ABSTRACT. The present study was conducted to assess the influence of fearful cues on human causal learning, specifically on extinction and spontaneous recovery of causal relationships. Two experiments employed a learning reversal procedure, in which two cues (i.e., X and Y) were first paired with two outcomes (i.e., O₁ and O₂), followed by a reversal of these relationships. In other words, the treatment consisted of X-O₁ and Y-O₂ pairings in Phase 1, followed by X-O₂ and Y-O₁ pairings in Phase 2. Experiment 1 manipulated the nature of the cues across groups according to a 2 (artificial vs. naturalistic stimuli) x 2 (low vs. high fear level) factorial design and found a reversal of the causal roles of X and Y cues, which was not affected by the nature of the cues. Experiment 2 replicated the main treatment of Experiment 1 involving artificial stimuli as cues and, additionally, found a noticeable effect of a 5-min interval on the causal roles of both X and Y cues (i.e., spontaneous recovery), a result that was seemingly stronger for the condition trained with fearful stimuli. In other words, although prior differences in the causal ratings of X and Y tended to disappear following the retention interval in both high and low fear conditions, this effect was found to be stronger in the high fear condition than in the low fear condition. Possible explanations for these findings as well as directions for future research are discussed.

Biological Significance in Human Causal Learning
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Pharmacological as well as cognitive-behavioral approaches have been the choice of treatment for many anxiety disorders such as posttraumatic stress disorder (Gogella, Caroni, Luthi, & Herry, 2009; Hermans et al., 2005). Due to the well-documented persistence of conditioned behavior following extinction, many animal and human causal learning studies have examined a multitude of reasons why previously extinguished conditioned responses tend to recover (Costanzi, Cannas, Saraulli, Rossi-Arnaud, & Cestari, 2011; Hermans et al., 2005; Huff, Hernandez, Blanding, & LaBar, 2009; Robbins, 1990; Yamamoto et al., 2009). However, most research in human causal learning has been conducted using preparations that employ biologically neutral stimuli, a limitation that makes it difficult to generalize the findings from the human learning laboratory to the treatment of anxiety-related problems. According to Domjan (2005), Pavlovian conditioning is more robust when biologically relevant cues are employed. Domjan’s functional perspective implies that Pavlovian conditioning is the most effective when it occurs under natural conditions, and utilizes cues that are both salient and ecologically relevant. For example, taste aversion learning can be achieved in a single trial even when the unconditioned stimulus (US) is delayed for an extended period of time. Accordingly, the present research sought to examine the role of biologically significant cues in human causal learning phenomena involving outcome inference. Specifically, our study used a computer-based task to assess if the emotional content of the cues can affect extinction of causal relationships, as assessed in a reversal of stimulus discrimination treatment, and spontaneous recovery thereof. By determining whether a relationship exists between the biological

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significance of stimuli and the interference effects, we aimed to provide insight for future studies and treatments regarding anxiety disorders.

Early research on human causal learning (Dickinson, Shanks, & Evenden, 1984; Shanks & Dickinson, 1987; Shanks, Holyoak, & Medin, 1996) revealed that the processes involved in the acquisition and expression of information about causal relationships mirror those processes involved in animal conditioning. Early animal conditioning paradigms revealed that nonhuman animals are just as sensitive as humans to event contingencies and perceived contingency in the detection of causal relationships, Shanks and Dickinson (1987) employed an experimental setup consisting of a computer “video-game,” in which participants were asked to rate the causal effectiveness of instrumental responses such as pressing various keys on a keyboard in producing an outcome. The outcome in their study was the presentation of an illuminated triangle on a computer screen. Notably, the stimuli used were biologically neutral, consistent with the early tradition in human learning research, in which biologically significant stimuli such as shocks and food were not usually employed.

