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Human fear conditioning depends on stimulus contingency instructions

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Highlights

- This study showed that contingency instructions influence human fear conditioning
- This was found with contingency awareness rates and psychophysiological measures
- Contingency reversal instructions rapidly reverse psychophysiological responses
- Contingency instructions should be reported in human fear conditioning studies

Abstract

Human fear conditioning is often seen as the result of a highly automatic process that is independent of higher cognitive functions and verbal instructions. However, cumulative research findings call this view into question. In the current preregistered study (N = 102), we investigated whether the number of participants who successfully show conditioned fear acquisition depends on the instructions given to them before the fear conditioning phase. Particularly, one third of the participants were instructed about the precise contingency between the conditioned stimulus (CS) and unconditioned stimulus (US). Another third was merely instructed that there would be a contingency. The last third did not get any instructions about the CS-US contingency. We found facilitated fear acquisition rate in the first and second group compared to the third group. Furthermore, contingency reversal instructions following the acquisition phase reversed both conditioned skin conductance and startle responses. These results highlight that researchers should systematically report the instructions given to participants in human fear conditioning studies.

Keywords: Fear Conditioning; Replicability; Instructions; Psychophysiology

1. Introduction

The fear conditioning procedure is a widely used translational paradigm to investigate the etiology and treatment of anxiety-related disorders (Vervliet, Craske, & Hermans, 2013). In this paradigm, a neutral Conditioned Stimulus (CS) gets paired with an aversive Unconditioned Stimulus (US), which typically results in fearful Conditioned Responses (CRs) towards the CS. It is generally believed that this paradigm models an important etiological pathway for the development of anxiety disorders (De Houwer, 2020; Field, 2006; Mineka & Zinbarg, 2006). Furthermore, it connects fundamental cognitive and psychopharmacological research in animals with clinical research in humans (Haaker et al., 2019). Hence, the fear conditioning procedure has been established as one core translational research paradigm in experimental clinical research.

A growing body of research has indicated that fear conditioning is substantially influenced by verbal instructions (for a review see: Mertens, Boddez, Sevenster, Engelhard, & De Houwer, 2018). For instance, simply providing participants with the instruction that a CS will be followed by a US is sufficient to install subjective, behavioral, and psychophysiological responses related to fear, without requiring any actual CS-US pairings (e.g., Deltomme, Mertens, Tibboel, & Braem, 2018; Javanbakht et al., 2016; Mertens et al., 2016; Raes, De Houwer, De Schryver, Brass, & Kalisch, 2014). Surprisingly, however, the type of CS-US instructions is often neglected in the reporting of fear conditioning research. To illustrate, we analyzed the method sections of 69 empirical articles reporting a fear conditioning study involving human

participants published in 2018.[1] We found that 39% of these articles failed to provide any information whatsoever about the instructions given to the participants. Furthermore, information about the instructions is often minimal (i.e., usually there is no verbatim description of the instructions).

Neglecting to report the instructions can be problematic for replicating research findings in the fear conditioning literature. Specifically, verbal instructions can have a considerable impact on the number of participants who show successful fear acquisition. Indeed, several studies have found that, compared to participants who did not receive instructions about the CS-US contingency, participants who did received such instructions showed stronger differential (i.e., CS+ > CS-) conditioned skin conductance responses (Atlas, Doll, Li, Daw, & Phelps, 2016; Javanbakht et al., 2016; Tabbert, Stark, Kirsch, & Vaitl, 2006), conditioned startle responses (Duits et al., 2017), US expectancy ratings (Raes, De Raedt, Fias, Koster, & Van Damme, 2009), and higher rates of contingency awareness in a post-conditioning assessment (Tabbert et al., 2006). Hence, verbal contingency instructions influence the strength of conditioned fear acquisition. This problem is further exacerbated by the fact that fear conditioning studies commonly exclude participants who fail to discriminate between the CSs that are followed by a US from the CSs that are not (sometimes up to 74% of the sample; see Lonsdorf et al., 2019). As such, different contingency instructions can result in a different sample selection, which can

