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PEDRO FONSECA ZUCCOLO

Investigação dos efeitos do comportamento verbal durante a extinção pós-recuperação sobre o retorno do medo

Investigation of the effects of verbal behavior during post-retrieval extinction on the return of fear

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(Versão corrigida)

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(Corrected version)

Tese apresentada ao Instituto de Psicologia da Universidade de São Paulo como parte dos requisitos para obtenção de título de Doutor em Psicologia

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Banca Examinadora

Prof. Dr. _____ Julgamento: _____

Instituição: _____ Assinatura: _____

Prof. Dr. _____ Julgamento: _____

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Prof. Dr. _____ Julgamento: _____

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RESUMO

Zuccolo, P. F. (2018). *Investigação dos efeitos do comportamento verbal durante a extinção pós-recuperação sobre o retorno do medo* (Tese de doutorado), Instituto de Psicologia, Universidade de São Paulo, São Paulo.

Estudos sobre extinção do condicionamento Pavloviano envolvendo estímulos aversivos (condicionamento de medo) são considerados como análogos experimentais das terapias por exposição, nas quais pacientes são confrontados com situações temidas (porém seguras) com o objetivo de reduzir respostas de medo. Nos experimentos sobre extinção, estímulos que eliciam respostas condicionais (estímulos condicionais, CSs) por terem sido previamente associados a estímulos aversivos incondicionais (estímulos incondicionais, US) são apresentados repetidamente na ausência do US. Como resultado, as respostas condicionais de medo diminuem. Um desafio nessa área é sustentar a redução do medo a longo prazo, visto que o retorno de respostas condicionais (retorno do medo) é comumente observado no laboratório e na clínica. Estudos recentes conseguiram impedir o retorno do medo por meio da extinção pós-recuperação (*post-retrieval extinction*, PRE), procedimento que consiste em extinção após a apresentação de um estímulo que estava presente durante o condicionamento (*retrieval cue*). Contudo, tentativas de replicação desse procedimento geraram resultados conflitantes. O objetivo desta tese é contribuir para o debate sobre as variáveis envolvidas no retorno do medo com o uso da PRE em humanos. Um experimento foi conduzido para verificar se o comportamento verbal emitido pelos participantes durante a PRE pode mudar a probabilidade de retorno do medo. Participantes adultos (n=57) foram submetidos a condicionamento Pavloviano diferencial no qual uma fotografia de uma face humana (CS+) foi pareada a um estímulo elétrico leve (US), enquanto que outra fotografia de face humana nunca foi pareada ao US. No dia seguinte, os participantes foram alocados em um de três grupos (n=19): Experimental atividade verbal relacionada (Exp R), Experimental atividade verbal não-relacionada (Exp N) e Controle. Todos os grupos passaram por extinção, mas para os grupos experimentais, esse procedimento foi antecedido em 10 min por uma pista (*retrieval cue*) que consistia na apresentação não-reforçada dos CSs. Durante o intervalo entre essa pista e a extinção, os participantes do grupo Exp R se engajaram numa atividade na qual tinham que fazer verbalizações relacionadas às contingências experimentais, enquanto que os participantes do grupo Exp N tinham que fazer verbalizações que não estavam relacionadas às contingências experimentais. O grupo controle foi submetido à extinção tradicional (sem apresentação de pista ou 10 min de intervalo antes da extinção). No terceiro dia, todos os participantes passaram por um teste que consistia em quatro apresentações do US seguidas de extinção (teste de restabelecimento). As respostas de condutância da pele frente ao CS e ao US foram usadas como medidas das respostas condicionais e incondicionais, respectivamente. Retorno do medo, medido pelo responder diferencial (discriminação entre CS+ e CS-) no teste, estava presente no grupo controle e em menor grau no grupo Exp R. Em comparação, sujeitos do grupo Exp N não apresentaram responder diferencial em função de diminuição nas respostas frente ao CS+ e aumento nas respostas frente ao CS-. Este estudo mostra que o comportamento verbal pode mudar os efeitos da PRE, o que tem implicações para a sua adaptação para uso clínico.

Palavras-chave: comportamento verbal, extinção pós-recuperação, reconsolidação, retorno do medo, condutância da pele

ABSTRACT

Zuccolo, P. F. (2018). *Investigation of the effects of verbal behavior during post-retrieval extinction on the return of fear* (Tese de Doutorado), Instituto de Psicologia, Universidade de São Paulo, São Paulo.

Studies on extinction of Pavlovian conditioning involving aversive stimuli (fear conditioning) have been considered experimental analogues of exposure treatments in which patients are confronted with feared but safe situations in order to reduce fear responses. In extinction experiments, stimuli that elicit conditioned fear responses (conditioned stimuli, CS) because they have been previously associated with aversive stimuli (unconditioned stimuli, US) are repeatedly presented in the absence of the US. As a result, conditioned fear responses tend to diminish. The challenge in this area is how to maintain fear reduction in the long term, as return of conditioned fear responses (return of fear) is commonly observed in laboratory and clinical settings. Recent studies were able to prevent return of fear by means of post-retrieval extinction (PRE), a procedure consisting of extinction after the presentation of a stimulus that was present during conditioning (*retrieval cue*). However, replications of this procedure have yielded mixed results. With this thesis, I attempted to contribute to the debate on the variables that determine the probability of return of fear after PRE in humans. An experiment was conducted to test if verbal behavior emitted by participants during PRE can change the probability of return of fear. Adult participants (n=57) underwent differential Pavlovian conditioning in which one photograph of a human face (CS+) was paired with a mild electrical stimulus (US), whereas another photograph of human face was not paired with the US. On the next day, participants were designated to one of three groups (n=19): Experimental related verbal activity (Exp R), Experimental non-related verbal activity (Exp N), and Control. All groups underwent extinction but for experimental groups, a retrieval cue consisting of a single unreinforced presentation of the CSs was carried out 10-min prior to extinction. During the interval between retrieval cue and extinction, participants from the Exp R group were required to engage in an activity directing their overt verbal behavior *towards* the experimental contingencies, whereas participants from the Exp N group were required to engage in an activity directing their overt verbal behavior *away* from the experimental contingencies. Control group underwent a standard extinction procedure (no retrieval cue or 10-min interval prior to extinction). On a third day, all participants underwent a test consisting of four presentations of the US alone followed by extinction (reinstatement test). Skin conductance responses to the presentations of the CSs and US were used as the dependent measure of conditioned and unconditioned responses, respectively. Return of fear, as measured through differential responding (discrimination between CS+ and CS-), was present in subjects from the control group and to a lesser extent in subjects from the Exp R group. In contrast, differential responding was abolished in subjects from the Exp N group, a result that was dependent both on decrease in responses to the CS+ as well as increase in responses to the CS-. This study shows that verbal behavior might change the effects of PRE, which can have implication for its adaptation for treating pathological fear.

Keywords: verbal behavior, post-retrieval extinction, reconsolidation, return of fear, skin conductance

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ABBREVIATIONS

ADIS-5	Anxiety and related disorders interview schedule for DSM-5
ASI	Anxiety Sensitivity Index
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
CS, CS+, CS-	Conditioned stimulus
DSM	Diagnostic Statistic Manual
FPS	Fear-Potentiated Startle
ITI	Inter-trial interval
PRE	Post-retrieval extinction
SCID-1	Structured Clinical Interview for DSM-IV Axis I Disorders
SCR	Skin conductance response
SPQ	Spider Phobia Questionnaire
STAI-S	State-Trait Anxiety Inventory – state scale
STAI-T	State-Trait Anxiety Inventory – trait scale
US	Unconditioned stimulus

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The pioneering works of Ivan Pavlov (1849-1936) have described an important learning process commonly known as *Pavlovian (or classical) conditioning*. During Pavlovian conditioning, an initially neutral stimulus (*conditioned stimulus*, CS) acquires the function of eliciting responses in the organism (*conditioned responses*) after being associated with another stimulus that already has eliciting function (*unconditioned stimulus*, US) (Catania, 1999; Pavlov, 1927). When the US is noxious or potentially harmful, the conditioned responses are characterized by a series of reactions implicated in the detection and response to threat (LeDoux, 2014), such as freezing (Bouton & Bolles, 1980) or physiological arousal of the autonomic nervous systems (Graeff, 2007). In this case, conditioned responses are termed *fear conditioned responses* and the learning process, *fear conditioning* (LeDoux, 2014).

The study of fear conditioning in the laboratory has been widely accepted as a model for understanding and, in some cases, guiding behavioral treatments for psychiatric disorders (LeDoux, 2014). For example, studies on the *extinction* of fear conditioning are thought to contribute for the improvement of treatments for psychiatric disorders characterized by significant fear and anxiety, such as Post-Traumatic Stress Disorder, Obsessive-Compulsive Disorder, and phobias (Foa & McLean, 2016; Kaczkurkin & Foa, 2015). During *extinction*[1], the CS is repeatedly presented in the absence of the US, which typically results in reduction of conditioned responses (Lattal & Lattal, 2012). This experimental setup allows us to understand the variables involved in the reduction or maintenance of fear responses and is thought to be an analog of exposure techniques utilized in clinical settings. Exposure techniques consist of exposing patients to feared situations, objects and images in the absence of aversive or threatening outcomes with the goal of reducing fear reactions to those stimuli (Foa & McLean, 2016; Vervliet, Craske, & Hermans, 2013).

One of the many challenges in this area is how to maintain fear reduction in the long term (Foa & McLean, 2016; Vervliet et al., 2013). Though exposure-based treatments have received empirical evidence of its efficacy in treating anxiety disorders or patients with a history of traumatic events (Foa & McLean, 2016; Powers, Halpern, Ferenschak, Gillihan, & Foa, 2010), the return of fear symptoms after successful completion of the treatment is common (Vervliet et al., 2013). Likewise, the return of conditioned fear responses after

[1] In the literature, the term "extinction" has been used to refer either to a procedure (the presentation of the CS without the US after conditioning) or a process (the progressive reduction of the magnitude of the CR due to this procedure) (Catania, 1999; Hermans, Craske, Mineka, & Lovibond, 2006; Lattal & Lattal, 2012). Here, we chose to use the term as a synonym for the procedure.

extinction (referred to as *return of fear*, Lonsdorf et al., 2017) has been extensively described in laboratory studies (Vervliet et al., 2013). Specifically, return of fear has been experimentally demonstrated to occur as a function of three general circumstances: 1) the passage of time since extinction (*spontaneous recovery*, Maren & Chang, 2006; Myers, Ressler, & Davis, 2006; Norrholm et al., 2008; Pavlov, 1927; Robbins, 1990; Schiller et al., 2008); 2) the presentation of the CS in a different context than the setting in which extinction took place (*renewal*, Alvarez, Johnson, & Grillon, 2007; Bouton & Bolles, 1979a; LaBar & Phelps, 2005; Milad, Orr, Pitman, & Rauch, 2005; Neumann & Longbottom, 2008; Schiller et al., 2008; Thomas, Larsen, & Ayres, 2003; Westbrook, Iordanova, McNally, Richardson, & Harris, 2002); and 3) the re-exposure to the US alone before testing (*reinstatement*, Bouton & Bolles, 1979b; Milad et al., 2005; Rescorla & Heth, 1975; Schiller et al., 2008; Westbrook et al., 2002)[2].

Inspired by experimental data on the brain mechanisms of memory, researchers have developed a laboratory procedure that was shown to prevent return of fear in spontaneous recovery, renewal and reinstatement tests (Monfils, Cowansage, Klann, & LeDoux, 2009; Schiller et al., 2010). This procedure, which is termed *post-retrieval extinction* (PRE), consists of exposing subjects to a stimulus that was present during conditioning (*a retrieval cue*), such as the CS without the US or the US alone, followed by extinction (Liu et al., 2014; Monfils et al., 2009; Schiller et al., 2010).

Given the potential for developing a behavioral procedure that prevents the return of fear, several laboratories have attempted to replicate and extend these findings, with mixed results. In the case of studies with human participants, whereas some laboratories were able to demonstrate diminished fear responses after PRE (Agren, Engman, et al., 2012; Asthana et al., 2015; Bjorkstrand et al., 2015; Johnson & Casey, 2015; Liu et al., 2014; Oyarzún et al., 2012; Schiller, Kanen, LeDoux, Monfils, & Phelps, 2013; Schiller et al., 2010; Thompson & Lipp, 2017), there are reports of failure in producing these effects (Fricchione et al., 2016; Golkar, Bellander, Olsson, & Öhman, 2012; Kindt & Soeter, 2013; Klucken et al., 2016; Meir Drexler et al., 2014; Soeter & Kindt, 2011). These discrepancies across studies show that much work is needed to fully understand the variables and specific parameters involved in the

[2] Like the term extinction, spontaneous recovery, renewal and reinstatement are terms that can either refer to procedures (for example, the presentation of the CS in a context different from where extinction took place, as in renewal) or processes (elicitation of conditioned responses after one of these procedures). These terms will also be used as synonyms for the procedures.

long-lasting reduction of fear, and the sources of failure to replicate this effect when exposing subjects to PRE.

With this thesis, I attempt to contribute to the debate on the variables that determine the probability of return of fear after PRE. This work has two parts. In the first part, I present the experimental data and hypothesis that originated the PRE procedure. This is followed by a review of experimental data with human participants, with a focus on methodological differences between studies and variables pointed as relevant to preventing return of fear. Since my interest was on basic behavioral processes, I focused mainly on studies in which the conditioned fear response was created in the laboratory and later diminished using PRE. In the second part of this work, I present an experiment that aimed at testing a variable that might change the effects of PRE and that has received little attention so far in the literature: verbal behavior emitted by participants during PRE. I close with some arguments in favor of this line of investigation, its implications for the study of pathological anxiety, and suggest some questions for future research.

Origins of post-retrieval extinction

Post-retrieval extinction originates in experiments on the brain mechanisms of memory (Agren, 2014; Beckers & Kindt, 2017; Lee, Nader, & Schiller, 2017; Nader & Hardt, 2009). In these studies, subjects are exposed to different learning situations (for example, Pavlovian conditioning) followed by the administration of procedures known to disrupt performance (amnesic treatments), such as strong electroconvulsive shocks, hypoxia, hypothermia, inhibitors of protein synthesis (Sara, 2000). In some cases, experimenters utilize drugs that enhance performance (for example, strychnine, Gordon, 1977).

Many experiments utilizing amnesic or enhancing treatments have found a time interval during which these procedures are effective in changing behavior (Nader & Hardt, 2009). Specifically, amnesic (or enhancing) treatments were shown to be effective when administered shortly after initial learning; when given after a delay (approximately 6 h after learning), no effects were seen on behavior (Lee et al., 2017; Nader & Hardt, 2009). These results led to the memory consolidation hypothesis, which states that there is a time-dependent stabilization of changes in synaptic efficacy following a learning situation (Nader & Hardt, 2009).

Of importance to the present discussion is a body of evidence showing that amnesic treatments can disrupt behavior even after a long time has elapsed since initial learning if they are applied shortly after exposing subjects to part of the environment in which learning took place. This exposure prior to amnesic treatment is called *retrieval cue* and can be a single presentation of a CS (in the case of fear conditioning experiments), exposure to the start box and the click of the opening of its door (in studies where subjects had to learn how to cross a complex maze), or other stimuli present at the occasion of learning (for a description of retrieval cues used in different learning tasks, see Sara, 2000). For example, Misanin, Miller, and Lewis (1968) fear-conditioned rats by pairing an auditory stimulus (a loud white noise, CS) with an electrical shock (US), which was followed by strong electroconvulsive shocks at different times following conditioning. Consistent with data that led to the consolidation hypothesis, conditioned fear responses were no longer elicited by the CS when the amnesic treatment was applied immediately after conditioning. Electroconvulsive shocks also diminished conditioned responses when applied 24 h after conditioning if this treatment was immediately preceded by a single presentation of the CS; no effects on the conditioned responses were seen when the electroconvulsive shocks were applied 24 h after conditioning, but this procedure was not preceded by the single CS.

The findings of Misanin et al. (1968) were replicated by other studies utilizing several amnesic treatments (Dębiec & Ledoux, 2004; Nader, Schafe, & LeDoux, 2000). These data have been interpreted as evidence that putting subjects into contact with retrieval cues can destabilize the memory, which requires a reconsolidation process to be restabilized (Agren, 2014; Kredlow, Unger, & Otto, 2016; Nader & Hardt, 2009; but see Beckers & Kindt, 2017 and Lee et al., 2017 for alternative explanations and problems with this hypothesis). For the present discussion, it is noteworthy that some studies demonstrated that amnesic treatments right after the presentation of a retrieval cue can reduce conditioned responses in a long-lasting way; for example Dębiec and Ledoux (2004) observed diminished conditioned fear responses one month after this treatment. There is also evidence to suggest that the longer the interval between the retrieval cue and the treatment is, the less behavior will be affected, with intervals longer than 6 h rendering the amnesic treatment completely ineffective (Nader et al., 2000; Sara, 2000). Therefore, experimental data suggest that behavioral responses are more likely to be changed in the 6 h following the presentation of retrieval cues.

The possibility of inhibiting conditioned fear responses in a long-lasting way can have profound clinical implications for the improvement of treatments for emotional disorders, and this potential has been increasingly recognized in the literature (Lee et al., 2017). However, with the exception of a few pharmacological treatments (such as administering propranolol), most interventions utilized after retrieval cues to diminish conditioned responses in animals are not safe for use in humans due to their high toxicity or serious side effects (Beckers & Kindt, 2017). Because of these limitations, some researchers have started to investigate whether behavioral treatments could also result in a long-lasting inhibition of fear responses when applied immediately after the presentation of a retrieval cue.

The first study to demonstrate the effects of behavioral treatments applied immediately after the presentation of a retrieval cue was conducted by Monfils et al. (2009). They exposed rats to parings of an auditory stimulus (a tone, CS) with an electrical shock (US). Twenty-four hours later, they exposed the rats to a retrieval cue consisting of a single presentation of the CS without the US. After this retrieval cue, instead of using pharmacological agents or other amnesic treatments, they conducted an extinction session consisting of several presentations of the CS without the US. The length of the interval between the presentation of the first CS (retrieval cue) and extinction training varied across five groups: 10 min (Group 1), 1 h (Group 2), 6 h (Group 3), 24 h (Group 4) and no interval, i.e., the retrieval cue was immediately followed by extinction training in a session in which the CS was presented every 180 s on average (Group 5). Except for Group 5, rats were removed from the experimental chamber

after the retrieval cue and returned to their cages, where they remained during the established interval, after which they underwent the extinction training in the experimental chamber. Subsequently, two tests were conducted, 24 h and one month after extinction, in which the CS was presented alone (without the US) and CS-induced freezing was measured. Conditioned responses were diminished in all groups in the first test (24 h after extinction training). However, one month after extinction, only subjects from Groups 1 and 2, whose interval between the retrieval cue and extinction training was 10 min and 1 h, respectively, showed diminished conditioned responses; the remaining groups showed a significant return of conditioned responses, i.e., spontaneous recovery. Similar results were obtained by conducting the test in a different context than the setting in which extinction took place (renewal test) or presenting the US in isolation before the test (reinstatement test) (Monfils et al., 2009).