More recently, however, some research conducted in the human learning laboratory has employed biologically significant stimuli. For example, Lovibond, Saunders, Weidemann, and Mitchell (2008) used a human avoidance-learning preparation in which participants were able to avoid electric shocks by pressing a particular button. Using a computer task, participants were told that, for every other trial, they would be presented with a colored square. This represented the neutral stimulus, which then played the role of a surrogate conditioned stimulus (CS) for 5 s, followed by a 10-s waiting period, and a possible shock lasting for 0.5 s, which served as the US. Additionally, participants were informed that response buttons might light up while a particular colored square was presented and that pressing a particular button might terminate the shock. Receiving a shock was contingent on whether participants pressed the right response button; pressing the correct response button canceled shocks, and pressing the wrong response button did not allow participants to avoid shocks. Those who received Pavlovian contingencies prior to instrumental conditioning learned the relationship between the stimulus and the outcome much faster than those who did not receive this training. These findings suggested that simply practicing an avoidance response could reduce anxiety by eliminating the expectancy of a fearful outcome. However, although the study found that shock expectancy and skin conductance declined as a function of the availability of an avoidance response, shock expectancy (i.e., fear) increased on trials with no available avoidance response.

Conditioned fear extinction and reinstatement have also been observed in humans through a fear-potentiated startle paradigm (Norrholm et al., 2006). This consisted of fear conditioning in which participants were presented with colored lights (i.e., the CSs) and a blast of air to the throat (i.e., the US), followed by extinction after a period of 24 hr. Reinstatement of fear was observed following unpaired presentations of the US. One of the first studies to demonstrate reinstatement of fear in human conditioning was Hermans et al. (2005). Similar to that of other researchers (Alvarez, Johnson, & Grillon, 2007; Norrholm et al., 2006), Hermans et al. (2005) employed an experimental treatment comprising several phases: stimulus selection, acquisition, extinction, reinstatement, and postreinstatement test phase. The study found a significant reinstatement effect on the ratings of the US expectancy. More specifically, participants in the study were presented with pictures of men and women with little to no emotional expression on the background of a computer screen. CS and US were selected based on individual fear ratings on an 11-point Likert-type scale ranging from 0 (not fearful at all) to 10 (very fearful). Additionally, the threshold of fear was determined when the experimenter attached electrodes to participants and gradually increased the level of the electrocutaneous stimuli until it was reported to be unpleasant by the participant. During the acquisition phase, participants were told that they would be presented with two stimuli, one of which would be followed by the electrocutaneous stimuli. In their study, the + and - signs referred to either the presence or absence of the US, following the presentation of the CS. Therefore, the CS+ was always followed by the aversive stimuli (or US), and the CS- was never followed by the US. Subsequent to this stage, participants were asked to identify

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contingencies between stimuli that had been previously presented. Upon correct identification of the two stimuli, participants had to determine whether the CSs were followed by the US, and they were then asked to rate the extent to which they expected the CS+. The association between the CS+ and US was then extinguished through 24 trials involving CS+ presentations without the US. US expectancy and fear ratings were assessed again following extinction. Half of the participants were presented with the US again and the other half were not. The postreinstatement test phase evaluated the impact of the reinstatement procedure. Similarly to the work of Norrholm et al. (2006), the results of Hermans et al. (2005) supported the view that extinction does not lead to the unlearning of a CS-US association.

In a similar vein, research by Effting and Kindt (2007) examining avoidance learning and renewal found that renewal of shock expectancy appears to be higher when testing takes place in the fear acquisition context (e.g., ABA renewal design) compared to testing in a novel context (e.g., ABC renewal design). Moreover, several other researchers examining the role of extinction in human causal learning have obtained similar findings (Bouton & Swartzentruber, 1991; Nelson, Gregory, & Sanjuan, 2012). Research examining human causal learning in anxiety disorders has shown that, despite cognitive and behavioral treatment success rates of approximately 85% for anxiety disorders, fear return occurs in as many as 30 to 50% of these individuals (Boschen, Neumann, & Waters, 2009; Yamamoto et al., 2009). Return of fear following extinction seems to be mostly attributed to passage of time (e.g., spontaneous recovery) or a change of context (e.g., renewal effect; Effting & Kindt, 2007; Hermans et al., 2005; McLean & Foa, 2011).

