Behavioral Neuroscience, 5*. doi:10.3389/fnbeh.2011.00024

Schiller, D., Cain, C. K., Curley, N. G., Schwartz, J. S., Stern, S. A., LeDoux, J. E., & Phelps, E. A. (2008). Evidence for recovery of fear following immediate

extinction in rats and humans. *Learning and Memory, 15*(6), 394-402. doi: 10.1101/lm.909208

Schiller, D., Kanen, J. W., LeDoux, J. E., Monfils, M. H., & Phelps, E. A. (2013). Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. *Proceedings of National Academyof Sciences of the United States, 110*(50), 20040-20045. doi:10.1073/pnas.1320322110

Schiller, D., Monfils, M. H., Raio, C. M., Johnson, D. C., LeDoux, J. E., & Phelps, E. A. (2010). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature, 463*(7), 49-54.

Seligman, M. E. (1971). Phobias and preparedness. *Behavior Therapy, 2*, 307-320.

Sevenster, D., Beckers, T., & Kindt, M. (2012). Retrieval per se is not sufficient to trigger reconsolidation of human fear memory. *Neurobiology of Learning and Memory, 97*, 338-345.

Soeter, M., & Kindt, M. (2010). Dissociating response systems: Erasing fear from memory. *Neurobiology of Learning and Memory, 94*, 30-41.

Soeter, M., & Kindt, M. (2011). Disrupting reconsolidation: Pharmacological and behavioral manipulations. *Learning and Memory, 18*, 357-366.

Spear, N. E. (1973). Retrieval of memory in animals. *Psychological Review*, 163-194.

Squire, L. R., Slater, P. C., & Chace, P. M. (1976). Reactivation of recent or remote memory before electroconvulsive theraphy does not produce retrograde amnesia . *Behavioral Biology, 18*, 335-343.

Appendix A

"Participant Evaluation Form" applied before first experimental session.

Katılımcı Bilgi Formu

AD-SOYAD:

TELEFON: E-MAIL:

CİNSİYET: YAŞ:

Aşağıdaki soruları yanıtlarken son bir haftanızı göz önünde bulundurarak size en uygun olan numarayı yuvarlak içine alınız.

(0 = hiç yorgun değil, 7 = çok yorgun)

1. Şu anda kendinizi ne kadar yorgun hissediyorsunuz?
   0-----1-----2-----3-----4-----5-----6-----7
2. Son 24 saat içinde kendinizi ne kadar yorgun hissettiniz?
   0-----1-----2-----3-----4-----5-----6-----7
3. Eğer kendinizi halsiz ve yorgun hissediyorsanız, bu durumunuz aşağıda verilen aktiviteleri ne kadar etkiledi?

(0 = hiç etkilemedi, 7 = çok etkiledi)

- **Günlük aktiviteler**

  0-----1-----2-----3-----4-----5-----6-----7

- **Ruh hali**

  0-----1-----2-----3-----4-----5-----6-----7

- **Yürüme eylemi**

  0-----1-----2-----3-----4-----5-----6-----7

- **Sosyal ilişkiler**

  0-----1-----2-----3-----4-----5-----6-----7

"Participant Evaluation Form" applied before first experimental session (cont.).

Aşağıdaki soruları yanıtlarken lütfen durumunuzu en iyi yansıtan seçeneğin yanına işaret koyunuz.

4. Aktif olarak kullandığınız eliniz hangisi?

☐Sağ ☐Sol

5. "Renk körlüğü hastalığınız var mı?

☐Evet ☐Hayır

Yanıtınız evet ise 6. soruya hayır ise 7. soruya geçiniz.

6. Hangi renkleri göremiyorsunuz?

……

7. Herhangi bir psikolojik rahatsızlık geçirdiniz mi?

☐Evet ☐Hayır

Yanıtınız evet ise 8. sorudan devam ediniz. Yanıtınız hayır ise 10. soruya geçiniz.

8. Bir ruh sağlığı çalışanı tarafından rahatsızlığınıza konulan tanı nedir?

……

9. Rahatsızlığınız için ilaç tedavisi uygulandı mı?

☐Evet ☐Hayır

10. Herhangi bir obje veya duruma karşı fobiniz var mı? (örn: belirli bir hayvan, yükseklik, kalabalık, dişçi vs.)

☐Evet, …….fobisi
☐Hayır

Yanıtınız evet ise 11. soruya, hayır ise 12. soruya geçiniz.