Post-retrieval extinction in humans

PRE procedures derived from Monfils et al. (2009) were first adapted for humans by Schiller et al. (2010), who used colored squares displayed on a computer screen as CSs, an electrical shock as US, and skin conductance response as the measure of conditioned and unconditioned responses. Participants were exposed to three experimental phases separated by 24 h: conditioning, extinction and testing. In the first phase, one of two squares was paired with the US in 38% of the trials (CS+), whereas the other square was never paired with the US (CS-). During extinction, participants were divided into three groups (10 min, 6 h, and no reminder groups) and were exposed to 11 CS+ and 11 CS- without the US. Two groups (10 min and 6 h groups) were exposed to an isolated presentation of the CS+ (retrieval cue), followed by an interval of 10 min during which participants watched a TV show episode previously selected by the researchers. After this interval, the 10-min group underwent extinction (i.e., were exposed to the remaining CSs). The 6-h group received the same treatment as the 10-min group, but extinction was conducted 6 h after the retrieval cue. The remaining groups (no reminder groups) began the session by watching the TV program for 10 min. For half of these subjects, extinction followed immediately after this 10-min period; for the other half, extinction was conducted 6 h after watching the TV show. During the test, all the participants were exposed to 11 presentations of each CS. The results showed that only subjects whose interval between the retrieval cue and extinction was 10 min did not show recovery of conditioned responses. The results were the same after one year, when 19 of the

65 original participants underwent a reinstatement test consisting of four unsignaled presentations of the US, followed by extinction.

Schiller et al. (2010) conducted a second experiment to assess the specificity of extinction with the procedure tested in their first experiment, i.e., to test whether interfering with the eliciting function of one CS would affect the eliciting function of another CS associated with the same US. Three CSs (colored squares) were used in a within-subject design. During the conditioning phase, two squares (CSa+ and CSb+) were paired with the US (in 38% of presentations), and the third was never paired (CS-). On the following day (extinction phase), participants were exposed to 11 presentations of each of the three CSs without the US. At the start of extinction session, one CSa+ and one CS- were presented (retrieval cues), followed by a 10-min interval, during which participants watched an episode of a TV program; after this interval, participants were exposed to 10 presentations of the CSa+ and CS- and 11 presentations of the CSb+. Testing occurred on a third day and involved the presentation of four USs (without CS), followed by a 10-min interval, during which participants watched the same television episode as on the previous day, and a new extinction procedure (11 presentations of each of the three CSs alone). Like their first experiment, conditioned responses in the test were only observed when the CSb+ was presented. This set of results suggests that even with humans, the effects of extinction can be enduring if the procedure is conducted after the presentation of a retrieval cue. These data also indicate that the timing of extinction relative to retrieval cues is important, with intervals shorter than 6 h allowing for the effect to occur.

Since the publication of Schiller et al. (2010), several laboratories have begun to investigate PRE in human participants. Table 1 shows the experimental manipulations and timeline of experiments in which the behavior of interest (conditioned fear responses) was created in the laboratory and diminished with PRE. These studies have at least three phases, usually separated by a 24-h interval (though this interval has been manipulated in some reports, as discussed in section **Possible boundary conditions to the reduction of conditioned fear responses using PRE: Time since conditioning**). In all studies, the participants underwent differential Pavlovian conditioning in the first phase. In differential Pavlovian conditioning, one or more CSs are paired with the US (CS+s), and there is another CS that is never paired with the US (CS-) (Haaker, Golkar, Hermans, & Lonsdorf, 2014). Table 2 shows the different stimuli used in these experiments. As is typical in studies on Pavlovian conditioning in humans, CSs were visual stimuli (pictures) presented on a computer screen. Mild electrical shocks were the US in all but four studies, which used

aversive auditory stimuli, air-blast directed at the larynx, or a hybrid of white noise and pictures of potentially dangerous animals.

Regarding the experimental design, while some experimenters utilized a between-subject design, in which different groups of subjects are exposed to different experimental conditions, there are several studies reporting within-subject design or both within- and between-subject comparisons in the same experiment. In within-subject designs, two CSs are paired, while a third CS is never paired with the US (CS-). In this case, experimental manipulations are conducted with only one CS+, and responses to both CS+s (manipulated and not manipulated) are compared in the test.

In all experiments, PRE procedures were conducted in the second phase (Table 1). Specifically, after a conditioning phase, participants were exposed to extinction, which consisted of several presentations of the CS in the absence of the US. This procedure was preceded by the presentation of a retrieval cue in some groups (or preceded by a retrieval cue for only one of the CS+s in within-subjects design). The interval between the retrieval cue and extinction was usually 10 min. In most studies, a single presentation of the CS alone was used as a retrieval cue. However, very recently, some experimenters have also used a single presentation of the US alone as a retrieval cue (Table 1). As discussed below, these differences in the type, duration and structure of retrieval cues have been shown to influence test results.

One important feature of most PRE studies in humans is that participants are tested for return of fear only if they show evidence of conditioning and extinction. Only a few studies do not report excluding subjects because of lack of conditioning or extinction, and some have conducted analyses with both the entire sample and only with subjects fulfilling these criteria (Table 3). Conditioning and extinction are assessed by comparing responses to the CS+ with responses to the CS- during the first experimental phase. Greater responses to the CS+ in comparison to responses to CS-, as well as an increase in responses to the CS+ from the beginning to the end of the session, are interpreted as evidence of fear conditioning. Responses are said to have been reduced after extinction when differences between CS+s and CS- are no longer significant or when results are in the opposite direction of the conditioning (i.e., greater responses to the CS- in comparison with responses to the CS+).

Regarding the test phase, most studies assess return of fear by means of a reinstatement procedure in which the US is presented alone several times, followed by presentations of the CS alone. However, it is also common for experimenters to utilize spontaneous recovery (presentations of the CS after some time has passed since extinction) or

renewal (presentations of the CS in a different context than the setting where extinction took place) tests. In these tests, return of fear is assessed by comparing responses to the CSs (CS+s and CS-) at the end of extinction (last trial or a block of trials at the end of the session) with responses to the CSs at the beginning of the test (first trial or first block of trials) (Asthana et al., 2015; Bjorkstrand et al., 2015; Kindt & Soeter, 2013; Klucken et al., 2016; Oyarzún et al., 2012; Schiller et al., 2013, 2010) or by comparing responses to the different CSs in the test (CS+ vs. CS-) (Fricchione et al., 2016; Golkar et al., 2012; Golkar, Tjaden, & Kindt, 2017; Thompson & Lipp, 2017). In some cases, only responses to the CS+ at the end of extinction are compared with responses to the CS+ at the beginning of the test (Johnson & Casey, 2015; Meir Drexler et al., 2014; Warren et al., 2014). In other cases, a differential score (CS+ - CS-) at the end of extinction is compared with differential scores at the beginning of the test (Agren, Björkstrand, & Fredrikson, 2017; Agren, Engman, et al., 2012; Agren, Furmark, Eriksson, & Fredrikson, 2012; Liu et al., 2014; Schiller et al., 2010; Soeter & Kindt, 2011; Steinfurth et al., 2014).

There are also studies reporting reconditioning (usually called *reacquisition*) and generalization tests. In reconditioning, the CS is paired again with the US and experimenters assess whether CS+ / CS- differentiation occurs again (Agren, Furmark, et al., 2012). In generalization tests, subjects are presented with visual stimuli that are matched in stimulus category, valence, and arousal with the CSs utilized in conditioning to assess whether reduction of fear conditioned responses after PRE generalizes to these stimuli. For example, Soeter and Kindt (2011) conditioned subjects using pictures of a spider (CS1+), a gun (CS2+), and a mug (CS-). In the test, subjects were presented different gun, spider, and mug pictures, and conditioned fear responses to these stimuli were assessed.

Possible boundary conditions to the reduction of conditioned fear responses using post-retrieval extinction

As is the case with any new phenomenon, one fundamental question is whether the findings suggesting its existence are sufficiently reliable and replicable. In the case of PRE, this is especially important because of its possible clinical exploitation and theoretical implications. As mentioned earlier, after the first experiments demonstrating diminished return of fear after PRE, several researchers tested this procedure to extend the findings and describe the variables that are responsible for the described effects. However, despite apparently manipulating the same variables, the results obtained diverged among the various studies. These contrasting results have been discussed as evidence that there might be

conditions under which PRE is not effective in preventing the return of fear (Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Lee et al., 2017; Nader & Hardt, 2009). In what follows, I will present a review of variables that have been experimentally tested as possible *boundary conditions* to the reduction of conditioned fear responses using PRE.

Time since conditioning

As previously discussed, studies demonstrating long-lasting reduction of fear in humans usually use a three-day procedure in which PRE is conducted one day after conditioning. As such, it is important to ask whether the procedure can be as effective when learning took place after more than 1 day (i.e., when memories are older). In humans, this has been tested by a study in which participants underwent a differential fear conditioning by pairing electrical shocks (US) with one of two colored squares (CS+s) (Steinfurth et al., 2014). Participants were divided into four groups, two of which underwent traditional extinction 1 or 7 days after conditioning (No Reactivation Day 1 and No Reactivation Day 7, respectively). The other two groups were exposed to PRE, i.e., a single presentation of the CS+ without the US, followed by a 10-min interval and then extinction. PRE was conducted 1 or 7 days after initial learning (Reactivation Day 1 and Reactivation Day 7, respectively). On a third day, all groups underwent a reinstatement test. Participants who underwent PRE showed no evidence of fear recovery as measured through SCR, regardless of the age of the memory.

Results from Steinfurth et al. (2014) suggest that PRE might be effective in reducing the return of older fears. This is consistent with an experimental study showing the successful reduction of avoidance responses to pictures of spiders after PRE in subjects with a history of fear of spiders (Björkstrand et al., 2016). However, evidence from experimental studies on rodents suggests that other factors might contribute to the effects of PRE in >1 d old conditioned fear responses. First, a series of rat studies failed to show reduction of conditioned fear responses with pharmacological blockage of protein synthesis after a retrieval cue when the number of CS-US pairings was increased during conditioning or when the interval between conditioning and post-retrieval pharmacological blockage was prolonged (eight weeks, Suzuki et al., 2004). However, if the duration of the retrieval cue was increased, then the same procedure was effective in both circumstances (Suzuki et al., 2004). Another series of studies in rats showed that boundary conditions related to the interval between initial learning and intervention can be transient. Specifically, pharmacological blockage of protein synthesis after retrieval cues did not reduce conditioned fear responses when conducted 7

days after conditioning; however, the same procedure, when conducted 30 or 60 days after conditioning, diminished fear responses (Wang, de Oliveira Alvares, & Nader, 2009).

One issue about the study of Steinfurth et al. (2014) is that only autonomic responses (skin conductance responses) were acquired in order to closely replicate animal studies. As will be discussed later, the inclusion of more than one method of assessment of conditioned responses, and especially verbal ratings, can change conditioning, extinction, and return of fear. Therefore, it remains to be seen whether results of Steinfurth et al. (2014) would be the same if multiple indices of conditioning are used.

Reinforcement rate and number of CS-US pairings

The reinforcement rate during conditioning (i.e., the proportion of CS trials ending with the US) has been referred to as a variable that might change the probability of return of fear after PRE (Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013; Oyarzún et al., 2012; Soeter & Kindt, 2011; Warren et al., 2014). The first two studies showing diminished return of fear after PRE utilized low reinforcement rates during conditioning (38% in Schiller et al., 2010, and Oyárzun et al., 2012), whereas some experiments reporting failure to produce these effects were conducted with higher reinforcement rates (75% in Kindt and Soeter, 2013, and 80% in Soeter and Kindt, 2011). This difference has led some authors to suggest that higher reinforcement rates might produce conditioning that is more resistant to change (Oyarzún et al., 2012). However, the experimental data are at odds with this hypothesis because return of fear with PRE has been prevented in studies using high reinforcement rates (for example, Agren, Engman, et al., 2012, and Thompson and Lipp, 2017, who utilized a reinforcement rate of 100%; or Asthana et al., 2015, who used 80%).

Golkar et al. (2012) pointed out some problems with the hypothesis that a higher proportion of CS-US pairings in post-retrieval might lead to stronger conditioning. First, none of the studies on PRE in humans utilized an independent measure of conditioning strength, such as resistance to extinction, which is the measure described in animal studies (Suzuki et al., 2004; Wang et al., 2009). Moreover, if resistance to extinction was to be used as a measure of conditioning strength, then experiments showing long-lasting fear reduction are the ones with stronger conditioning, since they utilized intermittent pairings, and studies have shown that under this condition, more extinction trials are required for conditioned responses to reduce (Bouton, 2004; LaBar, LeDoux, Spencer, & Phelps, 1995; Schiller et al., 2008). Another problem arises from the fact that studies reporting no return of fear after PRE included only participants who showed evidence of reduction of CR at the end of the

extinction phase; therefore, only subjects who did not present resistance to extinction were included (Agren, Engman, et al., 2012; Agren, Furmark, et al., 2012; Oyarzún et al., 2012; Schiller et al., 2013, 2010). One last point regarding the issue of conditioning strength is that animal studies produced resistance to extinction by increasing the number of CS-US pairings and not by manipulating the reinforcement rate. For example, Wang et al., (2009) showed that ten CS-US pairings needed more extinction trials for conditioned fear responses to decrease compared to a single CS-US pairing. An analysis of the studies with human participants (Table 2) shows that there is no apparent relation between reinforcement rate or number of CS-US pairings and fear reduction after PRE. In fact, a very recent study compared the effects of PRE when conditioning was conducted once and when it was conducted on three consecutive days and failed to show differences between the two groups (Kredlow, Orr, & Otto, 2018).

Nature of the CS

Given the possibility of using PRE as a clinical strategy for augmenting behavioral treatments for anxiety disorders, some authors have tried replicating Schiller et al's findings utilizing stimuli that resemble clinical situations. As previously described, Schiller et al. (2010) utilized colored squares as CSs, which are thought to be fear-irrelevant and less likely to be associated with anxiety in real life (Golkar et al., 2012, 2017; Kindt & Soeter, 2013; Kindt, Soeter, & Vervliet, 2009; Soeter & Kindt, 2011). Some authors utilized fear-relevant stimuli as CSs, such as pictures or high definition video-clips of potentially dangerous animals, such as tigers, dogs, snakes, spiders (Fricchione et al., 2016; Kindt & Soeter, 2013; Meir Drexler et al., 2014; Soeter & Kindt, 2011), or fearful male faces (Golkar et al., 2012). In these studies, PRE did not prevent return of fear, which led to the hypothesis that the nature of the CS would explain the divergence of results reported (and that PRE was able to prevent return of fear only when CSs were fear-irrelevant) (Fricchione et al., 2016; Kindt & Soeter, 2013; Soeter & Kindt, 2011).

It has been argued that failures to replicate the findings from Schiller et al. (2010) with fear-relevant stimuli might be related to stronger conditioning produced by these stimuli. Evidence supporting this hypothesis comes from experimental studies demonstrating that pairing aversive USs with fear-relevant CSs result in conditioning that is more resistant to extinction (Mineka & Öhman, 2002; Öhman, Fredriksson, Hugdahl, & Rimmö, 1976; Öhman & Mineka, 2001). However, as pointed out by Golkar et al. (2012), many PRE studies in humans report including only participants who showed evidence of extinction (Agren et al.,

2017; Agren, Engman, et al., 2012; Agren, Furmark, et al., 2012; Golkar et al., 2012; Liu et al., 2014; Oyarzún et al., 2012; Schiller et al., 2013, 2010; Steinfurth et al., 2014). In other studies, return of fear was prevented by PRE using fear-irrelevant stimuli and including the whole sample (i.e., no exclusion of non-extinguishers was reported) (e.g. Asthana et al., 2015). Lastly, there is one report of failure to replicate findings from Schiller et al. (2010) using both fear-relevant and fear-irrelevant stimuli (Golkar et al., 2012), suggesting that the fear-relevant properties of the CS are not sufficient to explain failures to replicate Schiller et al. (2010).

Recent data suggest that PRE might work on both fear-relevant and fear-irrelevant stimuli depending on the retrieval cue utilized and the extinction method (Golkar et al., 2017; Thompson & Lipp, 2017). Thompson and Lipp (2017) associated both fear-relevant (pictures of a spider or a snake) and fear-irrelevant (pictures of a blue or yellow square) stimuli with an electrical stimulus (US). Subsequently, subjects were divided into two groups. One group was exposed to a retrieval cue consisting of a presentation of the US at half the physical intensity used during conditioning, followed by a 10-min interval and then extinction training; participants in the other group (control group) were only exposed to extinction training after a 10-min period equivalent to the experimental group. The results showed spontaneous recovery and reinstatement of skin conductance responses to both CSs (fear-relevant and fear-irrelevant) in the control but not in the experimental group. As will be discussed later, this US-retrieval cue procedure has been shown to be effective and to have different effects than the traditional CS-retrieval procedure (see **Possible boundary conditions to the reduction of conditioned fear responses using PRE: Retrieval procedures and prediction error**)

In another very recent study, Golkar et al. (2017) paired two CSs (CSa+ and CSb+) with an electrical shock (US) and used a third CS, never paired with the US, as a control (CS-). On the second day, one of the previously reinforced CSs (CSa+) was presented once 10 min prior to a vicarious extinction procedure, which consisted of watching a 24-min video depicting a model in front of a computer screen reacting calmly to unreinforced presentations of CS. During the video presentation, subjects were connected to stimulation equipment but were never shocked. The recovery of fear was measured with fear-potentiated startle on the third day. In the test, subjects showed an increase in responses only to the CSb+, which was not presented prior to extinction training.

Retrieval procedures and prediction error

Evidence from studies using pharmacological interventions have shown that it is possible to reduce conditioned responses by changing the parameters or structure of retrieval cues. For example, it has been demonstrated that depending on the history of learning, the retrieval cue does not have to be an exact replica of the CSs used in conditioning (Soeter & Kindt, 2015). Soeter and Kindt (2015) tested this by exposing participants to differential fear conditioning with two CSs, one of which was paired with the US (CS+). For one group of participants, the CS+ and CS- were different pictures of spiders (perceptual group), whereas two stimuli of different categories served as CSs in the other group, specifically, a picture of a spider and a picture of a snake (categorical group). In a second phase of the experiment, both groups were exposed to a retrieval cue followed by the administration of propranolol. For the perceptual group, the retrieval cue used consisted of the word "SPIDER," while in the categorical group, the retrieval cue was a word referring to the category of the CS+ (that is, the word "SPIDER" for participants whose CS+ was a spider and the word "SNAKE" for participants whose CS+ had been a picture of a snake). The results of this experiment showed that the differential fear-potentiated startle response was eliminated only in the categorical group.

In rats, reduction in conditioned responses was observed when the US was used as a retrieval cue prior to administering a protein synthesis inhibitor (Dębiec, Díaz-Mataix, Bush, Doyère, & LeDoux, 2010). This observation was later replicated in a series of experiments on PRE in humans. Liu et al. (2014) conditioned participants using pictures of colored squares (CSs) and electrical shocks (US) and exposed them 24 h later to the presentation of a retrieval cue followed by extinction. They used a single presentation of the CS (without the US) or a single presentation of the US (at half the intensity utilized in conditioning) as retrieval cues. The results of Exp. 1 showed that a US-retrieval cue before extinction can be as effective as a CS-retrieval cue in reducing fear return in spontaneous and reinstatement tests. As previously shown, these experiments have been replicated very recently in humans using both fear-relevant and fear-irrelevant stimuli as CSs (Thompson and Lipp, 2017).

Other results from Liu et al (2014, Exp. 2 and 3) demonstrated that the US-retrieval procedure shows some differences in relation to the use of CS as retrieval cues. Specifically, they showed a long-lasting reduction of responses to all CSs previously associated with the US, whereas using a CS as a retrieval cue reduces only responses elicited by that specific CS (Liu et al., 2014, Exp. 2). They also demonstrated that a US retrieval cue before extinction

can prevent the return of conditioned responses to all CSs previously associated with it even when extinction was conducted with only one of the CSs (Liu et al., 2014, Exp. 3).

The issue of which properties of the retrieval cue are critical for producing long-lasting reduction of fear after PRE has been increasingly discussed in the literature. One hypothesis, raised to explain why some retrieval cues are more effective than others, comes from classic theories of conditioning (Sevenster, Beckers, & Kindt, 2013). Specifically, these theories state that changes in the strength of conditioned responses depend on the generation of a prediction error, that is, a discrepancy between actual and past events (Rescorla & Wagner, 1972). In line with this theory, some authors have proposed that a retrieval cue would allow post-retrieval intervention to effectively change behavior if it shows that there is something new to be learned (Sevenster, Beckers, & Kindt, 2012b). For this to happen, retrieval conditions cannot have fully predictable outcomes; that is, there must be a certain degree of prediction error.