In spite of the growing list of studies to employ biologically significant stimuli in the human causal learning literature, limitations remain when applying those findings into treatment settings. Due to limitations in human causal learning studies, clinical treatments have relied on evidence from some animal studies (Costanzi et al., 2011; Maren & Chang, 2006). For example, studies utilizing animal models of posttraumatic stress disorder have been able to show that conditioned fear is subject to spontaneous recovery following extinction (Costanzi et al., 2011; Robbins, 1990; Yamamoto et al., 2009). Specifically, some animal studies have shown that the time between training and extinction plays a pivotal role in causal learning and affects the efficacy of long-term extinction in animal models (Corley, Caruso, & Takahashi, 2012; Costanzi et al., 2011; Maren & Chang, 2006; Robbins, 1990; Schreurs, Smith-Bell, & Burhans, 2011; Yamamoto et al., 2009).

**Study Overview**

Determining how to minimize the return of fear following extinction is imperative to the development of more effective treatments for anxiety disorders (Huff et al., 2009). Although previous research has focused on the role of temporal proximity and contextual cues in the recovery of a response, some studies have examined the relevance of biologically significant stimuli in the return of fear or spontaneous recovery. Prior research has studied human avoidance learning through the use of electric shocks (Alvarez et al., 2007; Effting & Kindt, 2007; Hermans et al., 2005; Lovibond et al., 2008; Norrholm et al., 2006). The present research aimed to explore the possibility of studying these phenomena in human causal learning without any need of aversive stimulation (e.g., shocks) by employing a computer-based experimental task devised to provide the presentation of stimuli of either low- or high-biological significance. Instead, the preparation employed in the present experiments used cues consisting of pictures that, presumably, would elicit either a mild emotional reaction of fear (i.e., international symbols for hazardous materials and poisonous animals) or no fear reaction (i.e., geometrical figures and fruits). This experimental setup would allow for an economic, yet realistic, study of learning processes involved in anxiety/fear disorders through a human learning paradigm.

The present study aimed to ascertain whether the emotional content of the cues might determine the occurrence of outcome interference phenomena in human causal learning. To test this, the experiment employed a learning reversal procedure, in which pairings of X and Y cues with O1 and O2, respectively (i.e., X-O1 and Y-O2 pairings) were followed by a reversal of these relationships (i.e., X-O2 and Y-O1 pairings). X and Y cues referred to the type of stimulus presented to participants, and O1 and O2 referred to the possible outcomes associated with the cues. In our learning reversal task, O1 meant that a person was found dead, and O2 consisted of a person found alive. The study was conducted in two separate experiments, Experiments 1 and 2. In Experiment 1, the nature of the cues were manipulated across groups according to a 2 (artificial vs. high stimulus) x 2 (low vs.
high fear level) factorial design. Experiment 2 was virtually identical to Experiment 1, with the critical addition of a delayed test in order to test for spontaneous recovery of responding following extinction. However, Experiment 2 only included two of the conditions of Experiment 1, namely, those given artificial stimuli (i.e., it did not include the artificial vs. naturalistic stimuli manipulation of Experiment 1).

Method

Participants
Thirty undergraduate students (10 men and 20 women) from a psychology course at a northeastern university participated in Experiment 1. Their average age was 18.90 years ($SEM = 0.36$). Participants were randomly assigned to one of four groups (i.e., participants were asked to pick a piece of paper from a bag, which contained a number corresponding to their group assignation), which were artificial low fear (A-L), artificial high fear (A-H), naturalistic low fear (N-L), and naturalistic high fear (N-H). This random assignation resulted in nine, six, eight, and seven participants respectively. In Experiment 2, participants were 19 undergraduate students (6 men and 13 women), also from a psychology course at a northeastern university. Their average age was 20.47 years ($SEM = 0.32$). Random assignment of participants was 9 and 10 participants for groups A-L and A-H, respectively. In accordance with our a priori exclusion criterion, the data of those participants who failed to discriminate between X-O₁ and Y-O₂ relationships by the end of Phase 1 were removed from the analysis. Specifically, in order for the data to be included in the analysis, the participant had to rate X higher than Y in Test 1. In Experiment 1, five participants failed to meet our exclusion criterion; two from each of groups A-L and A-H, and one from group N-L. Thus, the final number of participants involved in our study was 25 A-L ($n = 7$), A-H ($n = 4$), N-L ($n = 7$), and N-H ($n = 7$). In Experiment 2, one participant from group A-L failed to meet this criterion. Thus, the resulting sample was composed of 18 participants: A-L ($n = 8$) and A-H ($n = 10$). Groups in our study were uneven due to our small sample size and the data removal of participants who failed to discriminate between X-O₁ and Y-O₂ during the first phase of the experiment.