[1]This was established with a search on PubMed (search syntax: (((fear conditioning) NOT rats) NOT mice) NOT animal). It provided 174 hits, of which 69 articles that included a differential cue fear conditioning procedure with adult human participants were selected for full text screening. We checked whether the articles provided any information about the instructions given to participants prior to the fear conditioning phase (i.e., articles did not necessarily have to state the exact instructions). 42 articles did provide some information on the instructions given to the participants and 27 articles did not provide any information (for an overview of these studies see https://osf.io/7j56p/).

further complicate the replication of prior research findings. However, no studies so far have directly tested the effects of different types of instructions about the CS-US contingency (see below) on conditioned fear acquisition.

Moreover, participants are also usually given instructions before other phases of fear conditioning studies, such as fear extinction (i.e., when a CS is not followed by a US anymore), generalization (generalization of CRs to similar CSs), and return of fear (return of CRs after fear extinction) (Lonsdorf et al., 2017). For example, in research from our group and other research groups investigating context renewal in a 2-day paradigm, participants were instructed to "think back to what you learned the previous day" on the second day (Landkroon, Mertens, Sevenster, Dibbets, & Engelhard, 2019; Milad, Orr, Pitman, & Rauch, 2005). Such instructions may facilitate context renewal (i.e., the return of conditioned fear due to a change in contextual features). However, few studies so far have tested whether contingency instructions affect conditioned responses beyond the acquisition phase.

The current study aimed to provide a direct empirical demonstration that contingency instructions can affect the rate of successful conditioning and can influence conditioned responses in later phases of the experiment. Contingency instructions at the start of a conditioning procedure generally take three different forms. First, participants can be informed at the start of the experiment about the precise contingency between the CS and US (e.g., Atlas et al., 2016; Bublatzky, Gerdes, & Alpers, 2014; Costa, Bradley, & Lang, 2015; Mertens & De Houwer, 2016). Second, participants can be informed that there is a contingency in the task and encouraged to discover this contingency, but without being told which of the CSs will be followed by the US (e.g., Golkar, Bellander, Olsson, & Ohman, 2012; Haesen & Vervliet, 2015; Mertens, Wagensveld, & Engelhard, 2019; van Uijen, Dalmaijer, Hout, & Engelhard, 2018).

Finally, participants are sometimes not provided any information at all about the contingencies in the task (i.e., uninstructed fear conditioning; e.g., Haaker et al., 2015; Leuchs, Schneider, & Spoormaker, 2018; Miskovic & Keil, 2013; Sjouwerman, Niehaus, Kuhn, & Lonsdorf, 2016). In the current study, we assessed the effect of these three different types of contingency instructions (i.e., precise contingency instructions, general contingency instructions, and no contingency instructions) on psychophysiological discrimination between the CS+ (i.e., the CS paired with the US) and the CS- (i.e., the CS not paired with the US) at the end of the acquisition phase, and contingency awareness rates as assessed with a retrospective questionnaire. We expected that participants in the precise contingency instructions and general contingency instructions conditions would show higher fear acquisition rates than participants in the no contingency instructions condition. Furthermore, we expected only a slight advantage in the precise contingency instructions condition compared to the general contingency instructions condition, because most participants in the general contingency instructions condition are expected to discover the contingencies as well (see Section 2.2).

An additional aim of this study was to demonstrate that contingency instructions can also influence conditioned responses later on in a conditioning procedure. For this purpose, we also provided participants with contingency reversal instructions after the acquisition phase. As observed in previous studies (e.g., Atlas et al., 2016; Mertens & De Houwer, 2016; Morriss, Saldarini, Chapman, Pollard, & van Reekum, 2019; Wilson, 1968), we expected that these contingency instructions would reverse conditioned psychophysiological responses.