Three experiments from the same laboratory support this hypothesis (Sevenster et al., 2012b, 2013; Sevenster, Beckers, & Kindt, 2014). In one study, participants underwent differential conditioning in which one of two pictures of spiders (CSs) was associated with an electrical shock (US) (Sevenster et al., 2012b). In a second phase of the experiment, participants were exposed to a retrieval cue consisting of one presentation of the CS without the US, followed by the administration of propranolol. For one group of participants (Propranolol group), the retrieval cue was conducted with shock electrodes attached, which is the usual procedure and is thought to induce prediction error, since a shock was to be expected based on the conditioning phase. For a second group, shock electrodes were not attached, and participants were instructed that the CS+ would not be followed by US (Propranolol No-Shock Expectation). This procedure was conducted to promote a situation in which the absence of US was unsurprising. There was also a third group, which was exposed to the retrieval cue with shock, but this was followed by the administration of a placebo (Placebo group). In line with their hypothesis, only the Propranolol group showed no fear-potentiated startle responses when exposed to presentations of the CS without the US 24 h later.

These authors expanded these results in another experiment in which two groups of participants were conditioned by associating one of two pictures (CSs) with an electrical shock (US) in all trials (100% reinforcement rate), and participants were explicitly instructed that the CS would be followed by the US (Sevenster et al., 2013). On the second day, both groups were presented with a retrieval cue followed by the administration of propranolol. For

one group, however, the retrieval cue was a single presentation of the CS+ without the US (Negative prediction error group), whereas the other was exposed to a presentation of the CS+ with the US, as with conditioning (No prediction error group). Experimenters also tested a third group (Positive prediction error group), for which conditioning was conducted by pairing the CS with the US in 33% of trials and by giving verbal instructions that did not describe which of the two CSs would be followed by a shock. For this group, the retrieval cue was a single presentation of the CS with the US. All participants were exposed to a test on a third day on which the CS was presented without the US several times, which was followed by a reinstatement test. According to the hypothesis that prediction error is necessary for post-retrieval amnesia to occur, the Negative prediction error group should present diminished return of fear in the test, since the omission of the US during the retrieval cue consisted in a mismatch between actual and past events. They also predicted that the Positive PE group would not show fear responses in the test because the partial reinforcement rate used in conditioning would not induce asymptotic learning and, as such, a reinforced presentation of the CS on the second day of the experiment should generate additional learning. In accordance with these predictions, participants from the Negative prediction error group reported a decrease in their expectancy to receive the US from the end of conditioning to the beginning of the test, whereas this expectancy increased in the positive Prediction error group, and remained similar in the No prediction error group. Furthermore, only the No prediction error group showed return of fear.

A third experiment from the same group was designed to test if different degrees of mismatch between conditioning, and the retrieval cue would change the results. In this study, participants underwent fear conditioning with a 50% rate of reinforcement in which CSs were paired on all even trials but not odd ones. Therefore, USs could be expected at every other trial. On Day 2, participants received either one, two or four presentations of the CS without the US, followed by the administration of propranolol. On Day 3, when the CS was presented, attenuation of fear was observed in the group presented with the CS twice in the second day of the experiment. These results suggest that retrieval cues are effective only when they induce an optimal degree of expectancy violation. Moreover, the transition from insufficient prediction error to sufficient prediction error appears to be subtle (Sevenster et al., 2014).

The results from studies in rats have also supported the notion that some degree of discrepancy between retrieval cues and the original learning condition are required for a persistent attenuation of fear to be observed in the test. In these studies, changes in the parameters of training and retrieval cues, such as the timing of the US relative to the CS offset

or the removal of the US during CS presentation, have been shown to result in drug-induced amnesia (Alfei, Ferrer Monti, Molina, Bueno, & Urcelay, 2015; Díaz-Mataix, Ruiz Martinez, Schafe, LeDoux, & Doyère, 2013; Pedreira, Pérez-Cuesta, & Maldonado, 2004). To the best of our knowledge, there are no reports in which the role of prediction error was directly tested using PRE procedures in humans. However, some authors analyzed that differences between the stimuli when used as conditioning and when used as retrieval cues might be a possible source of failure in showing persistent fear reduction (Agren, 2014; Golkar et al., 2012). For example, Golkar et al. (2012) raised the possibility that retrieval cues consisting of unreinforced (nonpaired) presentations of the CS prior to extinction were more likely to avoid return of fear if conditioning was done at a high reinforcement rate. This is because one unreinforced presentation of a CS after a history of 100% CS-US pairings would represent a greater mismatch than the same unreinforced presentation after a history of intermittent and infrequent pairings. However, as Golkar et al. (2012) pointed out, this hypothesis is at odds with the fact that several studies demonstrating no return of fear have utilized low reinforcement rates during conditioning (Liu et al., 2014; Schiller et al., 2013, 2010, Table 2). Another possible source of mismatch between the retrieval procedure and conditioning could be the duration of the CS. For example, Agren, Engman, et al. (2012), who showed no return of fear after PRE, used a 120 s presentation of the CS without the US as a retrieval cue (in conditioning, CSs had a 6 s duration). Nevertheless, most studies demonstrating no return of fear have used the exact same duration for CSs during conditioning and retrieval procedures (Johnson & Casey, 2015; Liu et al., 2014; Schiller et al., 2013, 2010; Steinfurth et al., 2014).

Some authors have pointed out that an analysis of the relation between prediction error and return of fear after PRE should consider the strength of the conditioning. According to this view, the amount of prediction error (i.e., the amount of discrepancy between the retrieval cue and the history of conditioning) might have to be greater when conditioning is stronger (Exton-McGuinness, Lee, & Reichelt, 2015; Fricchione et al., 2016). However, as discussed previously, confirmation of this hypothesis requires experimenters to present some sort of independent measure of strength. Future work should address this issue.

In sum, work is still needed to confirm this hypothesis and the limits of prediction error on the prevention of fear after PRE. Future studies should directly test the relation between the degree of similarity between retrieval cues and the history of conditioning. In addition, measures of strength also appear to be necessary here to make comparisons between the degree of prediction error and the amount of fear reduction. Lastly, it is necessary to consider the role of verbal instruction in prediction error.

Instructions during experimental phases

Verbal instructions have been shown to establish (Olsson & Phelps, 2004) or extinguish conditioned responses to aversive stimuli (Sevenster, Beckers, & Kindt, 2012a). In addition, there are studies demonstrating that extinction via instructions attenuates return of fear through reinstatement (Sevenster et al., 2012a). A recent study demonstrated that instructions about the CS-US contingency prior to fear conditioning enhanced fear responses during the conditioning and renewal test, whereas information about the absence of CS-US contingency prior to extinction and the test promoted diminished fear responses during these phases; information on the absence of the CS-US contingency only prior to extinction promoted diminished fear responses during this phase but did not prevent return of fear in a renewal test (Javanbakht et al., 2017). In view of this data, it is important to ask whether instructions might change the effects of PRE.

Although information on the instructions given to participants is not always reported, some analyses can be performed. In studies demonstrating no return of fear after PRE, participants were instructed to pay attention to the stimuli (CSs and US) and try to determine the relation between both during conditioning (Kredlow et al., 2018; Liu et al., 2014; Schiller et al., 2010). Experiments reporting negative results usually have more explicit instructions describing that one of the stimuli would be followed by the US (Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013; Soeter & Kindt, 2011). However, there are exceptions, such as Thompson and Lipp, 2017, who told participants that one of the CSs would be followed by the US and were able to prevent return of fear in the test, and Klucken et al. (2016), who told participants only to pay attention to the computer screen and showed negative results. One could ask if explicit instructions rendered conditioning stronger, as there is evidence from experimental studies that conditioned responses are enhanced when verbal instructions regarding CS-US contingencies are presented (Mertens et al., 2016).

In six out of eight studies reporting no reduction return of fear with PRE, participants were asked to "remember what they had learned" at the end of conditioning (Fricchione et al., 2016; Golkar et al., 2012; Kindt & Soeter, 2013; Meir Drexler et al., 2014; Soeter & Kindt, 2011). There are also exceptions here, such as Thompson and Lipp (2017) and Golkar et al., (2017), who showed long-lasting fear reduction, but their procedures are not entirely comparable (specifically, Thompson and Lipp used a US as retrieval cue, while Golkar et al. utilized vicarious extinction after memory reactivation). According to Fricchione et al. (2016), asking participants to remember the experimental session allowed for the verbalization of the learning experience. Participants' recollection was neither confirmed nor denied by the

experimenters, but it is possible that asking participants to review the contingences rendered the conditioning more resistant to PRE. As such, future studies should address the differences between PRE when one asks or does not ask for the explicit recollection of the experimental situation.

Subjects' pre-experimental characteristics

A possible source of variation between studies might be related to the pre-experimental characteristics of participants, such as genetic, demographic, or psychiatric factors. For example, Agren, Furmark, et al. (2012) explored the effects of genetic characteristics on PRE. Specifically, they assessed whether serotonin- and dopamine-related polymorphisms could affect the results of PRE. They compared two groups, one with a 10-min and another with a 6-h interval between the retrieval cue and extinction. They found that reacquisition one day after extinction was stronger in individuals whose interval between the retrieval cue and extinction was 6 h. In addition, reacquisition in the 6-h group was predominantly present in val/val homozygotes of the functional val158met polymorphism of the catechol O-methyltransferase (COMT) enzyme and in short-allele carriers of the serotonin-transporter length 5-HTTLPR polymorphism. Carriers of the met allele and long-allele homozygotes did not display reacquisition regardless of the timing of extinction. However, a recent study replicating these procedures in a different population did not find the same associations between COMT Val158Met-polymorphism and return of fear (Klucken et al., 2016). By contrast, another study has shown that persistence in reduction of conditioned responses after PRE was present only in the Met-allele carriers of the BDNF val66met polymorphism (Asthana et al., 2015).

Research on the influence of genetic characteristics on PRE shows great potential. Twin studies suggest that fear conditionability is moderately inheritable. In addition, there is evidence from experimental reports that both fear conditioning and extinction are influenced by individual differences in serotonin- and dopamine-related polymorphisms (Garpenstrand, Annas, Ekblom, Oreland, & Fredrikson, 2001; Lonsdorf et al., 2009). There are also studies showing that response systems are differentially affected by genetic characteristics: whereas individual differences in the conditioning and extinction of fear-potentiated startle were associated with polymorphisms in 5-HTTLPR and COMT Val158Met genes, the amount of conditioning and extinction of skin conductance was independent of genotype (Lonsdorf et

al., 2009). As pointed out by Klucken et al. (2016), it remains to be seen whether this dissociation between response systems and genotype also applies to the effects PRE.

There has been some exploration of the influence of other pre-experimental characteristics on the effects of PRE such as age and gender. Johnson and Casey (2015) analyzed the differences between adolescents and adults regarding the effects of PRE. Each age group was divided in two subgroups: a group exposed to a retrieval cue 10 min before extinction and a control group exposed to standard extinction. Adolescents showed diminished reduction of conditioned responses during extinction, but no differences were found in the test; both adolescents and adults showed diminished recovery of fear when a retrieval cue was presented 10 min prior to extinction. A recent meta-analysis of PRE found no relation between the age of participants and the direction of the results (Kredlow et al., 2016). Nevertheless, it would be interesting to explore whether the results are the same when older persons are assessed. Additionally, since both conditioning and extinction involving aversive stimuli may be affected by hormones and women's menstrual cycles (Merz et al., 2012; Milad, Igoe, Lebron-Milad, & Novales, 2009; Milad et al., 2010), some authors have performed analyses within their samples to determine whether sex could alter the results of PRE, but the results were negative (Liu et al., 2014; Meir Drexler et al., 2014). Further examination of these issues is warranted.

An important boundary condition might be related to the presence of psychiatric symptomatology. A meta-analysis of studies of fear conditioning in human subjects found evidence that individuals with anxiety disorders present increased Pavlovian conditioning and take longer to stop responding to CS presentations during extinction procedures than do healthy subjects (Lissek et al., 2005). A positive correlation has also been found between chronic anxiety levels, as measured by self-report (Kindt & Soeter, 2013; Spielberger, Gorsuch, & Lusthene, 1970), and an increase in skin conductance responses when CS- is presented after uncontrollable and unpredictable shocks (Kindt et al., 2009; Soeter & Kindt, 2010).

The demonstration of the effects of PRE in clinical populations is especially important if this procedure is to be used in applied situations. Some laboratories have begun exploring this issue. For example, Kredlow et al., 2018 compared the effects of PRE and standard extinction in a mixed sample of healthy and anxious individuals using skin conductance as dependent measure. These authors failed to show attenuation of return of fear, and no measure of anxiety served as a moderator of the effect of PRE. In another study, subjects reporting a predisposition to being afraid of spiders showed no reduction of return of fear after PRE when

the CSs were fear-relevant CSs (Fricchione et al., 2016). Despite these failures to show attenuation of return of fear created in the laboratory in these specific samples, there is evidence that PRE is effective in attenuating a pre-existing fear of spiders (Björkstrand et al., 2016). Therefore, the area would certainly benefit from other comparisons between healthy people and psychiatric patients or between two extremes of the same psychiatric disorder (e.g., high vs. low anxious individuals).

Response systems

Several methods have been used to measure conditioned responses in humans, including skin conductance responses (SCR), fear-potentiated startle (FPS)[3], US expectancy ratings[4], valence ratings [5], and reaction-time tasks (Haaker et al., 2014). Although these measures can covary, there is evidence to suggest that they do not necessarily work in conjunction (Kindt & Soeter, 2013). For example, conditioning of SCR was demonstrated with both aversive and non-aversive USs (electrical shock and a vibrotactile stimulus, respectively), whereas FPS was only observed when the US was aversive (Hamm & Vaitl, 1996). Another difference between SCR and FPS is that the conditioning of SCR was demonstrated only in participants that could describe the CS-US contingency (i.e., contingency-aware participants), whereas participants showed FPS conditioning in both aware and unaware participants (Hamm & Vaitl, 1996; Weike, Schupp, & Hamm, 2007). There is also evidence of a dissociation between US-expectancy ratings and FPS. One example is a

[3] Fear-potentiated startle is measured through electromyographic activity (EMG) of the orbicularis oculi muscle (Kindt & Soeter, 2013; Vervliet, Craske, & Hermans, 2013). In this type of measure, it is necessary to present not only the CS and the US (usually an image and an electrical stimulus, respectively) but also a startle stimulus, which in many studies is a brief sound stimulus (for example, 95 dB of white noise lasting 50 ms, as used by Weike, Schupp, and Hamm, 2007). The startle stimulus, when presented, produces a blink response of a certain magnitude and latency. This stimulus is presented either alone or superimposed on the CS. Next, the difference between the blinking magnitude in the absence and presence of the CS is estimated. If the magnitude of the blink response to the startle stimulus is greater when superimposed on the CS, a response elicitation is considered to have occurred (Kindt & Soeter, 2013; Weike et al., 2007).

[4] US-expectancy rating is assessed by presenting a scale whose values reflect the degree of certainty that a CS will be followed by a US. For example, in Kindt and Soeter (2013), in the presence of the CS, a scale appeared on a computer screen, ranging from -5 (certainty that the US would not occur) to +5 (certainty of the presentation of the US), and the subjects were asked to choose the scale value that corresponded to their belief that a CS would be followed by a US.

[5] Valence rating is assessed by presenting a scale whose value reflects the degree of pleasantness (or unpleasantness) of a stimulus. For example, in Thompson and Lipp (2017), participants were shown a 9-point scale (from 1 [unpleasant] to 9 [pleasant]) and were asked to rate the CS and US at different times throughout the experiment.

study analyzing the extinction and reinstatement of Pavlovian conditioning in which FPS responses and US-expectancy ratings were measured (Norrholm et al., 2006). In this study, though almost the entire sample indicated not to be expecting the US to follow the CS, nearly half of the participants still showed FPS in the final trials of extinction. Lastly, some studies have shown that administering propranolol prior to the presentation of a retrieval cue prevented the return of FPS responses but not skin conductance responses or US-expectancy ratings (Kindt et al., 2009; Soeter & Kindt, 2010, 2011).

Given this evidence of dissociations between response systems, some authors have questioned whether PRE might have the same effects on different measures of learning (Kindt & Soeter, 2013). Moreover, it has also been questioned whether the presence of multiple measures can change the results of PRE (Golkar et al., 2012; Kindt & Soeter, 2013; Oyarzún et al., 2012; Warren et al., 2014).

In most studies on PRE, conditioned responses have been assessed through SCR, but some experiments have also utilized FPS, retrospective or online US-expectancy ratings, or distress or valence ratings. Usually, only one measure was used, but some studies reported acquiring multiple measures (see Table 2). Diminished return of fear after PRE has been seen almost exclusively when SCR were utilized as a single measure. However, there have been failures to show diminished return of fear when only SCR was used. One study demonstrating diminished returned of fear with FPS was by Golkar et al. (2017), who used vicarious extinction after presentation of a retrieval cue. All other studies with FPS combined this measure with ratings and sometimes SCR, and the results were negative. The only study with multiple measures demonstrating long-lasting return of fear was Thompson and Lipp (2017), who associated both fear-relevant and fear-irrelevant pictures (CSs) with an electrical shock (US). The following day, they exposed one group of participants to a US-retrieval cue prior to extinction and another group to traditional extinction. The return of skin conductance responses in spontaneous recovery and reinstatement tests were observed only for the group exposed to traditional extinction. However, no differences between groups were found regarding CS-valence, with both groups rating CS+s as more negative than CS-s.

Failures to show diminished return of fear with FPS have been argued to be associated with two variables. First, when assessing FPS, some studies (Kindt & Soeter, 2013; Soeter & Kindt, 2010) utilized a 104 dB sound as startle stimuli. Some authors have argued that this stimulus is intrinsically aversive, and its presence therefore might have created a more threatening environment, increasing contextual conditioning and preventing fear attenuation (Oyarzún et al., 2012). Another point relates to the fact that FPS was associated with the US-

expectancy rating in several studies. There is evidence that the presence of a keypad for online assessment of US-expectancy enhances acquisition, spontaneous recovery, and reinstatement of FPS (Warren et al., 2014). Warren et al. (2014) examined the effects of PRE in the presence or absence of a three-button response keypad to report participant US-expectancy during each trial. They used a spontaneous recovery test followed by a reinstatement test. Online expectancy ratings enhanced the degree of FPS in both the acquisition and extinction phases. However, all groups displayed some level of spontaneous recovery of FPS, with keypad groups showing a greater degree of recovery.

In sum, SCR appear to be more sensitive to PRE than FPS, but this effect was not found in most studies with multiple measures of conditioned fear responses. In addition, failure to show persistent reduction in conditioned responses has also been reported in studies using only SCR as a dependent measure. There are studies showing long-lasting reduction of conditioned responses with FPS and when multiple measures were used (SCR and valence-ratings), but this was achieved through vicarious extinction or using the US as a retrieval cue (Golkar et al., 2017; Thompson & Lipp, 2017). As such, it appears that different post-retrieval procedures can engage different response systems (Kredlow et al., 2018).