Apparatus
The apparatus consisted of Lenovo™ R61 laptops with 2.00 GHz Intel® Core™ Duo processors to conduct our study. The computers were arranged in a relatively small ($12' \times 9'$), room at the psychology department at a northeastern university.

Procedure
Prior to conducting this study, approval was granted by the institutional review board at a northeastern university. The experimental preparation employed in this experiment (i.e., risk evaluation task) consisted of a simple task in which participants were asked to pretend that they were epidemiologists requested to study the information in a series of fictitious files in order to subsequently rate certain events as possible causes of death. The instructions presented to participants read as follows.

You are a renowned epidemiologist who has been assigned a case involving mysterious deaths in an apartment building in New York City. You have been granted access to the files of the case, which contain evidence carefully collected by the New York Police Department. You will first need to study this evidence to accurately identify the cause or causes of the deaths. However, because this is sensitive information, the judge has set some restrictions in your access to the files; they will be released one by one, and for a limited time only. Also, you are not allowed to take notes. Thus, you will need to pay attention and try to remember the evidence in order to complete your report later.

In your report, you will use numeric values in a scale from -10 to +10 to answer a series of questions regarding the evidence you previously reviewed. In this scale, -10 means not at all, 0 means not sure, and +10 means very much (you will be reminded of the meaning of these values again). Any value between -10 and +10 is a valid answer. Good luck!

The cues were presented within one of three horizontally arranged panels, which were constantly present at the upper half of the screen. The presentation of each of the cues in the panels was random on each trial (i.e., the randomization is determined by the experimental program). The size of these panels was 215 x 200 (h x w) pixels, and they were separated by 35 pixels between the closest borders of each panel. These panels were embedded into a larger panel measuring 705 x 300 (h x w) pixels. The outcome was presented...
in a separate panel measuring 705 x 215 (h x w) pixels, allocated at the bottom half of the screen. On each trial, the cues were presented for 4 s. The outcomes were presented for 4 s. The offset of the cue presentation coincided with the onset of the outcome presentation. The duration of the intertrial interval was also 4 s.

As can be appreciated in Table 1, the treatment in Phases 1 and 2 was identical in both Experiment 1 and 2. Phase 1 treatment consisted of the presentation on the computer screen of different trials involving the cues and outcomes in an observational stage. Specifically, all participants received five pairings of a cue, X, with O1 (i.e., the person was found dead), interspersed with five pairings of a second cue, Y, with O2 (i.e., the person was found alive). Based on previously learned observations, the participants had to determine the appropriate outcomes for the X and Y cues.

Of critical importance, the stimuli serving as X and Y cues were manipulated across groups according to a 2 (artificial vs. naturalistic stimuli) x 2 (low vs. high fear level) factorial design. Thus, there were four groups in this experiment: group A-L, in which the stimuli for X and Y were a circle and triangle; group A-H, in which X and Y were the symbols for radioactive and chemical materials; group N-L, in which stimuli for X and Y were berries and nipple fruits; and lastly group N-H, in which stimuli for X and Y were a snake and a scorpion. The stimuli serving as X and Y cues were counterbalanced within each group.

At the end of Phase 1 training, a causality test was given. In the test phase, the stimuli presented during training were presented on a single screen along with the following question: “How responsible is [stimulus name] for the observed cases of death?” Participants were asked to rate the causal relationships between X and Y cues with O1 (i.e., person found dead) using a numeric scale ranging from -10 to +10, in which -10 meant not at all, 0 meant not sure, and +10 meant very much. In Phase 2 training, the relationships between the cues and the outcomes were reversed. That is, Phase 2 training consisted of five X-O2 pairings interspersed with five Y-O1 pairings. This phase was also followed by a test of the causal status of X and Y in relation to O1.