2. Methods

2.1. Preregistration and data availability

This study was preregistered on the Open Science Framework at the following link: 10.17605/OSF.IO/7J56P. Raw and final datafiles can be obtained through this link as well.

2.2. Participants

One hundred and eight students from Utrecht University were recruited to participate in this study. Mostly English speaking international undergraduate students were recruited to reduce overlap with the target population of other ongoing (Dutch) fear conditioning studies from our research group. Participants were randomly allocated to one of the three different conditions in the experiment (i.e., precise contingency instructions condition, general contingency instructions condition, and no contingency instructions condition; n = 36 per condition). This sample size was determined using a power calculation. Particularly, under the assumption that 90% of the participants in the precise contingency instructions condition, 80% in the general contingency instructions condition, and 50% in the no contingency instructions condition show successful fear acquisition ($\omega = 0.384$), a total sample of 102 participants was required to detect a significant effect ($p = .025$; see below) with a power of > 0.9 (Faul, Erdfelder, Lang, & Buchner, 2007). A slightly larger sample was tested due to data exclusions (see Section 2.5.3.1). Participants were recruited through flyers and posters on campus and were screened for self-reported physical and mental health. All participants completed an informed consent form and were instructed that they could discontinue the experiment at any point without any negative consequences. The procedure of this study falls within a research line of fear conditioning studies, which has already received approval by the ethics committee of the Faculty of Social and Behavioral Science at Utrecht University (FETC16-054). Participants received financial compensation (€8) or course credit in exchange for their participation. Table 1 provides more

detailed demographic information, trait anxiety scores, selected US intensity, and US pain ratings regarding the participants in the three different conditions of this study (see below).

Table 1. Descriptive statistics of the demographic information of the participants in the three different conditions of the experiment.

	Precise contingency instructions (n = 35)	General contingency instructions (n = 33)	No contingency instructions (n = 34)	Group comparison
Mean age in years (SD)	23.14 (3.17)	23.09 (3.23)	23.53 (3.57)	$F(2, 99) < 1$
Gender distribution	25 females 10 males	21 females 12 males	24 females 10 males	$X^2(2) < 1$
STAI-T	42.23 (8.66)	41.55 (8.96)	40.18 (10.48)	$F(2, 99) < 1$
Mean US intensity in mA (SD)	4.11 (3.64)	4.20 (2.60)	5.46 (6.48)	$F(2, 99) < 1$
Mean US rated pain on a 0-10 scale (SD)	5.50 (0.92)	5.43 (0.96)	5.75 (1.07)	$F(2, 99) < 1$

2.3. Materials

2.3.1. Apparatus. The experiment was programmed in Inquisit (v4) and run on a HP Z230 desktop computer running Windows 8.1 Pro. The electrical simulation was generated with a Digitimer DS7A system. Skin conductance was measured using a Biosemi bio-amplifier and two Biosemi GSR electrodes filled with Signa electrode gel. Startle responses were measured with two BioSemi EMG electrodes attached below the left eye (Blumenthal et al., 2005). Psychophysiological measures were collected with Actiview and further analyzed offline with BrainVision Analyzer 2.0 software.

2.3.2. Questionnaires. Trait anxiety was determined with the Dutch translation of the State-Trait Anxiety Inventory – trait version (STAI-T, range: 20-80; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983; van der Ploeg, Defares, & Spielberger, 2000) to control for possible differences in trait anxiety between the conditions. Additionally, the short version of the Intolerance of Uncertainty Scale (Carleton, Norton, & Asmundson, 2007) and the Context Sensitivity Index (Bonanno, Maccallum, Malgaroli, & Hou, 2018) were completed by the participants for unrelated research questions, and will therefore not be reported here.

2.3.3. Stimuli. The unconditioned stimulus (US) was a 500-ms electrical stimulation administered through two electrodes attached to the index and middle finger of the right hand. The intensity of this stimulus was individually set for each participant with a work-up procedure (see the Procedure section).