Table 1 - Experimental manipulation and timeline in post-retrieval extinction experiments in humans

Study info	**Design**					
Reference	**Type of Comparison**	**CSs**	**Experimental manipulation and timing**	**Retrieval cue**	**Test Type**	**Diminished CR in test?**
Schiller et al. (2010, Exp.1)	Between	CS+, CS-	Cond → 24h → (Ret cue → 10min → Ext) OR (Ret cue → 6h → Ext) OR (Ext) → 24h→ Test	1 x CS+ (no US) for 4 s	spont rec, reinst	Y
Schiller et al. (2010, Exp.2)	Within	CSa+, CSb+, CS-	Cond → 24h → Ret cue → 10min → Ext → 24h → Test	1 x CSa+ (no US), 1 x CS- (4 s each)	reinst	Y
Soeter & Kindt (2011, exp. IIa and IIb)	Within	CS1+, CS2+, CS-	Cond→ 24h → Ret cue →10 min → Ext → 24h → Test	1 x CS1+ (no US), 1 x CS2+ (no US) and 1 x CS- (8 s each)	spont rec, reinst, recond, gen test	N
Agren, Engman, et al. (2012)	Between	CS+, CS-	Cond → 24h→ (Ret cue →10min → Ext) OR (Ret cue → 6h → Ext) → 24h → Test → 48h → Day 4: Re-test	1 x CS+ (no US) for 120 s	renewal, reinst	Y
Agren, Furmark, et al. (2012)	Between	CS+, CS-	Cond → 24h → (Ret cue → 10min → Ext) OR (Ret cue → 6h → Ext) → 24h → Test	1 x CS+ (no US) for 120 s	reinst, recond	Y
Oyarzún et al. (2012)	Within	CSa+, CSb+, NS	Cond → 24h → Ret cue → 10min → Ext → 24h → Test	1 x CSa+ (no US), 1 x CS (no info on duration)	reinst	Y
Golkar et al. (2012, Exp.1)	Within	CS+r, CS+nr, CS-	Cond → 24h → Ret cue → 10min → Ext → 24h → Test	1 x CSr+ (no US) for 4 s	reinst	N
Golkar et al. (2012, Exp.2)	Within	CS+r, CS+n, CS-	Cond → 24h → Ret cue → 10min → Ext → 24h → Test	1 x CSr+ (no US) for 4 s	reinst	N
Kindt & Soeter (2013)	Between	CS1+, CS2-	Cond → 24h → (Ret cue →10min → Ext) OR (Ext) → 24h → Test	1 x CS1, 1 x CS- (8 s each)	spont rec, reinst, recond	N
Schiller et al. (2013)	Within	CSa+, CSb+, CS-	Cond → 24h → Ret cue → 10min → Ext → 24h → Test	2 x CSa+ (4s each)	reinst	Y
Warren et al. (2014)	Between and Within	CSa+, CSb+, CS-	Cond → 24h → Ret cue → 10min → Ext → 24h → Test	1 x CSa+, 1x CS (6 s each)	spont rec, reinst	N
Steinfurth et al. (2014)	Between	CS+, CS-	Cond → (24h) OR (7 days) → (Ret cue → 10min → Ext) OR (Ext) → 24h → Test	1 x CS+ for 4 s	reinst	Y
Meir Drexler et al. (2014)	Between	CS1+, CS2-, CS3+, CS4-, CS5-	Cond → 24 h → (Ret cue → Ext in different context) OR (Ext in different context) → 24 h → Test in conditioning context	1 x CS1+ for 30 s without context	spont rec, renewal	N
Liu et al. (2014, Exp.1)	Between	CS+, CS-	Cond → 24h → (Ret cue → 10min → Ext) OR (Ret cue → 24h → Ext) OR (Ext) → 24h → Test	1 x weak US (half of the intensity used in cond) for 0.2 s	spont rec, reinst	Y
Liu et al. (2014, Exp.2)	Between	CS1+, CS2+, CS-	Cond → 24h → (CS- Ret cue → Ext) OR (US- Ret cue→ Ext) → 24h → Test	(1 x weak US (half of the intensity used in cond) for 0.2 s) OR (1 x CS1+ for 4 s)	spont rec, reinst	Y

Table 1- ***Continued***

Liu et al. (2014, Exp.3)	Between	CS1+, CS2+, CS-	Cond → 24h → (US- Ret cue →10min→ CS1+ Ext) OR (CS1 and CS2 Ret cue → 10min → Ext CS1+) → 24h → Test	(1 x weak US (half of the intensity used in cond) for 200 ms) OR (1 x CS1+, 1 x CS2+ (for 4 s each))	spont rec, reinst	Y
Liu et al. (2014, Exp.4)	Between	CS1+, CS2+, CS-	Ext 6-7 months after test (participants from experiment 3)	(1 x weak US (half of the intensity used in cond) for 0.2 s) OR (1 x CS1+, 1 x CS2+ (for 4 s each))	spont rec, reinst	Y
Liu et al. (2014, Exp. 5)	Within	CS1+, CS2+, CS-	Cond → 2 weeks → US Ret cue →10min → CS1+ Ext → 24 h → Test	1 x weak US (half of the intensity used in cond) for 0.2 s	spont rec, reinst	Y
Liu et al. (2014, Exp.6)	Within	CS1+, CS2+, CS-	Cond (CS1+ with US1, CS2+ with US2, CS-) → 24h → (US1 Ret cue → 10min → Ext) OR (US2 Ret cue →10min--> Ext) → 24h → Test	1 x weak US1 (half of the intensity used in cond) for 0.2 s	spont rec, reinst	Y
Johnson & Casey (2015)	Between	CS+, CS-	Cond in context A → 24 h → (Ret cue → 10min Ext in context B) OR (Ext in context B) → 24h → (Test in context B)	1 x CS+ (in context B) for 7 s	reinst	Y
Björkstrand et al. (2015)	Between	CS+, CS-	Re-cond (participants from Agren et al., 2012)	N/A	recond	Y
Asthana et al. (2015)	Between	CS+, CS-	Cond → 24h → (Ret cue → 10min → Ext) OR (Ext) → 24h → Test	1 x CS+ for 4s	spont rec	Y
Fricchione et al. (2016)	Within	CS+R, CS+NR, CS-	Cond in context A → 24h → Ret cue → Ext in context B → 24h → Test in context A → 1 month → Re-test	1 x CS+R for 12 s	renewal, reinst, spont rec, recond	N
Klucken et al. (2016)	Within	CS+rem, CS+nonrem, CS-	Cond → 24h → Ret cue → 10min → Ext → 24h → Test → 6 months → Day 4: Test	1 x CSr+, 1 x CS- (8 s each)	reinst	N
Thompson & Lipp (2017)	Between and within	CSa+, CSa-, CSb+, CSb-	Cond → 24h → (Ret cue → 10min → Ext) OR (Ext) → 24h → Test	1 x weak US (half of the intensity used in cond) for 0.2	spont rec, reinst	Y
Golkar et al. (2017)	Within	CSa+, CSb+, CS-	Cond → 24h → Ret cue → 10min → Vic Ext → 24h → Test	1x CSa+ for 8 s	reinst	Y
Agren et al. (2017)	Between	CS+, CS-	Cond → 24h → (Ret cue → 10min - in vivo Ext) OR (Ret cue → 6h - in vivo Ext) OR (Ret cue → 10min - imaginary Ext) OR (Ret cue → 6h - imaginary Ext) → 24h → Test	1 x CS+ for 120 s	reinst	Y
Kredlow et al. (2018)	Between	CS+, CS-	Cond → 24h → [(Ret cue → 10 min → Ext) OR (Ext) in **healthy participants**] OR [(Ret cue → 10 min → Ext) OR (Ext) OR (Cond → 24h → Cond → Ret cue → 10 min → Ext) **in anxious subjects**] → 24h Test	1 x CS+ for 8 s	reinst	N

Cond: conditioning; Ext: extinction; Ret cue: retrieval cue; Vic Ext: vicarious extinction; spont rec: spontaneous recovery test; reinst: reinstatement test; gen test: generalization test; recon: reconditioning; Y: yes; N: no

Table 2 - Stimuli and dependent variables in studies on post-retrieval extinction in humans

Study info	Stimuli						Measures	
Reference	**CS Description / duration**	**CS is fear-relevant?**	**US (intensity / duration)**	**Reinforcement rate (number of pairings)**	**Number of acquisition trials**	**Number of extinction trials**	**Dependent variable (behavioral only) ***	**Diminished CR in test?**
Schiller et al. (2010, Exp.1)	Colored squares / 4s	N	electrical shocks (10-60 V / 200ms)	38% (6)	16	11	SCR	Y
Schiller et al. (2010, Exp.2)	Colored squares / 4s	N	electrical shocks (10-60 V / 200ms)	38% (5)	13	11	SCR	Y
Soeter & Kindt (2011, exp. IIa and IIb)	Pictures of spider or gun (CS+), or picture of mug (CS-) / 8s	Y	electrical shocks (1 mA minimum /2 ms)	80% (4)	5	10	FPS, SCR, Retrospective Expectancy Ratings, Distress ratings	N
Agren, Engman, et al. (2012)	Photo of lamp lit either in red or blue presented in neutral environment / 6s	N	electrical shocks (up to 5 mA / 500 ms)	100% (16)	16	8	SCR	Y
Agren, Furmark, et al. (2012)	Photo of lamp lit either in red or blue presented in neutral environment / 6s	N	electrical shocks (up to 5 mA / 500 ms)	100% (16)	16	8	SCR	Y
Oyarzún et al. (2012)	Colored squares / 4s	N	loud shrill sounds (98 dB / 1.7 or 2.4 s)	38% (6)	16	10	SCR	Y
Golkar et al. (2012, Exp.1)	Fearful male faces / 6s	Y	electrical shocks (no info / 100 ms)	50% (6)	12	12	FPS, SCR	N
Golkar et al. (2012, Exp.2)	Colored squares / 6s	N	electrical shocks (no info / 100 ms)	50% (6)	12	12	SCR	N
Kindt & Soeter (2013)	Pictures of spiders	Y	electrical shocks (1 mA minimum / 2ms)	75% (6)	8	12	FPS, SCR, Online US-expectancy Ratings	N
Schiller et al. (2013)	Colored squares / 6s	N	electrical shocks (20-60 V / 200 ms)	38% (5)	13	11	SCR	Y
Warren et al. (2014)	Geometric shapes / 6s	N	air-blast (140 PSI / 250 ms)	100% (12)	12	24	FPS, Online US-expectancy Ratings	N

Table 2 – *continued*

Steinfurth et al. (2014)	Colored squares / 4s	N	electrical shocks (20-60 V / 200 ms)	50% (8)	16	20	SCR	Y
Meir Drexler et al. (2014)	Pictures of dog, spider, snake, and tiger presented in two zoo frames / 8s	Y	electrical shocks (no info / 100 ms)	75% (12)	16	8	SCR, Online US-expectancy Ratings	N
Liu et al. (2014, Exp.1)	Colored squares / 4s	N	electrical shocks (5-50 V/ 200 ms)	38% (6)	16	10	SCR	Y
Liu et al. (2014, Exp.2)	Colored squares / 4s	N	electrical shocks (5-50 V/ 200 ms)	38% (6)	16	10	SCR	Y
Liu et al. (2014, Exp.3)	Colored squares / 4s	N	electrical shocks (5-50 V/ 200 ms)	38% (6)	16	10	SCR	Y
Liu et al. (2014, Exp.4)	Colored squares / 4s	N	electrical shocks (5-50 V/ 200 ms)	N/A	N/A	10	SCR	Y
Liu et al. (2014, Exp. 5)	Colored squares / 4s	N	electrical shocks (5-50 V/ 200 ms)	38% (6)	16	10	SCR	Y
Liu et al. (2014, Exp.6)	Colored squares / 4s	N	electrical shocks (to the right inner wrist and right eyelid) (5-50 V/ 200 ms)	38% (6)	16	10	SCR	Y
Johnson & Casey (2015)	Picture of blue or yellow windows (CSs) showed on 2 different backgrounds (kitchen or child's room / 7 s	N	hybrid of white noise and a 1000-Hz tone + pictures of potentially dangerous animals (fanged snake, spider, snarling dog, shark) (94-102 dB / 1s)	50% (8)	16	16	SCR	Y
Björkstrand et al. (2015)	Photo of lamp lit either in red or blue presented in neutral environment / 6s	N	electrical shocks (up to 5 mA / 500 ms)	N/A	N/A	N/A	SCR	Y
Asthana et al. (2015)	Colored squares / 4s	N	auditory stimulus (code 276 from IADS) (102 dB / 2 s)	80% (12)	16	16	SCR	Y
Fricchione et al. (2016)	High-definition video clips depicting one of three tarantulas in one of three contexts (kitchen, bedroom, and office) / 12 s (of which, 4s context only and 8s CS+ in context)	Y	electrical shocks (up to 4 mA / 500 ms)	62.5% (5)	8	10	SCR	N
Klucken et al. (2016)	Colored squares / 8s	N	electrical shocks (up to 5 mA / 100 ms)	50% (8)	16	11	SCR	N

Table 2 – *continued*

Thompson & Lipp (2017)	Picture of spider and snake (CSa + and -); and picture of colored squares (CSb+ and -) / 6s	Y	electrical shocks (no info / 200 ms)	100% (8)	8	10	SCR, Valence rating to CSs	Y
Golkar et al. (2017)	Picure of gun (CS+) and mug (CS-) / 8s	Y	electrical shocks (minimum 1 mA / 2ms)	no info	10	N/A	FPS	Y
Agren et al. (2017)	Blue or green lamp presented on neutral environment / 6s	N	electrical shocks (up to 5 mA / 100 ms)	100% (16)	16	8	SCR	Y
Kredlow et al. (2018)	Colored shapes (yellow circle or white square) / 8 s	N	Electrical shock to the second and third fingers of participants' dominant hand (0.2 to 4 mA / 50 pulses per s/ 500ms or 1000ms) and auditory stimulus (scream noise 1s / 95dB)	60% (6 for weak conditioning group and 18 for strong conditioning group)	10	11	SCR	N

* Only behavioral measures are shown, but note that is some studies, there are neuroimaging or genetic data also. Y: yes; N: no; N/A: not applicable; SCR: skin conductance response; FPS: fear-potentiated startle.

Table 3 - Participants' information in studies on post-retrieval extinction in humans

Participants				Inclusion criteria	
Reference	**n (n per group)**	**Age (Range or mean in years)**	**% females**	**Psychiatric / Medical***	**Conditioning / Extinction**
Schiller et al. (2010, Exp.1)	65 (20-23 per group) 19 in the 1-year test (8-11 per group)	Range 18-48	63%	no info	Successful conditioning and extinction[a]
Schiller et al. (2010, Exp.2)	18	Range 18-34	55%	no info	Successful conditioning and extinction[a,b]
Soeter & Kindt (2011, exp. IIa and IIb)	40	Range 18-32	72%	Absence of current or previous medical or psychiatric condition contraindicating participation; score < 26 on Anxiety Sensitivity Index (ASI, Peterson & Reiss, 1992)	No info
Agren, Engman, et al. (2012)	22 (11 per group)	M= 24.0 (SD±0.48)	50%	no info	Successful conditioning[c]
Agren, Furmark, et al. (2012)	66 (aprox. 33 per group)	M = 24.6 (SD±-4.0)	58%	no info	Successful conditioning[c]
Oyarzún et al. (2012)	17	M = 23.4 (±5.11)	66%	No history of psychiatric or neurological disease	Successful conditioning and extinction[d]
Golkar et al. (2012, Exp. 1)	19	M= 27.2 (±-9.55)	53%	no info	Analysis with entire sample and with subjects showing successful conditioning and extinction[d]
Golkar et al. (2012, Exp. 2)	15	M = 26.26 (±-7.52)	58%	no info	Analysis with entire sample and with subjects showing successful conditioning and extinction[d]
Kindt & Soeter (2013)	40	Range 18-33	67%	Absence of current or previous medical condition contraindicating participation; ASI < 26	No info
Schiller et al. (2013)	19	Range 18-34	53%	No use of medication for psychiatric or neurological reasons	Measurable SCR on all 3d and successful conditioning and extinction[a, b]
Warren et al. (2014)	55 (10-20 per group)	M = 20.8 (±1.7)	64%	Absence of current or past psychiatric illness (Structured Clinical Interview for DSM-IV Axis I Disorders, SCID-1, First, 1997) and no use of illicit drug or alcohol abuse or dependency	No info
Steinfurth et al. (2014)	80 (20 per group)	M = 23.21 (±18-57)	57%	no info	Reliable SCR response during conditioning and successful conditioning and extinction[a]

Table 3 – *continued*

Meir Drexler et al. (2014)	39 (aproxim. 20 per group)	Range 19-30	49%	Absence of somatic / endocrine disease, history of psychiatric/neurological treatment or medication use	No info
Liu et al. (2014, Exp.1)	54 (16 -19 per group)	Range 18-29	50%	no info	Successful conditioning and extinction[e]
Liu et al. (2014, Exp.2)	36 (18 per group)	Range 17-29	50%	no info	Successful conditioning and extinction[e]
Liu et al. (2014, Exp.3)	37 (18-19 per group)	Range 18-31	57%	no info	Successful conditioning and extinction
Liu et al. (2014, Exp.4)	24 (11-13 per group)	Range 19-29	58%	no info	Successful conditioning and extinction
Liu et al. (2014, Exp. 5)	15	Range 20-27	60%	no info	Successful conditioning and extinction
Liu et al. (2014, Exp.6)	19	Range 21-28	47%	no info	Successful conditioning and extinction
Johnson & Casey (2015)	74 (18-19 per group)	Range 12-17 and 18-32	50%	Absence of hearing impairment, color blindness, diagnosed animal phobias and neurological and psychiatric disorders	Reliable SCR response and successful conditioning
Björkstrand et al. (2015)	20	no info	no info	No info	Successful conditioning
Asthana et al. (2015)	91 (aproxim. 22 per group)	no info	46%	Absence of neurological or psychiatric illnesses; pregnancy; students with psychology as major	Successful conditioning[h]
Fricchione et al. (2016)	21	Range 18-28	48%	Presence of Non-phobic fear of spiders (scores above the mean on the Spider Phobia Questionnaire, SPQ-15(Olatunji et al., 2009) and absence of specific spider phobia criteria; absence of current psychiatric disorders (SCID-I), serious medical or neurological conditions, brain injury, and current or past substance abuse	Successful conditioning[g]
Klucken et al. (2016)	70	M = 23.93 (±-4.15)	55%	Absence of current or past mental illness, chronic diseases, or consumption of psychotropic drugs	Analysis with entire sample and with subjects showing reliable SCRs and successful conditioning[i]
Thompson & Lipp (2017)	56 (28 per group)	M = 23.54 (±-7.05) (experimental group); M = 25.25 (±-8.36) (control group)	55%	Absence of cardiovascular disease, seizure disorder, or pregnancy	no info
Golkar et al. (2017)	18	Range 19-23	78%	Absence of medical conditions	Successful conditioning[j]

Table 3 – ***continued***

Agren et al. (2017)	86 (20-23 each group)	Range 19-39	67%	no info	Successful conditioning[c]
Kredlow et al. (2018)	92 (43-49 each group)			Absence of current medical disease contraindicative of fear conditioning, pregnancy, anticholinergic medications, clonidine, benzodiazepines, or psychotropic medications. Half of the sample was composed by healthy adults and the other half by anxious adults (Beck Anxiety Inventory, BAI score > 15, Beck & Steer, 1990; and Fear Questionnaire score > 37, Marks & Mathews, 1979). Anxious subjects could not have present bipolar or psychotic disorder, substance-related disorder in the last three months (other than caffeine or nicotine use disorder) (ADIS-5, Brown & Barlow, 2014), endorsed current suicidality, homicidality, or self-destructive acts or urges; or be engaged in exposure therapy the week prior to or during study procedures.	Adequate conditioning and extinction[k]

***** Psychiatric and / or medical inclusion info is only described when the authors explicitly reported having excluded participants base on this data.