Experiment 2 aimed to replicate the findings of Experiment 1 with the addition of a third causality test given after a retention interval. Before the additional test, a 5-min rest period was introduced, during which time participants were requested to leave the experimental room and wait in the hallway before being allowed to proceed with the experiment (for an identical manipulation in a study on latent inhibition, see Pineño, De la Casa, Lubow, & Miller, 2006). Therefore, Experiment 2 also aimed to study the discrimination reversal as indicated by the causal ratings in Tests 1 and 2, while testing for spontaneous recovery of causal judgments following the discrimination reversal (i.e., Test 3). However, given the secondary character of the purpose of Experiment 2 (i.e., replication of Experiment 1 with a retention interval), it only included two of the groups from Experiment 1, namely, groups A-L and A-H. All statistical tests were conducted and significance was set at the .05 level.

Results
The results of the Experiments 1 and 2 are shown in Figures 1 and 2, respectively. As can be seen in Figure 1, the causal roles of the cues in Experiment 1 were reversed from Test 1 to Test 2, with no apparent differences across groups. This finding was confirmed by a 2 (artificial vs. naturalistic stimuli) x 2 (low vs. high fear level) x 2 (X vs. Y cue) Analysis of Variance (ANOVA), which revealed only a significant Test x Cue interaction, F(1, 21) = 147.38, p < .001, MSeffect = 6464.60. The main effects and the remaining interactions were not significant.

In Experiment 2, the retention interval affected the causal ratings of X and Y in both groups, although such impact seemed stronger for group A-H than for group A-L. Unfortunately, an ANOVA could not be performed due to zero variance in the ratings of X in Test 1 and of Y in Test 2 for both groups (all participants gave ratings of 10). For the sake of simplicity, instead of conducting nonparametric analyses, we performed a series of t tests on the remaining comparisons of

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<td><strong>Design of Experiments</strong></td>
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Note. X and Y were the cues, which were paired with the outcomes, O1 and O2. The nature of the outcomes was identical in all groups, whereas the nature of the cues was varied across groups. The arrow (→) means “followed by,” and the delay of Experiment 2 consisted of a 5-min interval during which the participants were requested to leave the experimental room and wait in the hallway before being allowed to proceed with the experiment (see text for details).
relevance. There were no significant differences between groups on the causal ratings of Y in Test 1 and on the causal ratings of X in Test 2 (ps > .19). Therefore, the results of Experiment 2 replicated the main finding of Experiment 1, namely, that the learning reversal was not affected by the nature of the cues.

More importantly, the results of Test 3 in Experiment 2 revealed that the retention interval had a stronger impact for group A-H than for group A-L. Between-group comparisons found no difference in the causal ratings of X, t(16) = 0.86, p = .39, and a marginally significant difference for the ratings of Y, t(16) = 2.07, p = .054. Interestingly, for group A-L, causal ratings of X were significantly lower than the causal ratings of Y, t(14) = 2.46, p = .02, whereas such difference was completely abolished for group A-H, t(18) = 1.16, p = .25. A close inspection of Figure 2 indicates that, although both groups experienced a decline in the ratings of Y combined with an increase in the ratings of X, these changes were more marked for group A-H than for group A-L. Thus, it seems that spontaneous recovery was stronger for group A-H.

**Discussion**

The present study aimed to ascertain if the occurrence of outcome interference phenomena such as extinction could be modulated by the use of cues of intrinsic biological significance in a human causal learning preparation. The results of the present study were inconclusive, given our failure to observe any detectable influence of the presumed biological significance of the cues on outcome interference. In both Experiments 1 and 2, the reversal of discrimination was comparable in all experimental groups regardless of the nature of the stimuli employed for X and Y cues.