11. Bir ruh sağlığı çalışanı tarafından bu fobinizle ilgili bir tanı aldınız mı?

☐Evet ☐Hayır

12. Dün akşam kaç saat uyudunuz?

☐ 5 saatten az
☐ 6-8 saat
☐ 8 saatten fazla

"Participant Evaluation Form" applied before first experimental session (cont.).

13. Yakın zamanda (son 1 sene dahil) başka bir psikoloji deneyine katıldınız mı?

☐Evet ☐Hayır

Yanıtınız evet ise 14. sorudan, hayır ise 12. sorudan devam ediniz.

14. Hangi deneye katıldınız?

……

15 ve 16. Soruları yalnızca kadın katılımcılar yanıtlayacaktır.

15. Adet döneminde misiniz?

☐Evet ☐Hayır

16. Hamile misiniz?

☐Evet ☐Hayır

Aşağıdaki soruları yanıtlarken lütfen durumunuzu en iyi yansıtan seçeneğin yanına işaret koyunuz.

17. Bugün labaoratuvara gelmeden önce sigara ya da herhangi bir tütün mamülü tükettiniz mi?

☐Evet ☐Hayır

18. Bugün labaoratuvara gelmeden önce çay, kahve, kola vb. kafein/tein içeren içeceklerden tükettiniz mi?

☐Evet ☐Hayır

19. Bugün labaoratuvara gelmeden önce alkollü içeceklerden tükettiniz mi?

☐Evet ☐Hayır

20. Herhangi bir kalp rahatsızlığı tanısı aldınız mı?

☐Evet ☐Hayır

Yanıtınız evet ise 21. sorudan, hayır ise 22. sorudan devam ediniz.

21. Size konulan tanıyı belirtiniz:……
22. Herhangi bir ameliyat/operasyon geçirdiniz mi?

☐Evet ☐Hayır

Yanıtınız evet ise 23. sorudan, hayır ise 24. sorudan devam ediniz.

"Participant Evaluation Form" applied before first experimental session (cont.).

23. Geçirdiğiniz ameliyatı/operasyonu lütfen belirtiniz.
    Ameliyat/operasyon:……Ameliyat/operasyon tarihi:……..…....
24. Vücudunuzun herhangi bir yerinde protez/implant var mı?

    ☐Evet ☐Hayır

    Yanıtınız evet ise 25. sorudan, hayır ise 26. sorudan devam ediniz.
25. Lütfen protezin/implantın nerede olduğunu ve özelliğini belirtiniz.
    Protez/implant:……Protez/implantın yapı maddesi:…….….
26. Düzenli/sürekli olarak kullandığınız ilaçlar var mı?

    ☐Evet ☐Hayır

    Yanıtınız evet ise 27. sorudan, hayır ise 28. sorudan devam ediniz.
27. Lütfen kullandığınız ilaç(lar)ı ve ilaç(lar)ın kullanım amacını belirtiniz.
    İlaç(lar):……..…Kullanım amacı:……..……..…..
28. Ailenizde herhangi bir kap rahatsızlığı tanısı almış olan/ kalbinden herhangi bir operasyon geçirmiş biri(leri) var mı?

    ☐Evet ☐Hayır

    Yanıtınız evet ise 29. soruya, hayır ise formun son bölümüne geçiniz.
29. Ailenizde kalp rahatsızlığı tanısı almış, kalbiyle ilgili herhangi bir operasyon geçirmiş kişi/kişilerin size yakınlığı ve aldıkları tanı/geçirdikleri operasyonu belirtiniz.
    Yakınlık:……..…Tanı/operasyon:……..……..…..
30. Önceden beslediğiniz ya da beslemekte olduğunuz bir evcil hayvan var mı?

    ☐Evet ☐Hayır

    Var ise hayvanınızın türünü belirtiniz:……..

"Participant Evaluation Form" applied before first experimental session (cont.).

Aşağıdaki belirtileri bugün de dahil olmak üzere son bir hafta içinde ne ölçüde yaşadığınızı göz önünde bulundurarak yanıt veriniz.