[a] Criteria for conditioning and extinction were based on differential responses to the CS+ and CS- during the second half of the conditioning and extinction sessions (averaged), respectively. During conditioning, the difference between skin conductance responses to the CS+ and the CS- had to be equal or larger than 0.1μS. During extinction, this difference had to be smaller than 0.1μS or in the opposite direction (i.e., CS- > CS+).
[b] Conditioning and extinction to CSa+ and CSb+ had to be equivalent (difference ≤ 0.1 μS during conditioning and extinction).
[c] Criteria for conditioning were based on differential responses to the CS+ and CS- during conditioning. Authors calculated difference scores (CS+1 - CS-1, CS+2 - CS-2...CS+16-CS-16). The average delta scores were tested against zero using a one-tailed t-test with statistical cutoff offset at p<.10 as the within subject conditioning criterion.
[d] Conditioning and extinction criteria based on differential responses to the CS+s and CS-. Skin conductance responses to CS+s had to be greater than responses to the CS- during conditioning. During extinction, responses to CS+s could not be greater than responses to the CS-.
[e] Criteria for conditioning and extinction were based on differential responses to the CS+ and CS- during conditioning and extinction sessions. During conditioning, subjects had to present more than two delta scores higher than 0.05 μS. During extinction, participants had to present more than two delta scores lower than 0.1 μS.
[f] Responses to the CS+ had to be greater than responses to the CS- during acquisition.
[g] Difference between average responses to both CS+s and average responses to the CS- during conditioning had to be at least 0.1 μS.
[h] Responses to CS+ had to be greater than responses to the CS- during trials of conditioning. Also, responses to the CS+ during conditioning had to be greater than responses to the CS+ during habituation.
[i] Mean differential skin conductance responses (CS+ minus CS-) had to be at least >.02 μS.
[j] Larger differentiation on the last two trials of conditioning than during the first two trials of conditioning for each CS+ relative to the CS-.
[k] The criteria for adequate conditioning were: 1) average unconditioned skin conductance response of at least 0.1 μS (untransformed) and 2) average differential conditioned skin conductance response (CS+ minus CS-) across acquisition trials 2–10 of at least 0.1 μS (untransformed; CS + > CS-). Criterion for extinction was differential SCR smaller than 0.1 μS (square-root transformed and standardized) in the last four trials of extinction.

Testing a new variable: opportunity for verbal covert or overt behavior during experimental phases

As discussed in previous sections, several variables have been identified as sources of variation in the effectiveness of PRE to prevent the return of fear in human participants. However, when analyzing the current literature, there is one important variable that has received little attention so far: verbal behavior emitted by participants during PRE.

Studies showing prolonged reduction of fear in humans have relied on extinction preceded by a retrieval cue. Exposure to a single CS without the US or a single presentation of the US (at half the intensity used in conditioning) have been used as retrieval cues, which are presented 10 min to 6 h before extinction. Several of these reports describe disconnecting participants from US-delivering devices after the presentation of the retrieval cue and reconnecting the devices just before extinction. During this interval between the retrieval cue and extinction training, participants were exposed to a television show or video clip (Golkar et al., 2012; Johnson & Casey, 2015; Klucken et al., 2016; Meir Drexler et al., 2014; Oyarzún et al., 2012; Schiller et al., 2013, 2010; Steinfurth et al., 2014) or were offered magazines to read (Golkar et al., 2017; Kindt & Soeter, 2013; Soeter & Kindt, 2011; Thompson & Lipp, 2017). When Schiller et al. (2010, Experiment 1) used an interval of 6 h between the retrieval cue and extinction training, participants watched a television program in the experimental room for 10 min, but no information was given about where they were or what they did for the remainder of the 6 h interval.

These different procedures utilized during the interval between retrieval cue and extinction training might not control for verbal covert behavior that is related to the CS, the US, or the relation between both, which could theoretically change the effects of PRE. For example, some participants might engage in covert verbal behaviors, such as “remembering” the CS, the US, or the CS-US contingency. If this occurs during the manipulated interval, it could maintain (at a covert level) contingencies that are supposed to be experimentally suppressed, counteracting the effects of PRE. There are experimental data supporting this proposal. First, classic work has shown that rehearsal, defined as the covert or overt repetition of information, increases the number of stimuli remembered in memory tasks (Atkinson & Shiffrin, 1971; Rundus & Atkinson, 1970). Second, recent studies have demonstrated that conditioned responses take longer to reduce with extinction after the covert repetition of the CS-US contingency (Joos, Vansteenwegen, Vervliet, & Hermans, 2013). Additionally, a relationship between expectancy report and conditional responses has been reported: fear return, measured by FPS, was higher when subjects were asked to report on a keypad by

button-press whether they expected the US after the CS at every presentation of the CS (Warren et al., 2014). Most importantly, reduction of conditioned responses can be achieved by imagining the CS without the CS, a procedure called imaginal extinction, which shows that imagining a stimulus is equivalent to being exposed to the actual stimulus (Agren et al., 2017).

The possible role of covert (or overt) verbal behavior in PRE might be especially important when the retrieval cue is a single presentation of the CS. From a procedural point of view, the difference between standard extinction and this PRE procedure is that in the latter, the first and subsequent presentations of the CS are separated by a greater interval during which participants are disconnected from stimulating and assessment devices. Therefore, it is logical to suppose that events occurring during this interval might be relevant for changing the effects of the intervention. Are these changes in experimental context or responses emitted by participants during this interval relevant in determining the function of the first CS presentation?

Research objective

In what follows, I describe an experiment that aimed at exploring the effects of overt verbal behavior emitted during post-retrieval extinction on the return of fear. Specifically, I examined the effects of directing participants' verbal behavior towards or away from the experimental contingencies during the interval between retrieval cue and extinction training on the return of fear. Participant's verbal behavior was directed towards the experimental contingencies by means of an interview that required them to describe the CS, the US, and to think about the relation between these two stimuli. This procedure was compared with a control condition in which participants had their verbal behavior directed away from the experimental contingencies by means of a verbal fluency task that prevented them from thinking (or verbalizing) content related to the experimental procedures and stimuli. I also tested a third group of subjects who underwent standard extinction.

Materials and methods

Participants

Participants were adults recruited from the undergraduate and graduate populations of three universities in Sao Paulo, Brazil, and from the community through online and paper advertisements on and off campuses. Volunteers were included in this study if they met the following criteria: (1) age between 18 and 45 years; (2) no theoretical knowledge on behavior analysis; (3) no reported medical condition that would contraindicate participation in the study (cardiovascular disease, epileptic seizures, cutaneous lesions in the areas of electrode attachment, presence of a pacemaker or any other metal implant, pregnancy); (4) not currently using psychoactive medications; and (5) did not fulfil criteria for current Axis-I psychiatric disorders according to the DSM-IV, screened through the Portuguese version of the Structured Clinical Interview for DSM-IV Axis I Disorders, SCID-1 (Del-Ben et al., 2001).

From a total of 110 participants eligible for the study, 57 were included in the final analysis (34 males and 23 females; 18-41 years old, mean 23.42, sd±4,65; 11-23 years of formal education, mean 14.74, sd±2.58). Forty-six participants had to be excluded because they did not meet three standard criteria used in studies on post-retrieval extinction with skin conductance as dependent measure: (1) measureable skin conductance responses in all experimental phases (n=2), (2) evidence of conditioning (n=30); and (3) evidence of extinction (n=14) (see Criteria for conditioning and extinction for details). These criteria have been used by several laboratories in the area because it is only possible to assess return of a conditioned fear response if it has been conditioned in the first place and diminished by extinction (Schiller et al., 2013). Additionally, 5 participants were excluded due to technical problems (loss of data due to computer system failure, electrical shutdown that prevented data from the last session to be acquired), and 2 refrained from the experiment (dropout).

The participants gave informed consent prior to the experiment (Appendix 1). They were not paid for their participation but expenses with transportation were reimbursed. All procedures were approved by the Ethics Committee of the Institute of Psychology of the University of Sao Paulo (process CEP CAAE number: 50814615.0.0000.5561, Appendix 2).

Procedure

The experiment consisted of three consecutive stages conducted approximately 24 h apart: day 1: conditioning; day 2: extinction; day 3: test. All stages were conducted in a room

located in the Biobehavioral Analysis Laboratory (Psychology Institute, University of Sao Paulo). This room, which was equipped with a window, desk, and chair, offered ventilation and illumination deemed adequate for human comfort. During the experiment, the window was kept closed and except for breaks during extinction (detailed ahead), the room was dimly illuminated. At the beginning of each experimental stage, participants were asked to sit in a chair in front of the computer screen and electrodes for skin conductance monitoring and US administration were installed (see Skin conductance assessment and US administration for details). Participants remained seated and with the exception of the breaks (during extinction), they were attached to stimulation and monitoring electrodes throughout the session.

During conditioning (day 1), all participants underwent differential Pavlovian conditioning. Two 14 cm x 14 cm black-and-white photographs of human faces presented on a 21.5" computer monitor with a black background were used as conditioned stimuli (CS). The unconditioned stimulus (US) was a mild and brief electrical shock delivered to participants' inner right wrist (200-ms, 50 pulses / second). Skin conductance responses (SCR) to the presentations of the CSs and US were used as the dependent measure of conditioned and unconditioned responses, respectively.

Prior to conditioning, a procedure to set the intensity of the US was carried out (Schiller, Raio, & Phelps, 2012). This was achieved by asking participants to indicate their tolerance level to the stimulus, which should be felt as "uncomfortable but not painful". The initial level of the stimulation was 10 V, which was increased until the participant indicated that his tolerance level had been reached. Maximum level of stimulation was 60V. Once the intensity of the US was calibrated, it remained unaltered for the rest of the experiment.

After setting US intensity, the experimenter gave the following instructions: "The objective of this experiment is to assess how your sweat glands react to sensory, visual, and tactile stimuli. You don't have to do anything with your hands nor use the mouse or keyboard; you just have to look at the computer screen and pay attention to the things you are seeing and to the things you are feeling. Any questions?"

Once the participant said he /she understood the instructions, conditioning was initiated. Each CS was presented six times with a duration of 6 s, with a total of 12 presentations. After each CS presentation, the computer screen went black, corresponding to an inter-trial interval (ITI). ITIs varied randomly between 12-14 s. One of the CSs (hereafter termed CS+) was always paired with the US (100% reinforcement rate), whereas the other (CS-) was never paired with the US. CS-US pairing corresponded to the presentation of the US in the last 0,2s of the CS+ presentation, so that they co-terminated. Assignment of the

photographs as CS + and CS- was counterbalanced across participants, that is, photographs serving as CS+ for half of the participants were assigned as CS- for the other half.

During extinction (day 2), participants were designated to one of three groups (n=19): Experimental related verbal activity (Exp R), Experimental non-related verbal activity (Exp N), and Control. All groups underwent extinction training in which the CS+ and CS- were repeatedly presented without the US. For experimental groups, a retrieval cue consisting of a single unreinforced presentation of the CS+ and the CS- was carried out 10-min prior to extinction. During the interval between retrieval cue and extinction, participants from the Exp R group were required to engage in an activity directing their overt and covert verbal behavior towards the experimental contingencies, whereas participants from the Exp N group were required to engage in an activity directing their overt and covert verbal behavior away from the experimental contingencies. Control group underwent a standard extinction procedure (there was no retrieval cue or 10-min interval prior to extinction). The details of experimental procedures for each group are described below.

Exp R group: once the experimental setup was complete, the experimenter turned off the lights and gave the following instructions: “Similar to yesterday, you do not have to do anything with your hands nor use the mouse or keyboard. You just have to look at the computer screen and pay attention to the things you are seeing and to the things you are feeling. Any questions?” Following these instructions, there was a single unreinforced presentation of the CS+ and CS- (6 s, ITI 12-14s), after which the experimenter turned the room lights on, and disconnected the participants from stimulation and monitoring equipment. Afterwards, participants underwent an activity that aimed at directing their verbal covert and overt behavior towards the experimental contingencies. Specifically, an interview originally designed to determine whether participants are able to describe the CS-US contingency was carried out (Bechara et al., 1995; Weike, Schupp, & Hamm, 2007). Questions were adapted for the purposes of this experiment and aimed at forcing participants to emit verbal behavior related to the CS, to the US, and to the relation between these stimuli (see Appendix 3). The following instructions were given “Now we will have a 10-min break. During this interval, I want to ask you some questions about the experiment”. The following questions were used: 1) “I want you to think about our last experimental session. Did you know when you were going to receive an electro-tactile stimulus?”; 2) “How many different photographs did you see yesterday? Could you describe each one for me? Try to give me as many details as you can”; 3) Did you notice any difference between the faces? What were these differences?”; 4) “Now

that we've talked about the faces, I would like you to try to name each one based on characteristics you described"; 5) "Can you describe to me what the electrical shocks you received yesterday felt like? Please try to remember as many details as possible. Try also to estimate how long each shock lasted"; 6) "How many times did the photograph of the male face with [description of participant in question 4] was followed by the electrical shock?". The interview ended with a multiple-choice question: 7) "the presentation of the electrical shocks followed: (a) the male face of [name given by participant for one photograph], (b) the male face of [name given by participant for the other photograph], (c) there was no relation between faces and electrical shocks, and (d) it is not possible to answer this question". Participants were required to answer all questions regardless of providing a complete and correct answer to the first question. Each answer was repeated out loud by the experimenter while he wrote it down. This procedure was adopted because the purpose of the activity was to maintain verbal behavior towards experimental contingencies. Participants remained seated during the interview.

Verbal activity was conducted throughout the 10-min break, after which participants were given the following instructions: "We have finished the 10-min break. I will now reconnect these devices, and we will complete the remainder of the session". Subjects were reconnected to stimulation and monitoring devices. The session was resumed with 14 unreinforced presentations of each CS (6s, ITI 12-14 s).

Exp N group: procedures were the same as described for Exp R group with the difference that instead of the interview during the interval between retrieval cue and extinction training, participants underwent an activity that aimed at directing their verbal covert and overt behavior away from the experimental contingencies. Specifically, they were instructed to engage in a task adapted from controlled oral association tests (verbal fluency tests) originally designed to evaluate the production of words under restricted search conditions (Strauss, Sherman, & Spreen, 2006). In these tasks, subjects must produce orally as many words as possible beginning with a specified letter or belonging to a specified category during fixed periods of time. The following instructions were given: "Now we will have a 10-min break. During this interval, we will engage in an activity that requires some concentration. I will say a letter of the alphabet. Then I want you to give me as many words that begin with that letter as quickly as you can. For example, if I say the letter 'b', you might give me 'ball, bed, beach…'. There are only two rules you have to follow. First, you cannot give me proper names such as 'Bruno or Barbara'. Also, I do not want you to use words you've already given

me to create new ones, such as ‘bond, bonding, bonded’. Any questions?”. Once it was clear that the participant understood the task, the experimenter gave the following instructions: “Now tell me as many words as you can remember that begin with the letter ‘F’. Name them as quickly as you can.” The experimenter began timing with his smartphone chronometer. Participants were allowed a one-minute period for each letter. After the letters F, A, and S were applied, the experimenter gave the following instructions: “We will continue with this task but the rules will be a little bit different now. Instead of giving me words beginning with letters, I want you to give me as many words as you can remember that belong to a category, regardless of the letter. I want you to tell the names of as many animals as you can. Name them as quickly as possible”. The experimenter began timing with his smartphone chronometer. Participants were allowed a one-minute period for each category. Experimenter applied the categories animals, fruits, masculine, and feminine names. Words produced by participants were written down as they were produced. These categories were chosen because they were likely to elicit words that are not related to the experimental contingencies. Participants remained seated during this activity.

Control group: after the experimental setup and initial instructions, participants underwent 15 unreinforced presentations of each CS (6 s duration, ITI 12-14s), with a total of 30 stimulus presentations.

During the test (day 3), all groups underwent a reinstatement test consisting of four presentations of the US alone followed by a new extinction procedure. Once the experimental setup was complete, the experimenter turned off the lights and gave the following instructions: “Similar to yesterday, you do not have to do anything with your hands nor use the mouse or keyboard. You just have to look at the computer screen and pay attention to the things you are seeing and to the things you are feeling. Any questions?” Following these instructions, the US was presented alone four times while the computer screen remained black. The interval between each US presentation was 12-14 s. After the last ITI ended, the CSs were presented 15 times each (6 s, ITI 12-14 s), for a total of 30 CS presentations.

For each session, five stimuli presentation sequences were programed and counterbalanced across participants such that the CS+ and CS- were pseudorandomly presented within each phase and no more than two trials of each CS occurred consecutively. In the test phase, the CS- was always the first stimulus to be presented and responses to this

first presentation were not included in the analysis[6]. Except for the experimental setup conducted at the beginning of each session and the breaks given to experimental groups during extinction, the experimenter remained outside the room during data collection, and monitored participants through his smartphone by using an IP camera inside the experimental room.

The experimental design is summarized in Figure 1.

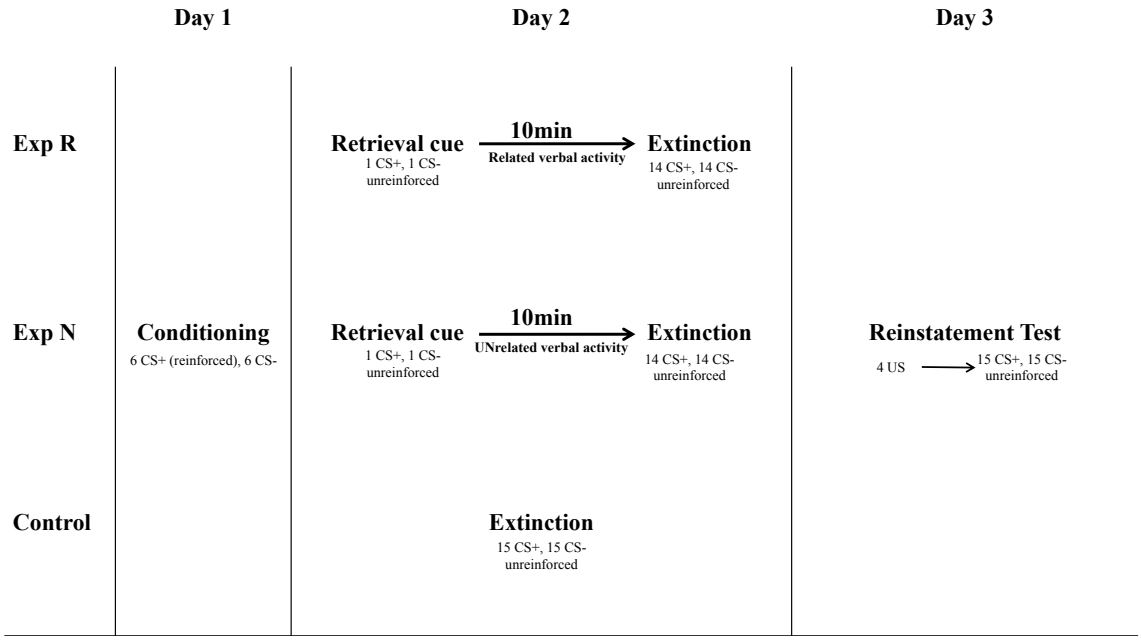

Figure 1 - Experimental design and timeline

Skin conductance assessment and US delivery

Skin conductance was recorded using two disposable adhesive Ag-AgCL electrodes with conductive gel connected to a GSR100C Biopac Systems skin conductance module (Goleta, California, USA). Electrodes were attached to the distal phalanges of the middle and index fingers of the left hand. Analyses were conducted offline using the AcqKnowledge 4.2 software. Skin conductance data was filtered using a low-pass filter (Blackman window, cutoff frequency 31Hz) and smoothed in order to eliminate artifacts and interferences in the signal (LaBar & Phelps, 2005).

[6] As in previous studies in the area, this procedure was utilized because the first stimulus presentation usually elicits an increase in skin conductance regardless of its nature, that is, regardless of whether it was a CS+ or CS- (orienting response, Boucsein, 2012; Schiller et al., 2013; Schiller et al., 2010; Schiller et al., 2012).

The amplitude of skin conductance responses was used to assess conditioned and unconditioned responses to the stimuli. This measure was obtained by taking the base-to-peak difference for the largest waveform (in micro Siemens, μS) initiating in the 0.5 - 5.8 s period after stimulus onset (Schiller et al., 2010). The minimal response criterion was 0.01 μS. Increases in signal lower than 0.01 μS, decrease in skin conductance, or stable recording during these time-windows were scored as zero and were included in the analysis.