Although our main assumptions were not supported, the findings of Experiment 2 suggested that our experimental task may actually be appropriate for use in research studying fear relapse, renewal, or extinction processes. Although spontaneous recovery occurred in both conditions, it was found to be more robust in group A-H (e.g., hazard symbols) than in group A-L (e.g., geometrical figures), a result that might be due to the hazard symbols evoking a slightly higher level of fear than that of the geometrical figures. Preconditioned associations between hazard symbols and presumed danger levels might or might not have influenced the intensity of spontaneous recovery observed within this particular group. Pineño and Miller’s (2005) comprehensive literature review alluded to Pavlov’s (1927) study of phenomena such as extinction and counterconditioning (i.e., CS-US pairings followed by pairings of the CS with a different US, typically of different motivational values such as tone-shock pairings followed by tone-food pairings). Of critical importance to the current study, the interpolation of a retention interval between counterconditioning and testing also resulted in partial recovery of the first-learned CS-US association. This effect was explained by Pineño and Miller (2005) as potentially due to an integration of the memories of conditioning and extinction, thereby resulting in an increase in responding due to the combined
action of both memories into performance (i.e., relative to extinction performance prior to the temporal or contextual manipulations).

Some limitations of the current research included the use of a small sample, which could have influenced our findings. It is important to consider the fact that students from specific psychology classes were offered an opportunity to participate in the study as opposed to a more diverse group of participants, which could have influenced the findings of this research. Intra- and interindividual differences or experiences in general might have contributed to whether participants found stimuli to be of either low or high fear. For example, pre-conditioned associations between hazard symbols and presumed danger levels might have influenced the intensity of spontaneous recovery observed in group A-H. Moreover, a portion of data was withdrawn from the final analysis due to the failure of some participants to properly discriminate between X-O₁ and Y-O₂ relationships. This was a necessary prerequisite to exclusively conduct our analysis on the data from those participants who correctly learned the cue-outcome relationships. Such a protocol was determined prior to starting the data collection, but might have negatively impacted the study’s external validity. Perhaps the pre-determined protocol for the removal of data based on a criterion might have imposed a ceiling effect on our findings, rendering the variation in the distribution of our data insufficient, meaning that the participants who were not eliminated by the criterion were probably the ones who responded in a very similar fashion. An increase in our sample size could have minimized such a possible effect.

Moreover, the findings of this research might or might not have been unique to the specific class group that participated and could raise questions about our ability to generalize these findings to other populations and subpopulations.

Future research regarding spontaneous recovery and extinction can be studied more effectively by engaging specific populations affected by anxiety disorders, while simultaneously incorporating stimuli that are meaningful to that group. In addition, as previously mentioned, the stimuli used in the current experiment might not have been powerful enough to elicit the level of fear that was originally anticipated. Therefore, this implies that future research regarding causal learning through the use of human learning tasks would presumably benefit from incorporating a greater variety of biologically relevant cues, as well as possibly incorporating more graphic and gruesome pictures. Considering the type of biologically significant stimuli employed in our study, another possible direction for future research regarding stimuli selection would include the use of more intrinsically emotional cues that elicit an immediate mental state or reaction.

Relapse and/or spontaneous recovery of conditioned fear continues to be a major concern for clinicians treating a plethora of anxiety disorders (Huff et al., 2009). In our study, we sought to determine whether the emotional significance of cues played a role in relapse following the extinction of a previously learned association. We found that the emotional significance of stimuli did not influence the rate of extinction in a learning reversal procedure, as evidenced by its comparable occurrence across all experimental conditions, regardless of the nature of the cue. However, in Experiment 2, spontaneous recovery was found to be more vigorous within group A-H (e.g., with hazard symbols as cues) than in group A-L (e.g., with geometrical figures as cues). Our findings simply suggested that, although biological significance of stimuli failed to have a strong impact on outcome interference phenomena (e.g., extinction), its influence was apparent in spontaneous recovery of an extinguished response, which is something that should be accounted for by clinicians designing new treatments for anxiety disorders.

Our findings concurred with previous research showing that extinction does not lead to the unlearning of the CS-US association (Hermans et al., 2005, Norrholm et al., 2006). Clinical implications of our findings suggested that previously extinguished responses can recover whether the cues are biologically significant or neutral. In other words, emotionally neutral stimuli are not immune to the effects of spontaneous recovery or relapse. Such an implication is imperative for clinical treatment approaches to anxiety disorders. Successful advances in anxiety treatment should incorporate therapies that account for the influence of time posttreatment. Additionally, the present research implied that clinicians treating anxiety disorders should be mindful of all related stimuli that can serve as a potential trigger, regardless of its biological significance, while ensuring that their approaches are individually tailored to help protect against the risk of unsuccessful treatment or relapse.

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