	Hiç	Hafif	Orta	Ağır
Bedeninizin herhangi bir yerinde uyuşma/karıncalanma				
Sıcak/ateş basmaları				
Bacaklarda halsizlik, titreme				
Gevşeyememe				
Çok kötü şeyler olacak korkusu				
Baş dönmesi/sersemlik hissi				
Kalp çarpıntısı				
Dengeyi kaybetme korkusu				
Dehşete kapılma				
Sinirlilik				
Boğuluyormuş gibi olma duygusu				
Ellerde titreme				
Titreklik				
Kontrolü kaybetme korkusu				
Nefes almada güçlük				
Ölüm korkusu				
Korkuya kapılma				
Midede hazımsızlık/rahatsızlık hissi				
Baygınlık				
Yüz kızarması				
Terleme (sıcağa bağlı olmayan)				

Appendix B

"Participant Information Form" given before first experimental session.

KATILIMCI BİLGİLENDİRME FORMU

Bu çalışmanın amacı, laboratuar koşullarında ekolojik ve keyfi uyarıcılar aracılığıyla geliştirilen fizyolojik korku tepkilerinin, belleğin yeniden-yapılanma evresinde ve dışında uygulanacak söndürme işlemi sonrasında geri gelmesine ilişkin etkilerinin incelenmesidir.

Çalışma sürecinde bilgisayar ekranından -belirli aralıklarla- birtakım uyarıcılar sunulacaktır. Bu uyarıcılardan bazıları, sağ kol bileğinize bağlanacak olan elektrotlar aracılığıyla verilen hafif bir elektriksel uyarım ile sonuçlanacaktır. Elektrotlardan verilecek olan elektriksel uyarımın şiddetini araştırmanın başında -sizi rahatsız edecek, fakat canınızı yakmayacak bir düzeyde olacak biçimde- sizin belirlemeniz istenecektir. Bilgisayar ekranından sunulan uyarıcılara verdiğiniz fizyolojik tepkiler, sol elinizin iki parmağına ve yüzünüzün dört noktasındaki belirli kaslara bağlanacak elektrotlar aracılığıyla ölçülecektir.

Çalışmada kapsamında katılımcılardan elde edilen veriler isim kullanılmaksızın analizlere dahil edilecektir; yani çalışma sürecinde size bir katılımcı numarası verilecek ve isminiz araştırma raporunda yer almayacaktır.

Katılımınız araştırma hipotezinin test edilmesi ve yukarıda açıklanan amaçlar doğrultusunda literatüre sağlayacağı katkılar bakımından oldukça önemlidir. Ayrıca katılımınızın psikoloji alanın gelişmesi açısından da bir takım faydaları bulunmaktadır.

Çalışmaya katılmanız tamamen kendi isteğinize bağlıdır. Katılımı reddetme ya da çalışma sürecinde herhangi bir zaman diliminde devam etmeme hakkına sahipsiniz. Eğer görüşme esnasında katılımınıza ilişkin herhangi bir sorunuz olursa, araştırmacıyla iletişime geçebilirsiniz.

Appendix C

"Consent Form" given before first experimental session.

KATILIMCI İZİN FORMU

Çalışmanın amacını ve içeriğini ..... denek numarasına sahip katılımcıya açıklamış bulunmaktayım. Çalışma kapsamında yapılacak işlemler hakkında katılımcının herhangi bir sorusu olup olmadığını sordum ve katılımcı tarafından yöneltilen bütün soruları yanıtladım.

Tarih: Araştırmacının İmzası:

..... / ..... / ....

Araştırmacının Telefon Numarası:

.....

Çalışmanın amacı ve içeriği hakkında açıklamaların yer aldığı "Katılımcı Bilgilendirme Formu"nu okudum. Araştırmacı çalışma kapsamındaki haklarımı ve sorumluluklarımı açıkladı ve kendisine yönelttiğim bütün soruları açık bir şekilde yanıtladı. Sonuç olarak, uygulama esnasında şahsımdan toplanan verilerin bilimsel amaçlarla kullanılmasına izin verdiğimi ve çalışmaya gönüllü olarak katıldığımı beyan ederim.

Tarih: Katılımcının İmzası:

..... / ..... / ....