The scorer was blind to stimulus type, since the record only allowed for the experimenter to identify that a stimulus occurred but not the type of stimulus (CS+, CS-, or US). The exception was the conditioning session because reinforced trials were tagged and given our 100% criteria, it was obvious which stimulus had been presented at this stage. Raw SCR values were square root transformed to normalize distributions. Each resulting value was divided by the mean square root transformed US response. This last procedure was conducted to obtain a relative measure of conditional response based on each participant's unconditional response (Olsson, Ebert, Banaji, & Phelps, 2005).

Electrical shocks serving as unconditioned stimuli were delivered through a bipolar electrode measuring 8mm in diameter and 21mm of separation between poles, with a ribbon strap. A conductive gel was applied between the electrode and the skin. The experimenter installed the stimulating electrodes on the participant's right wrist, attaching it with the ribbon strap in a position parallel to the forearm and verifying that both poles were in contact with the participant's skin. Electrical shocks were generated by a Grass Medical Instruments S48 stimulator connected to a SIU5 stabilizer (West Warrick, Rhode Island, USA).

The experiment was programmed using SuperLab 4.5.3 software (Cedrus, San Pedro, California, USA) running on a separated PC computer with Intel processor and connected to the SCR module and shock stimulator via C-POD (Cedrus, San Pedro, California).

Subjective psychiatric symptom assessment

State and trait anxiety, and depression symptoms were measured prior to the experiment on Day 1 by using the Brazilian versions of the Spielberger State-Trait Anxiety Inventory (STAI-T and STAI-S, Biaggio & Natalício, 1979; Spielberger et al., 1970) and the Beck Depression Inventory (BDI, Gorenstein & Andrade, 1998).

Criteria for conditioning and extinction

The conditioned response was defined as the differential SCR, which was calculated by subtracting responses to the CS- from responses to the CS+ in corresponding trials. Criteria for conditioning and extinction were based on the differential responses as described in previous studies Schiller et al. (2012). Specifically, participants were classified as being conditioned if they presented a differential response ≥0.1μS in the second half of the conditioning session (average of last three trials). For extinction, the criterion was the opposite, that is, participants were required to show average differential response <0.1μS in the last three trials of extinction.

Statistical analysis

To analyze differences in conditioned responses across experimental stages and between groups, we averaged trials into blocks. Conditioning was assessed by comparing the mean differential responding of the first half of the conditioning session (early conditioning phase) with that of the second half of the conditioning session (late conditioning phase). Extinction was assessed by comparing the mean differential responding of the first three trials of the extinction session (early extinction phase) with that of the last three trials of extinction phase (late extinction phase). We also compared the late phase of conditioning with the late phase of extinction. Return of fear was assessed by comparing data from the first test trial with the mean of the late phase of extinction.

Each experimental stage was assessed through repeated measures ANOVA with phase (early phase, late phase) as within-subjects factor and groups (Exp R, Exp N, Control) as between-subjects factor. Follow-up ANOVAS with post-hoc Tukey tests were used to compare groups at specific points of time, and t-tests were used to compared responses to CS+ and CS- within each group at specific points of time. The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for Mac, version 20.0 (IBM Corp. Released, 2011). An alpha level of 0.05 was set for all statistical comparisons.

Results

Table 1 presents demographic information and scores on self-report measures of anxiety and depression symptoms by group. Groups did not differ significantly in age or years of formal education, as shown by separate one-way ANOVAs ($p>0.05$). Likewise, they did not differ in gender according to a chi-square test ($p>0.05$). Participants from the control group reported slightly less symptoms of anxiety but these differences did not reach statistical significance. The groups did not differ in reported symptoms of depression.

Mean US intensity (in volts) chosen by participants are also shown in Table 1. Groups were equivalent in the selected intensity of the electrical shocks.

Table 4 - Participant characteristics

Variable	Control	Exp R	Exp N	Significance test
Age {Mean (SD) years}	23.52 (3.92)	23.74 (4.81)	23.00 (5.35)	$F_{2, 54} = 0.122$, p=0.885
Education {Mean (SD) years}	14.65 (2.34)	15.00 (2.66)	14.56 (2.83)	$F_{2, 50} = 0.143$, p=0.867
Gender (% female, n)	36.8%, n=7	47.4%, n=9	36.8%, n=7	$\chi^2_{2, n=57} = 0.587$, p=.837
STAI-S {Mean (SD)}	37.95 (8.75)	41.32 (10.23)	40.58 (10.12)	$F_{2, 54} = 0.630$, p=0.537
STAI-T {Mean (SD)}	40.94 (9.51)	43.22 (12.15)	43.74 (10.10)	$F_{2, 52} = 0.357$, p=0.702
BDI {Mean (SD)}	9.89 (6.51)	10.89 (8.83)	9.53 (7.10)	$F_{2, 54} = 0.167$, p=0.846
US intensity {Mean (SD) volts}	5.35 (1.28)	5.45 (0.89)	4.87 (1.08)	$F_{2, 54} = 1.550$, p=0.222

Figure 2 presents the mean differential SCR during conditioning (late phase), extinction (late phase), and test (first trial) for each experimental group. As expected given our exclusion criteria, all groups showed similar results in conditioning and extinction: differential SCR in the late phase of conditioning was positive and above 0.1 μS, but close to zero or negative in the late phase of extinction. In the test phase, the pattern differed between groups: a positive differential SCR was observed for the control group, as well as for the Exp

R group. In this last group, however, return of conditioned responses was lower than the control group. In contrast, mean differential SCR was close to zero for the Exp N group. These differences between groups were analyzed through a repeated measures ANOVA with phase (late conditioning, late extinction, first test trial) as within-subjects factor, group (Exp N, Exp R, and Control) as between-subjects factor, and differential SCR as dependent measure. There was a significant effect of phase, $F_{1.5,81.5}=31.815$, $p<0.001$ as well as a significant phase x group interaction, $F_{3,\ 81.5}=4.947$, $p=0.003$. A separate ANOVA for each phase showed that differential SCR was equivalent between the groups in conditioning and extinction ($F_{2,53}=0.358$, $p=0.701$, and $F_{2,54}=1.578$, $p=0.216$, respectively). In the test however, differences were statistically significant, $F_{2,54}=5.291$, $p=0.008$. Post-hoc Tukey test showed that differences between Control and Exp N groups were significant ($p=0.006$) and differences between Exp N and Exp R groups were marginally significant ($p=0.123$); however, differences between Exp R and Control groups were not significant ($p=0.444$).

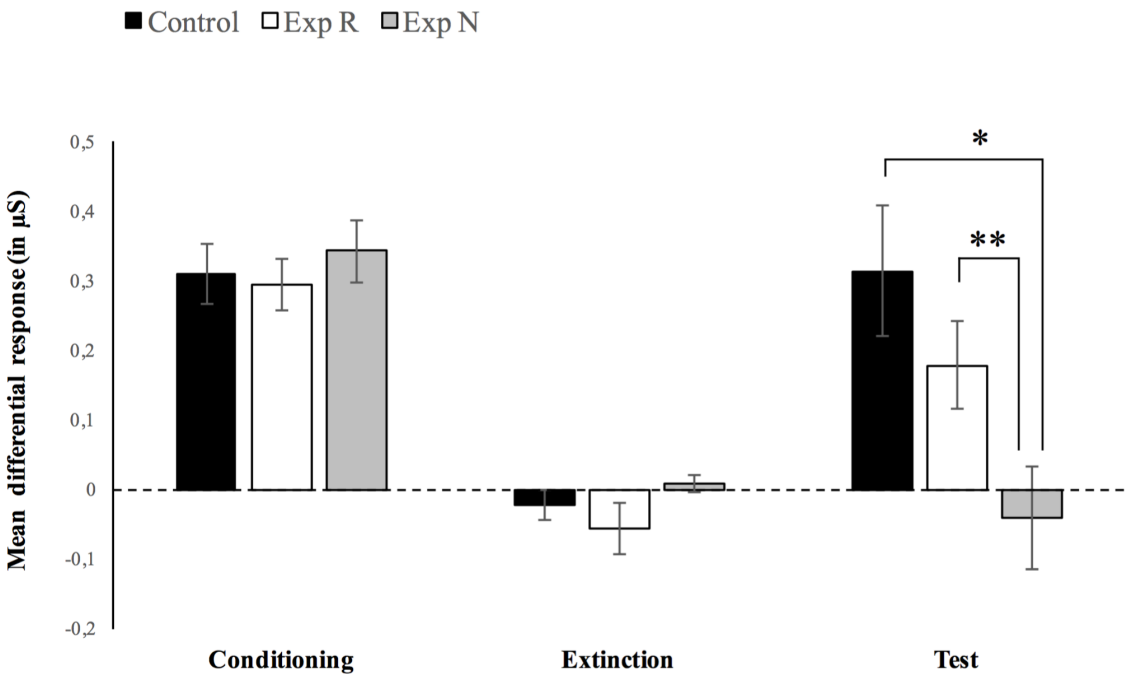

Figure 2 - Mean differential responding [(SCR to the CS+) - (SCR to the CS-)] during conditioning (late phase), extinction (late phase), and test (first trial) for each experimental group. Control group underwent standard extinction, whereas both experimental groups were presented with a retrieval cue (single unreinforced presentation of the CS+ and CS-) 10-min prior to extinction. Exp R group underwent a verbal activity related to the experiment during the interval between the retrieval cue and extinction; Exp N group underwent a verbal activity that was not related to the experiment during this interval. Error bars represent standard error of the mean. * p=0.006 ** p=0.123

Figure 3 presents the mean SCR amplitudes (in μS) for conditioned stimuli (CS+ and CS-) throughout experimental stages for each group (Control, Exp R, and Exp N). All groups presented a similar pattern in conditioning, that is, greater responses to the CS+ as compared to responses to the CS-, with these differences increasing from the beginning to the end of conditioning. A repeated measures ANOVA with group (Control, Exp R, and Exp N) as between-subjects factor, phase (early conditioning, late conditioning) as the within-subjects factor, and differential SCR as dependent variable showed that increases in differential responding were equivalent between the groups during conditioning: there was a significant phase effect, $F_{1,53}=15.00$, $p<0.001$, but not a phase x group interaction, $F_{2,53}=0.33$, $p=0.717$. Follow-up t-tests confirmed that responses to the CS+ were greater than responses to the CS- in all groups in the second half of conditioning (Control: $t_{18}=7.398$, $p<0.001$; Exp R: $t_{17}=7.596$, $p<0.001$; Exp N: $t_{18}=7{,}667$, $p<0.001$).

At the early phase of extinction (mean of the first three trials), all groups presented greater responses to the CS+ in comparison with responses to the CS- (Control: $t_{18}=5.054$, $p<0.001$; Exp R: $t_{18}=3.108$, $p=0.006$; Exp N: $t_{18}=3.773$, $p=0.001$). For the Exp R group, this difference was gone by the fifth trial, whereas for Exp N and Control groups this difference between CS+ and CS- took longer to diminish. At the late phase of extinction (mean of last three trials), however, differences between responses to CS+ and CS- were gone in all groups (Control: $t_{18}=-1.052$, $p=0.307$; Exp R: $t_{18}=-1.506$, $p=0.149$; Exp N: $t_{18}=0.777$, $p=0.447$).

In the first trial of the test, both Control and Exp R groups presented a pattern similar to conditioning, that is, responses to the CS+ greater than responses to the CS- (Control: $t_{18}=3.340$, $p=0.004$; Exp R: $t_{18}=2.858$, $p=0.010$). In contrast, Exp N group presented equivalent responses to the CS+ and CS-, $t_{18}=0.551$, $p=588$. In other words, no discrimination between CS+ and CS- occurred for this group.

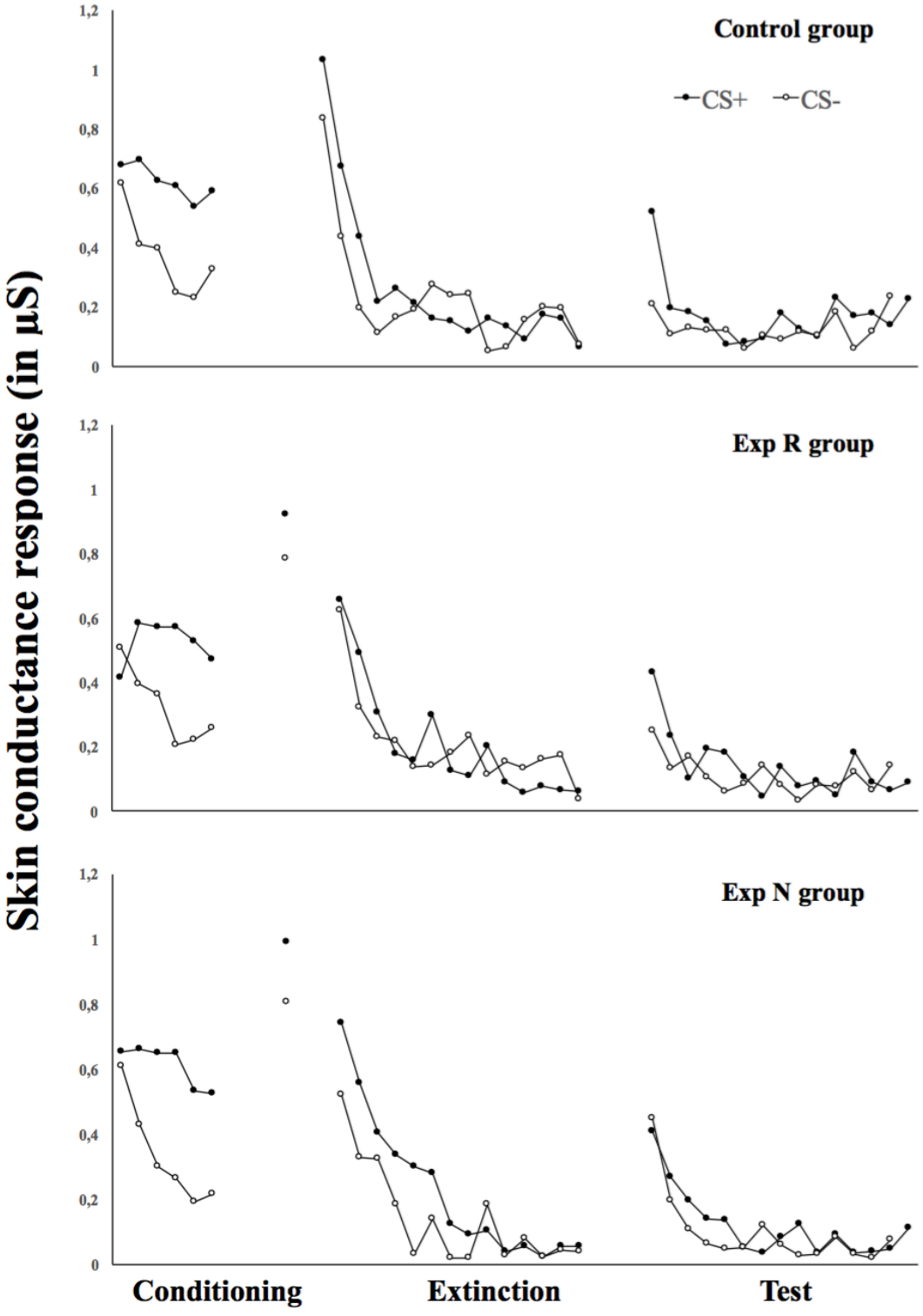

Figure 3 - Mean SCR amplitudes (in µS) for the CS+ and CS- throughout conditioning, extinction, and test for each experimental group (Control, Exp N, and Exp R).

Figure 4 presents a comparison between mean responses to the CS+ and CS- presentations during conditioning (late phase) and test (first trial) for each experimental group. Participants from the Control group presented equivalent responses to the CS+ in the test as compared with conditioning (mean CS+ in late conditioning = 0.57 μS x mean CS+ in first test trial = 0.52, t_{18}=0.621, p=0.542). In participants from groups Exp N and Exp R, there was a decrease in responses to the CS+ from conditioning to the test (Exp N from 0.56 to 0.40μS; Exp R from 0.52 to 0.41μS). However, this difference was statistically significant only for the Exp N group (t_{18}=2.285, p=0.035); in the Exp R group, differences were only marginally significant (t_{17}=1.668, p=0.114). The comparison between responses to the CS- during conditioning with responses to the CS- during the test did not reach statistical significance for the Control group (mean CS- in late conditioning = 0.26 μS x mean CS- in first test trial = 0.20, t_{18}=0.929, p=0.365. In contrast, group Exp R showed a non-significant increase in responses to the CS- from conditioning to the test (mean CS- in late conditioning = 0.22 μS x mean CS- in first test trial = 0.25, t_{18}=-0.354, p=0.728). Participants from Exp N showed a significant increase in responses to the CS- from conditioning to the test (mean CS- in late conditioning = 0.22 μS x mean CS- in first test trial = 0.44, t_{18}=-2.771, p=0.013).

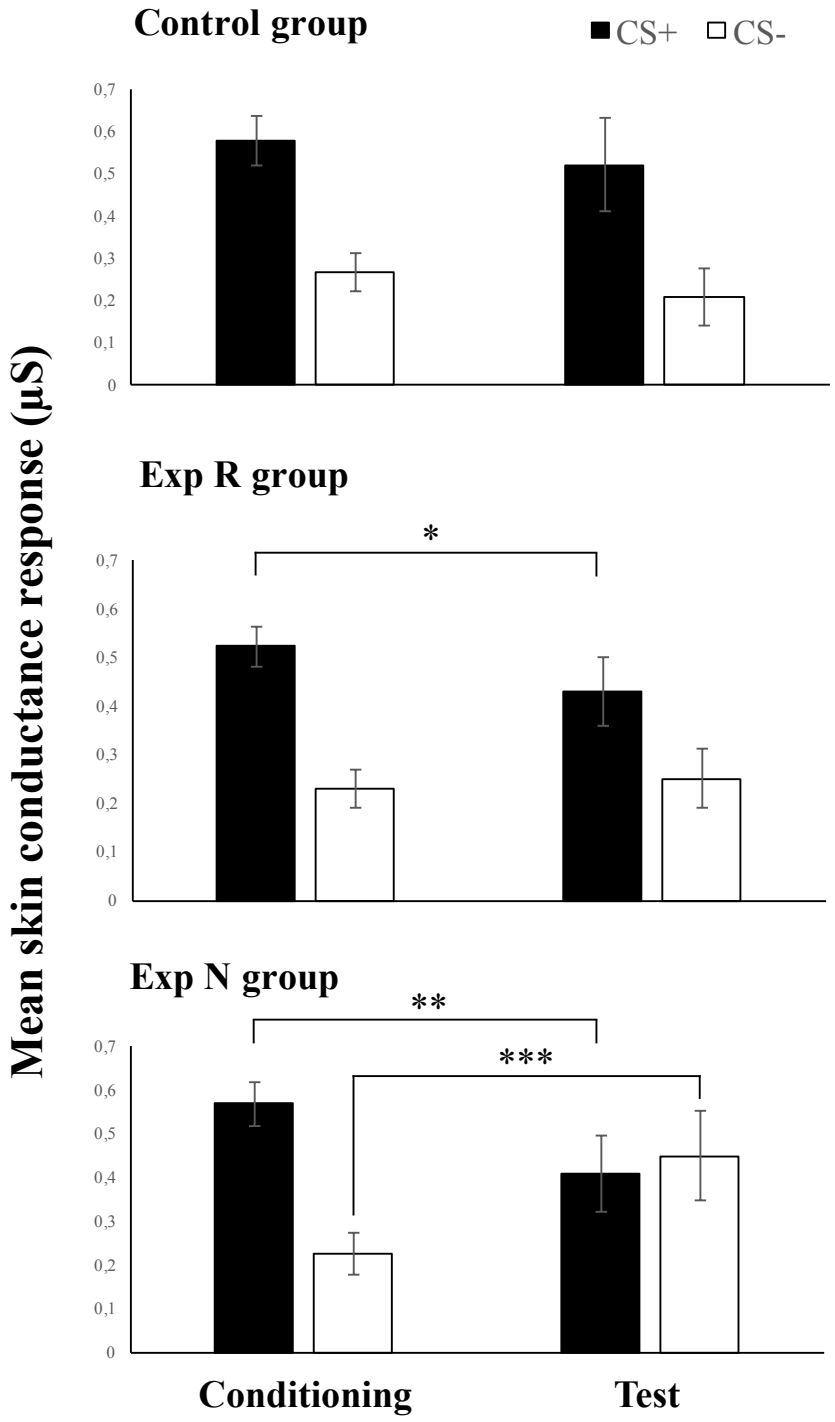

Figure 4 - Mean SCR (in µS) for CS+ and CS- presentations during conditioning (late phase) and test (first trial) for each experimental group. Error bars represent standard error of the mean. * p=0.114 **p=0.035 ***p=0.013

Figure 5 presents mean unconditioned responses (in μS) to US presentations during conditioning and test. The mean amplitude of SCR to the US during conditioning and test were equivalent between groups, as shown by separate ANOVAs (Conditioning $F_{2,54}$=1.171, p=0.318; Test $F_{2,54}$=1.332, p=0.272).

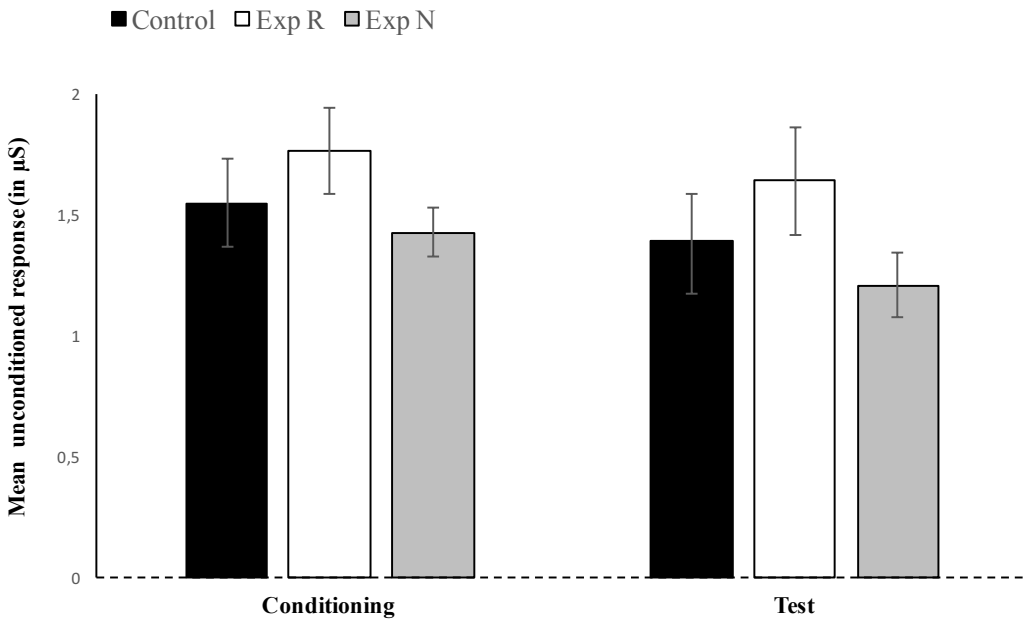

Figure 5 - Mean SCR (in μS) for the UR during conditioning and test. Error bars represent standard error of the mean.

Discussion

Results from the experimental test suggest that verbal behavior emitted during the interval between a retrieval cue and extinction training might change the effects of PRE in human participants. Return of fear, as measured through differential responding in a reinstatement test, was present in subjects who were required to emit verbal behavior related to the experimental contingencies during this interval, though responding in this group was lower as compared with a control group submitted to standard extinction (or compared with differential responding in conditioning). In contrast, differential responding was close to zero in subjects whose verbal behavior was directed away from experimental contingencies during this interval. This abolished discrimination between CS+ and CS- was dependent both on a decrease in responses to the CS+ as well as an increase in responses to the CS-.

Several questions can be raised based on these results. First, why would PRE be only partially effective when subjects engage in verbal behavior related to experimental contingencies during the interval between a retrieval cue and extinction training? The interview conducted after the retrieval cue is comparable to procedures utilized in studies on the effects of repetitive thought on Pavlovian conditioning and extinction (Arntz, Spit, & Merckelbach, 1997; Jones & Davey, 1990; Joos, Vansteenwegen, & Hermans, 2012a, 2012b; Joos et al., 2013). In these studies, participants were instructed to imagine or actively repeat at a covert level some part of the conditioning experience after its occurrence. Latter, the effects of this intervention were tested by exposing participants to extinction. In a series of experiments, Joos and colleagues have demonstrated that US-expectancy and conditioned suppression take longer to reduce during extinction after the covert repetition of the CS-US contingency (Joos et al., 2012a, 2012b, 2013). In other experiments, instructing participants to think about the US and their reactions to it resulted in maintenance or even larger SCR in extinction tests, as compared with rehearsing a non-aversive event or a non-related aversive event (Davey & Matchett, 1994; Jones & Davey, 1990).

Despite failures to replicate the findings described by Schiller et al. (2010) and growing body of evidence regarding limiting conditions, there is experimental evidence to suggest that PRE works to diminish return of fear. Given our 100% reinforcement rate during conditioning, the first unreinforced presentation of the CS should be discrepant enough to produce new learning (prediction error, Sevenster et al., 2013); on the other hand, the interview might have worked as rehearsal, which could theoretically strengthen conditioning (Joos et al., 2013). Therefore, one possibility is that directing participant's attention towards the experimental contingencies might have counteracted the effects of PRE. This might be the

case as subjects whose verbal behavior was directed away from experimental contingencies (group Exp N) did not present a return of fear as measured through differential conditioning.

It is important to discuss the fact that differential responding in the Exp R group was present in the test but it was lower compared with differential responding in the Control group. This result was dependent on a marginally significant decrease in responses to the CS+ (as compared with responses to the CS+ during conditioning). One could argue that this decrease in responsivity to the CS+ is not dependent on PRE itself but is due to some other aspect of the intervention during the interval between the retrieval cue and extinction. For example, our interview included questions requiring participants to think about the CS (Question 2: "How many different photographs did you see yesterday? Could you describe each one for me? Try to give me as many details as you can"; Question 3: "Did you notice any difference between the faces? What were these differences?"; and Question 4: "Now that we talked about the faces, I would like you to try to name each one based on characteristics you described"). There is experimental data suggesting that imagining the CS can be equivalent to being presented with the actual CS, as shown by experiments in which reduction of conditioned responses was achieved by imaginal extinction (Agren et al., 2017). The equivalence between imagining and having a direct contact with the actual stimulus or feared situation is the rationale for imaginal exposure used in clinical settings (Foa & McLean, 2016). Thus, it is possible that these questions entail additional extinction trials. However, one argument against this hypothesis is that our interview included questions about the CS-US contingency and about the US and their reactions to it. Whereas rehearsing only the CS could not prevent extinction in previous experiments (Joos et al., 2013), rehearsing the CS-US contingency or the US and reactions to it have been shown to maintain or even increase conditioned responses, as measured through US-expectancy rating (Joos et al., 2012a, 2012b), suppression of operant responses (Joos et al., 2013), or skin conductance responses (Davey & Matchett, 1994; Jones & Davey, 1990). In fact, most of the interview utilized in the present experiment addressed questions that supposedly strengthen conditioning, as four out of seven questions pertained to the US and the CS-US contingencies. Moreover, in studies demonstrating reduction of conditioned responses through instructed extinction, participants are explicitly told that shocks will not follow the US (Hugdahl, 1978; Javanbakht et al., 2017); in an experiment on imaginal extinction, participants were connected to stimulating electrodes and actually experienced not being presented with the US while being told to keep their eyes closed and imagine the CS (Agren et al., 2017).

It is also important to analyze data from the Exp N group, whose verbal behavior was directed away from the experimental contingencies during the interval between the retrieval cue and extinction training. As in most studies on PRE in humans, we utilized a differential conditioning paradigm in which there are two or more CSs, one of which is never paired to the US (CS-). This procedure is utilized to control for unspecific reactivity affecting responses to all stimuli (namely, sensitization effects and orienting responses) (Haaker et al., 2014). When using this procedure, conditioned responses are assessed through the difference between responses to the CS+ and responses to the CS-. When this difference is positive, meaning that participants' responses are greater in the presence of CS+ as compared with responses to the CS-, conditioning is said to have occurred. In this sense, the absence of significant differences between responses to the CS+ and CS- in the Exp N group can be interpreted as evidence that PRE abolished conditioning. However, it is important to note that in this group, differential responding in the test was dependent not only on a significant decrease in responses to the CS+ but also on increased responsivity to the CS-. Notably, these results were obtained even though the first response to the CS-, which was always the first stimulus to be presented in the test, was excluded from the analysis.

In several studies demonstrating diminished return of fear after PRE, results were mainly dependent on reduced responses to the CS+ during the test (Agren, Engman, et al., 2012; Agren, Furmark, et al., 2012; Schiller et al., 2013, 2010), although there are cases in which data on responses to the CS- are not reported, making a direct comparison with this results difficult (Johnson & Casey, 2015; Steinfurth et al., 2014). However, a recent experiment using equivalent methods reported increased responses to the CS- in the test phase (Agren et al., 2017). In this study, twenty-four hours after fear conditioning, participants underwent in vivo or imaginal extinction. Half of the subjects from each of these groups were given extinction 10-min after the presentation of a retrieval cue consisting of a single unreinforced presentation of the CS+; the other half underwent extinction 6 h after the presentation of the same retrieval cue. In groups receiving extinction 10 min after the retrieval cue (10-min imaginal extinction or 10-min vivo extinction), discrimination between the CS+ and CS- was abolished in a reinstatement test conducted 24 hours later. This lack of discrimination was dependent on an increased responsivity to the CS- in the group submitted to imaginal extinction; in the group submitted to in vivo extinction, results were dependent both on increased responsivity to the CS- as well as decreased responsivity to the CS+ in comparison with the conditioning session. The similarities with the present results are evident.

Agren et al. (2017) tried to explain their results according to the idea that both excitatory and inhibitory processes are produced in differential conditioning, as participants learn that the CS+ is followed by the US (excitatory process) and that the CS- is not followed by the US (inhibitory process). In this view, responses to the CS- reflect not only unspecific skin conductance responses but also a learned safety memory. Post-retrieval extinction is thought to decrease return of fear because the retrieval cue reactivates the fear memory, which becomes unstable and requires a re-consolidation process for stabilization to occur (Lee et al., 2017). Since extinction is conducted during this reconsolidation period, then it is likely to alter the original memory, preventing the return of fear. Agren et al., (2017) raised the possibility that upon reactivation of the fear memory by presenting the CS+ as a retrieval cue, the safety memory was also reactivated by virtue of proximity in the same context. Disruption of this safety memory was then demonstrated by a failure to inhibit responses to the CS-.

The same authors recognize that this hypothesis is at odds with data from PRE studies using more than one CS+. In these studies, only one CS+ was presented as retrieval cue but extinction was conducted with all CS+s (Oyarzún et al., 2012; Schiller et al., 2010, Exp.2). If the hypothesis of reactivation by proximity in same context was correct, then return of fear should be absent for both CS+s. However, that was not the case, as experiments with this design showed the opposite: return of fear was only present for CS+s used as retrieval cue prior to extinction (Schiller et al., 2010, Exp.2), although in Oayarzún et al. (2012), there was a non-significant increase in responses to the CS- in the test. Still, it is interesting to note that the present study replicated findings reported in Agren et al. (2017) in relation to responses to the CS- in a reinstatement test.

Another explanation to increased reactivity to the CS- might be related to the type of test used. A generalized return of fear, defined as an increase in responses to the CS+ and the CS- to the same degree, has been observed in several studies using standard extinction procedures (Haaker et al., 2014). Although it has been reported in spontaneous recovery (Norrholm et al., 2008) or renewal (Vervliet et al., 2013), it is most frequently reported in reinstatement tests (Dirikx, Vansteenwegen, Eelen, & Hermans, 2009; Haaker et al., 2014; Kull, Müller, Blechert, Wilhelm, & Michael, 2012; Sokol & Lovibond, 2012), which is the type of test used both in Agren et al. (2017) and in the present study.

The variables involved in increased responsivity to the CS- in reinstatement tests has been a matter of debate in the literature (Vervliet et al., 2013). Some experiments have associated this phenomenon with higher levels of self-reported trait anxiety (Kindt et al., 2009; Soeter & Kindt, 2010). According to Vervliet et al. (2013), increased responses to the

CS- after reinstatement tests in anxious participants might be related to two mechanisms: 1) generalization of the conditioned fear responses to the CS+ or 2) conditioning to the entire context after presentations of the US, so that any stimulus presentation will induce increased reactivity. These mechanisms were hypothesized in light of experimental data showing that discrimination between CS+ and CS- is more difficult to occur in anxious subjects (Grillon, 2002). Moreover, anxious participants are more likely to present generalization of conditioned responses to stimuli sharing physical features with the CS+ (Kaczkurkin et al., 2016). The results from the present experiment, however, cannot be explained by the presence of participants with high levels of self-reported anxiety because these participants were equally distributed among all groups and only the Exp N group showed increased responsivity to the CS-. Additionally, the results were not only driven by increased responses to the CS- but also decreased responses to the CS+.

According to Haaker et al., (2014), when a reinstatement test is conducted on a different day than conditioning and there are no CS presentations prior to the administration of reinstating USs, response enhancement might be related not only to associative processes (that is, to a history of a pairing between the CS and the US) but also to orienting responses and other phenomena. Considering this issue, they recommend including a re-extinction phase right before the reinstatement manipulation to allow for a measure that is unconfounded by sensitization effects (Haaker et al., 2014). Thus, one possibility is that the presentations of the US at the beginning of the session might have been paired with the context. In the absence of discrimination, which was abolished by PRE, subjects responded equally to all stimuli in this aversive context.

The use of reinstating shocks right before re-extinction in the present study was driven by the fact that this design is used by several experiments on PRE in humans (Agren et al., 2017; Agren, Engman, et al., 2012; Golkar et al., 2012, 2017; Johnson & Casey, 2015; Klucken et al., 2016; Kredlow et al., 2018; Oyarzún et al., 2012; Schiller et al., 2013, 2010; Steinfurth et al., 2014). Nevertheless, given considerations from Haaker et al., (2014) and results from both the present study and Agren et al. (2017), a replication of our verbal manipulations during PRE using tests with less confounding variables (spontaneous recovery or renewal tests) is warranted.

Conclusions and future directions

The general purpose of this thesis was to contribute to the debate on the variables implicated in the long-lasting reduction of conditioned fear responses after post-retrieval extinction. When combined in a recent meta-analysis, the results from studies in human participants show small-to-moderate effects for preventing the return of conditioned fear responses relative to standard extinction using the above procedure (Kredlow et al., 2016). However, given reports of failure to replicate these effects, it is clear that work is still needed to describe variables and parameters that allow PRE to be effective.

The discrepancies across studies have been viewed as evidence that post-retrieval extinction is sensitive to subtle methodological variations and that there might be conditions under which this procedure is not effective (boundary conditions). In the first part of this work, I reviewed the experimental manipulations conducted in studies on PRE in order to analyze the variables that have been tested and possible methodological issues meriting experimental testing. In the literature, there are several variables pointed as possible boundary conditions, such as conditions thought to produce stronger conditioning (time since conditioning, the percentage and/or number of CS-US pairings, the nature of CS, or the instruction during experimental phases), the retrieval procedures (the type of retrieval cue and the likelihood of generating prediction error), the response systems assessed, and pre-experimental characteristics of participants (genetic or psychiatric).

Uncovering these boundary conditions is important because critical differences between clinical disorders and laboratory experiments include many of the factors that have been highlighted as variables that might limit the efficacy of PRE in reducing conditioned fear responses (Golkar et al., 2012). For example, in Post-Traumatic Stress Disorder, patients present conditioning that supposedly is strong (due to the association between environmental stimuli with very intense USs) (Golkar et al, 2012) and associated with more complex CSs (and / or more CSs). Additionally, the period between fear conditioning in real life (the traumatic experience) and the beginning of a treatment is necessarily longer, as symptoms are required to be present for at least one month for the diagnosis of Post-Traumatic Stress Disorder to be made (DSM-V, American Psychiatric Association, 2013). Therefore, to improve treatments in applied situations, it will be necessary to thoroughly analyze the behavioral manipulations that are needed to prevent the return of fear in the laboratory.

In my review of experiments with human participants, I identified that little attention has been given to the possible role of (overt or covert) verbal behavior emitted by participants during the experimental phases. This proposal was based on the fact that most PRE studies

utilize a presentation of the CS (in the absence of the US) as a retrieval cue prior to extinction. In this case, the main difference between standard extinction and this PRE is the interval between the first and subsequent presentations of the CS. During this interval, participants might engage in covert verbal behaviors, such as "remembering" the CS, the US, or the CS-US contingency, a behavior which could strengthen conditioned fear responses, as shown by studies in which participants are required to imagine or actively repeat at a covert level some part of the conditioning experience after its occurrence (Joos et al., 2012a, 2012b, 2013). This question prompted an experimental test in which participants' verbal behavior was directed either towards or away from the experimental contingencies during the interval between retrieval and extinction. The results suggest that this variable plays a role in the return of fear after PRE.

The data from the experimental test suggest that PRE might be less effective in preventing return of fear when participants repetitively think about the conditioning experience. One possibility is that the interview to which participants were submitted worked as a form of rehearsal that counteracted the effects of PRE. If confirmed, this hypothesis can have clinical implications. In real life, the behavioral processes induced by fear conditioning (such as the physiological responses elicited by the US) extend beyond the aversive event, as humans tend to reflect upon their experiences, its causes and consequences, after its occurrence (Joos et al., 2012a). To put this in another way, not only the conditioning experience but also the way in which a person engages in thinking about this experience has an impact on conditioned responding. This idea is consistent with experimental data demonstrating that self-report measures of worry, defined as a "chain of thoughts and images, negatively affect-laden and relatively uncontrollable" (Borkovec, Robinson, Pruzinsky, & DePree, 1983) (p.9), have been positively correlated with fear conditioning in non-clinical samples (that is, in individuals who do not fulfill criteria for psychiatric disorders, Joos, Vansteenwegen, & Hermans, 2012c; Otto et al., 2007). Moreover, recent theories about anxiety disorders have highlighted that anxious individuals ruminate or rehearse the traumas relevant to their conditions, which results in maintenance or exacerbation of anxiety symptoms (Davey & Matchett, 1994).

The results presented by participants whose verbal behavior was directed away from the experimental contingencies right after the retrieval cue also merit some comments. In this group, differential responding in the test was close to zero. At first sight, this result indicates that post-retrieval extinction can abolish fear conditioning when participants do not rehearse the experimental contingencies during the procedure, a result that is closer to the main goal of

this line of research. However, inspection of the data showed that these results were dependent not only on a decrease in responses to the CS+ but also because responses to the CS- were significantly increased. From a clinical perspective, responding indiscriminately to the environmental after a threat stimulus does not seem a good outcome. In fact, recent theories point that the generalization of fear conditioned responses is a mechanism involved in pathological anxiety (Kaczkurkin et al., 2016; Lissek et al., 2008, 2010). As discussed earlier, additional experiments with different types of test, such as spontaneous recovery or renewal, are still needed to confirm whether these results are replicated. Therefore, although research on PRE has been considered promising for the development of behavioral treatments of pathological fear, much work is needed to understating the variables involved in the reduction of fear and filling the translational gap between basic research and clinical application.

In conclusion, this study raised several questions that could be investigated by future experiments. First, the hypothesis that rehearsal might counteract the effects of PRE should be confirmed. For example, the interview used to direct participants verbal behavior towards experimental contingencies included questions regarding the CS, the US, and the relation between these stimuli. Which of these components are responsible for the observed effects? Experimental studies suggest that questions about the CS-US contingency or about the US strengthen conditioning but this should be experimentally tested during PRE. Another issue is that the interview required participants to remember their responses to these stimuli, a procedure that has been shown to produce an increase in skin conductance responses (Lang, Kozak, Miller, Levin, & McLean, Jr., 1980). We did not measure physiological activity during the interview and therefore cannot draw any conclusion based on the present data in this regard. However, future studies should address if physiological responses during the interval between a retrieval cue and extinction are important for rehearsal to change the effects of PRE.

Another possible line of investigation relates to the timing of the verbal behavior intervention. We directed participants' verbal behavior in the interval between the retrieval cue and extinction training because our main question was whether verbal events during this time might change the effects of PRE. This decision was related to the fact that the apparent difference between standard extinction and PRE with unreinforced CS as retrieval cue is the interval between the first and subsequent presentations of the CS. Future studies should address the effect of these interventions at other moments, such as during conditioning or

previously to the test. Would the results be the same if verbal interventions were conducted, say, right before the presentation of the retrieval cue?

One final point is derived from an argument frequently used in the literature to justify failures to show diminished return of fear after PRE. It has been hypothesized that PRE might not be effective when conditioning is strong. As discussed by Golkar et al. (2012), this hypothesis is problematic because none of the studies utilized an independent measure of strength of conditioning. In animal studies, conditioning strength is assessed through resistance to extinction (Suzuki et al., 2004; Wang et al., 2009). These measures should be incorporated in investigations on PRE in humans. This would be especially useful when testing for the supposedly strengthening effects of verbal behavior on fear conditioned responses and whether this variable actually counteracts the effects of PRE.

The variables involved in the reduction of conditioned fear responses by means of PRE are not fully described and much work is still required to describe them. The possibilities are innumerous and I hope that this thesis can trigger other researchers into this exciting area of scientific inquiry.

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Appendix 1 – Informed consent (page 1)

INSTITUTO DE PSICOLOGIA – DEPARTAMENTO DE PSICOLOGIA EXPERIMENTAL (PSE) DA USP – LABORATÓRIO DE ANÁLISE BIOCOMPORTAMENTAL

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

DADOS DE IDENTIFICAÇÃO DO PARTICIPANTE DA PESQUISA

1. NOME: .:..... .....
DOCUMENTO DE IDENTIDADE Nº : ..... SEXO : .M □ F □
DATA NASCIMENTO: ...../...../.....
ENDEREÇO ..... Nº ..... APTO: .....
BAIRRO: ..... CIDADE .....
CEP:..... TELEFONE: DDD (.....) .....

DADOS SOBRE A PESQUISA

PESQUISADOR: PEDRO FONSECA ZUCCOLO

TÍTULO DA PESQUISA: Reocorrência de respostas condicionais em função do intervalo entre apresentações do estímulo condicional na extinção e das atividades durante esse intervalo

Caro voluntário,

Gostaríamos de convidá-lo a participar de um estudo que faz parte do doutorado do aluno Pedro Fonseca Zuccolo, sob orientação da Prof[a]. Dra. Maria Helena Leite Hunzinker. Esta pesquisa visa investigar como as pessoas lidam com eventos desagradáveis em suas vidas. Para tanto, vamos realizar um experimento no qual voluntários se submeterão a uma situação levemente desconfortável e observaremos como eles reagem a ela, logo depois da sua ocorrência e após 24/ 48 horas.

Para criar essa situação de desconforto será utilizada uma breve e pouco intensa estimulação elétrica aplicada no pulso direito dos participantes. A intensidade dessa estimulação será determinada pelos próprios participantes antes do experimento. Para isso, eles experimentarão uma estimulação elétrica muito suave, que será aumentada gradativamente até ele determine que é desconfortável,

Appendix 1 – Informed consent (page 2)

porém não dolorosa. Independentemente do julgamento que o voluntário fizer, a estimulação não passará de um grau máximo previamente estipulado pelo pesquisador com base em estudos científicos. A faixa de intensidade utilizada nesse estudo está demonstrada que não causa qualquer tipo de dano ou prejuízo, de ordem física ou psicológica. Portanto, caso aceite fazer parte deste estudo, você pode esperar se expor apenas a um pequeno desconforto durante sua participação, porém não haverá quaisquer danos à sua saúde. Tal procedimento foi aprovado pelo Comitê de Ética em Pesquisa com Seres Humanos do Instituto de Psicologia da Universidade de São Paulo, que fica situado na Av. Professor Mello Moraes, 1721 – Bloco G, 2o andar, sala 27 CEP 05508-030 - Cidade Universitária - São Paulo/SP E-mail: ceph.ip@usp.br - Telefone: (11) 3091-4182. Eventuais dúvidas éticas em relação projeto podem ser dirimidas no endereço acima citado.

Os participantes terão de um a quatro encontros com o pesquisador. No primeiro encontro, será feita uma entrevista para levantar dados sobre o voluntário tais como idade, escolaridade e presença de condições médicas que contraindiquem sua participação na pesquisa (presença de marca-passo cardíaco ou outros implantes metálicos, cardiopatia diagnosticada, uso de certas medicações psicoativas ou, no caso das mulheres, suspeita de gravidez). Também serão levantadas informações sobre o funcionamento emocional do voluntário, quais são suas fontes de medos e ansiedades, e em que grau essas questões afetam suas atividades cotidianas. Se durante essa entrevista forem constatados problemas de ordem emocional clinicamente relevantes (por exemplo, se forem observadas evidências de transtornos psiquiátricos ou neurológicos), o participante será informado disso. Nesse caso, o experimentador explicitará qual é o provável diagnóstico, de que modo ele se manifesta no presente caso e quais as opções de tratamento mais utilizadas para o problema (por exemplo, psicoterapia, tratamento farmacológico etc). O experimentador aconselhará que o participante procure um profissional da área para uma avaliação (psicólogo, psiquiatra ou neurologista, a depender dos dados de triagem) e oferecerá sugestões de locais onde esses serviços são prestados, levando em conta o contexto sócio-cultural e local de moradia do voluntário. Se o participante optar por fazer uma avaliação com um profissional da saúde, o experimentador entrará em contato com o profissional escolhido e passará todas as informações que levaram ao encaminhamento. Além disso, o experimentador manterá contato com o profissional para se informar sobre o andamento do caso, se foi confirmado o diagnóstico e o tipo de tratamento proposto.

Nos outros três encontros, será feita uma tarefa que dura em torno de 20 minutos. Enquanto fazem essa atividade, os participantes receberão a estimulação elétrica descrita anteriormente de tempos em tempos, sendo registradas suas reações por meio de um equipamento conectado à mão esquerda. Não haverá procedimentos alternativos ou mudanças no protocolo de pesquisa: uma vez iniciado o estudo, será feito exatamente o que está descrito acima, sem adições ou subtrações.

Appendix 1 – Informed consent (page 3)

A pesquisa será conduzida no Laboratório de Análise Biocomportamental do Instituto de Psicologia da Universidade de São Paulo, no endereço Av. Prof. Melo Moraes Bloco, 1721, Bloco A, Sala C6 - Butantã. Em qualquer etapa da pesquisa, você terá acesso ao profissional responsável pela pesquisa para esclarecimento de eventuais dúvidas, pelo endereço onde será realizada a pesquisa, ou pelos telefones (11) 2648-0177 ou (11) 9.95047489.

Ao concordar em participar desta pesquisa, você terá os seguintes direitos assegurados:

- você estará totalmente livre para desistir de realizar os testes em qualquer momento da pesquisa. Isso pode ser feito sem qualquer necessidade de explicação ou prejuízo à sua pessoa;

- caso você tenha despesas para participar da pesquisa (tais como, transporte), elas serão cobertas pelo pesquisador;

- caso você se sinta lesado de alguma maneira pela sua participação na sua pesquisa, você terá o direito de pedir indenização por eventuais danos;

As informações obtidas serão analisadas em conjunto com os dados de outros participantes, sem que cada indivíduo possa ser identificado no momento da divulgação da pesquisa. Você receberá uma via deste termo de consentimento e terá o direito de ter acesso às informações colhidas no estudo quando do seu término. Para tanto, basta procurar o pesquisar no endereço fornecido acima ou pelos telefones de contato.

A sua participação não lhe trará qualquer benefício direto, financeiro ou de outra natureza. Contudo, é importante que saiba que a sua colaboração representa uma contribuição inestimável que vai possibilitar a maior compreensão das reações humanas a situações desagradáveis.

Acredito ter sido suficientemente informado a respeito dos objetivos, procedimentos, riscos e benefícios relacionados à pesquisa “Reocorrência de respostas condicionais em função do intervalo entre apresentações do estímulo condicional na extinção e das atividades durante esse intervalo”.

_____ Data: ____/____/_____

Assinatura do participante

Appendix 1 – Informed consent (page 4)

CONSENTIMENTO

Eu discuti com Pedro Fonseca Zuccolo sobre a minha decisão em participar neste estudo. Ficaram claros para mim quais são os propósitos da pesquisa, os procedimentos a serem realizados, seus desconfortos e riscos, as garantias de confidencialidade e de esclarecimentos permanentes. Ficou claro também que minha participação é isenta de despesas. Concordo voluntariamente em participar deste estudo e poderei retirar o meu consentimento a qualquer momento, antes ou durante o mesmo, sem penalidades ou prejuízo ou perda de qualquer benefício que eu possa ter adquirido.

Este TCLE elaborado em duas vias, as quais serão rubricadas em todas as suas páginas e assinadas na última página pelo participante da pesquisa e pelo pesquisador, devendo cada parte ficar de posse de uma via.

_____ Data: ____/____/_____

Assinatura do participante

(Somente para o responsável do projeto)

Declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste participante para sua colaboração neste estudo.

_____ Data: ____/____/_____

Appendix 2 – Approval of experimental procedures by the Ethics Committee of the Institute of Psychology of the University of Sao Paulo (page 1)

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Reocorrência de respostas condicionais em função do intervalo entre apresentações do estímulo condicional na extinção e das atividades durante esse intervalo

Pesquisador: Pedro Fonseca Zuccolo
Área Temática:
Versão: 3
CAAE: 50814615.0.0000.5561
Instituição Proponente: UNIVERSIDADE DE SAO PAULO
Patrocinador Principal: UNIVERSIDADE DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 1.602.709

Apresentação do Projeto:
Estudo da área de Psicologia Experimental no nível de doutorado que propõe a realização de um experimento com 90 voluntários adultos, recrutados entre alunos da Universidade de São Paulo ou outras universidades, balanceados em relação a sexo, idade e escolaridade; serão investigados os efeitos de duas variáveis independentes (contexto e atraso na segunda apresentação do estímulo condicional) na reocorrência de respostas condicionais após uma fase de extinção.

Objetivo da Pesquisa:
Esta pesquisa tem por objetivo específico investigar se a probabilidade de reocorrência de respostas condicionais após extinção pode ser alterada em função de duas variáveis: 1) apresentação isolada do CS seguida de um intervalo de 10 min previamente à extinção e 2) atividades que o sujeito executa durante esse intervalo. Dentre essas atividades, serão propostas duas envolvendo comportamento verbal, porém com conteúdos diferentes (relacionados ou não relacionados com a contingência de reforçamento), e uma com atividade livre. Assim, seis condições serão testadas: a) apresentação isolada do CS sem US, seguida de atividade livre por 10 min e depois extinção (grupo experimental atividade livre, EL); b) apresentação isolada do CS sem US, seguida de atividade verbal relacionada ao experimento, especificamente, da descrição de contingências da sessão de condicionamento por 10 min, e depois extinção (grupo experimental

Endereço: Av. Prof. Mello Moraes,1721 - Bl. "G" sala 27
Bairro: Cidade Universitária **CEP:** 05.508-030
UF: SP **Município:** SAO PAULO
Telefone: (11)3091-4182 **E-mail:** ceph.ip@usp.br

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atividade verbal relacionada, ER); c) apresentação isolada do CS sem US, seguida de atividade verbal sem relação com o experimento por 10 min e depois extinção (grupo experimental atividade verbal não relacionada, EN); d) atividade livre por 10 min, seguida de extinção (grupo controle atividade livre, CL); e) atividade verbal relacionada ao experimento (descrição de contingências da sessão de condicionamento) por 10 min, seguida de extinção (grupo controle atividade verbal relacionada, CR); e f) atividade verbal sem relação com o experimento por 10 min, seguida de extinção (grupo controle atividade verbal não relacionada, CN).

Avaliação dos Riscos e Benefícios:

O pesquisador descreve de forma clara que a situação experimental incluirá uma breve e pouco intensa estimulação elétrica aplicada no pulso direito dos participantes. Para determinar a intensidade dessa estimulação, os participantes começarão sentindo uma estimulação elétrica muito suave, que será aumentada gradativamente até eles determinarem que é desconfortável, porém não dolorosa. Independentemente do julgamento que os participantes fizerem, a estimulação não passará de um grau máximo previamente estipulado pelo pesquisador com base em estudos científicos. A faixa de intensidade utilizada nesse estudo está demonstrada que não causa qualquer tipo de dano ou prejuízo, de ordem física ou psicológica. Portanto, os participantes podem se expor apenas a um pequeno desconforto durante sua participação, porém não haverá quaisquer danos à sua saúde.

Explicitou quais os possíveis desconfortos e riscos ao participante, inclusive no TCLE, bem como quais providências e cautelas serão empregadas para evitar e/ou reduzir efeitos e condições adversas que possam causar dano.

O pesquisador descreve que a participação na pesquisa não trará qualquer benefício direto, financeiro ou de outra natureza para o participante, sendo sua contribuição na compreensão de fenômenos de aprendizagem básicos que tem fornecido subsídios para o desenvolvimento de intervenções clínicas.

Comentários e Considerações sobre a Pesquisa:

O projeto encontra-se muito bem descrito, citando literatura relevante e relativamente recente, em especial no que se refere aos estudos com humanos e estimulação aversiva, e fornecendo os subsídios para entender a pergunta de pesquisa e a metodologia proposta. O projeto destaca a relação entre a pesquisa desse tipo e a compreensão e desenvolvimento de técnicas para o tratamento de fenômenos clínicos como ansiedade ou medo. O pesquisador descreve de forma

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detalhada os procedimentos de administração da estimulação elétrica, os cuidados que serão tomados para realizar o procedimento com segurança, os critérios de inclusão e exclusão dos participantes, e documentação adicional para realizar a avaliação dos aspectos éticos da pesquisa.

Considerações sobre os Termos de apresentação obrigatória:

O pesquisador apresenta os documentos adequadamente e procedeu às modificações solicitadas por este CEPH:

- corrigiu as informações sobre o número de participantes no formulário de informações básicas;
- incluiu a informação sobre a entrevista de triagem no TCLE;
- apresentou o roteiro da entrevista clínica estruturada a ser utilizada.
- explicitou de forma clara como irá lidar com casos em que identificar problemas que inviabilizem a participação na pesquisa.

Recomendações:

Sem recomendações.

Conclusões ou Pendências e Lista de Inadequações:

Sem pendências ou inadequações.

Considerações Finais a critério do CEP:

Se o projeto prevê aplicação de TCLE, todas as páginas do documento deverão ser rubricadas pelo pesquisador e pelo voluntário e a última página assinada por ambos, conforme Carta Circular no 003/2011 da CONEP/CNS.

Salientamos que o pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado. Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEPH de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Lembramos que esta modificação necessitará de aprovação ética do CEPH antes de ser implementada. De acordo com a Res. CNS 466/12, o pesquisador deve apresentar a este CEP/SMS o relatório final do projeto desenvolvido, conforme preenchimento de Protocolo disponível na página do Comitê de Ética em Pesquisa com Seres Humanos do IPUSP, do site do IPUSP. Em seguida, o protocolo preenchido deverá ser enviado ao CEPH pela Plataforma Brasil, ícone Notificação, logo que o mesmo estiver concluído.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Endereço: Av. Prof. Mello Moraes,1721 - Bl. "G" sala 27
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UF: SP **Município:** SAO PAULO
Telefone: (11)3091-4182 **E-mail:** ceph.ip@usp.br

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Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_427900.pdf	30/05/2016 10:32:04		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Pedro_Zuccolo.doc	30/05/2016 10:31:34	Pedro Fonseca Zuccolo	Aceito
Outros	carta_ao_cep_resposta2.pdf	30/05/2016 10:30:11	Pedro Fonseca Zuccolo	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_doutorado_PedroZuccolo_CEP_corrigido2.docx	30/05/2016 10:29:33	Pedro Fonseca Zuccolo	Aceito
Outros	carta_cep_resposta.pdf	28/12/2015 12:24:52	Pedro Fonseca Zuccolo	Aceito
Outros	Entrevista_SCID.pdf	28/12/2015 12:18:27	Pedro Fonseca Zuccolo	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_doutorado_PedroZuccolo_CEP_corrigido.docx	28/12/2015 12:16:45	Pedro Fonseca Zuccolo	Aceito
Folha de Rosto	folha_de_rosto_2.pdf	04/11/2015 19:41:45	Pedro Fonseca Zuccolo	Aceito
Cronograma	cronograma.docx	28/10/2015 09:45:34	Pedro Fonseca Zuccolo	Aceito
Declaração de Pesquisadores	declaracao_pesquisador_responsavel.pdf	28/10/2015 09:41:38	Pedro Fonseca Zuccolo	Aceito
Declaração de Instituição e Infraestrutura	declaracao_infraestrutura.pdf	28/10/2015 09:40:34	Pedro Fonseca Zuccolo	Aceito
Outros	pareceres_mestrado.pdf	13/10/2015 12:46:36	Pedro Fonseca Zuccolo	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO PAULO, 20 de Junho de 2016

Assinado por:
Jose de Oliveira Siqueira
(Coordenador)

Endereço: Av. Prof. Mello Moraes,1721 - Bl. "G" sala 27
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Appendix 3 – Post-retrieval interview

Post retrieval interview
Weike & Schupp & Hamm (2007)

Instrução: "Agora vamos fazer uma pequena pausa no experimento" [iniciar rodagem do cronômetro]. "Vou desligar os aparelhos, a única coisa que eu vou deixar é o eletrodo de condutância da pele porque a gente vai usar ele depois" [desligar aparelhos].

"Nós vamos fazer um intervalo de 10 min e durante esse tempo eu gostaria de conversar algumas coisas com você a respeito do experimento" [preparar papel e caneta].

1- Você sabia quando você ia receber um estímulo elétrico? Por favor descreva. [escrever a resposta do sujeito no papel, falando em voz alta para que ele te escute]

2- Quantas faces diferentes você viu? Descreva cada uma delas. Por favor, dê o maior número de detalhes possíveis. [idem pergunta 1]

3- Você percebeu alguma diferença entre as faces? Quais eram essas diferenças? [idem pergunta 1]

4- Agora que você descreveu essas faces, eu gostaria de você tentasse dar um nome para cada uma delas, com base em caraterísticas que você descreveu [idem pergunta 1]

5- Você consegue me descrever como era o choque? Por favor, dê o maior número de detalhes possíveis. Você consegue se lembrar a duração dos choques? [idem pergunta 1]

6- Quantas vezes a fotografia X foi seguida de estímulo elétrico? [idem pergunta 1]

7- A apresentação do estímulo elétrico seguia

(1) a face masculina… (descrição da face 1)
(2) a face masculina… (descrição da face 2)
(3) não havia relação sistemática
(4) não é possível responder a essa pergunta