Conditioned stimuli (CSs) were geometrical shapes (a circle and a square), which are common stimuli in fear conditioning procedures (Lonsdorf et al., 2017). These were presented with a resolution of 300 by 300 pixels on a 23-inch screen (screen resolution: 1920 by 1080 pixels).

2.4. Procedure

2.4.1. Startup and work-up procedure. Upon arrival in the lab, participants washed their hands and were then asked to read the information letter about the experiment, provide informed consent and complete the STAI-T. Next, skin conductance and electrical stimulation electrodes were attached. Participants went through a work-up procedure in which the US intensity was determined. They were asked to select an intensity level that was unpleasant but tolerable (Mertens & De Houwer, 2016). To operationalize the intensity, participants were asked

to score the intensity of the US on a 0 to 10 scale (0 = *no pain at all*, 10 = *maximum level to voluntarily tolerate*). The work-up procedure stopped when participants rated the intensity as 7 or higher. The final intensity level was used in the experiment, unless participants indicated before reaching 7 that they did not want to increase the intensity further. In these latter cases, the maximal tolerable intensity was used (Mertens & De Houwer, 2016).

2.4.2. Instruction manipulation. After the work-up procedure, participants were randomly allocated to one of the three conditions. Particularly, in the *precise contingency instructions* condition, they received the following instructions on the computer screen (translated from Dutch): "*In the following experiment you will see two different shapes appear on the screen: A square and a circle. The square*[/circle] *will sometimes be followed by an electrical shock and the circle*[/square] *will never be followed by an electrical shock.*" Participants in the *general contingency instructions* condition received these instructions: "*In the following experiment you will see two different shapes appear on the screen: A square and a circle. One of the shapes will sometimes be followed by an electrical shock and the other shape will never be followed by an electrical shock. Your task is to learn to predict when the shock will be presented.*" Finally, participants in the *no contingency instructions* condition received the following instructions: "*In the following experiment you will see two different shapes appear on the screen: A square and a circle. You will also sometimes receive an electrical shock.*" Following the instruction manipulation, participants were told to press the spacebar to continue with the experiment.

2.4.3. Startle habituation and fear conditioning phase. Then participants habituated to the startle probe (50 ms, 95 dB). They heard the probe 10 times with a 7 s inter-trial interval

(ITI), which was immediately followed by the fear conditioning phase. This phase consisted of eight presentations of the circle and the square. Counterbalanced, either the circle or square was followed by the electrical stimulation on six out of the eight trials (75% reinforcement rate). Each shape was shown for 8 s. In each trial, a startle probe was presented 7 s after CS onset. In case of a reinforced trial, the US was administered immediately at CS offset. The ITI was either 12, 14 or 16 s. The order of CS presentations was semi-random with the restriction of maximally two identical consecutive trials.

2.4.4. Questions regarding contingency awareness. After the acquisition phase, participants were asked about their awareness of the CS-US contingencies with the following two questions about each CS (translated into Dutch): (1) "Was the square[/circle] followed by the electric shock?", response options: "Yes", "No"; and (2) "How certain are you about your answer?"; response options: "completely certain", "fairly certain", "fairly uncertain", and "completely uncertain". A comparable procedure of assessing contingency awareness has been used in other fear conditioning studies (e.g., Singh et al., 2013; Tabbert et al., 2006; Wegerer, Blechert, Kerschbaum, & Wilhelm, 2013).

2.4.5. Contingency reversal instructions and reversal phase. Following the acquisition phase and contingency awareness assessment, participants were instructed that stimulus contingencies would be reversed in the following phase ("*In the next phase of the experiment, the relationship between the shapes and the electric shock will be reversed: The square[/circle] WILL now NOT be followed by the electric shock. The circle[/square] WILL now SOMETIMES be followed by the electric shock.*"). This instruction was identical for participants in all the conditions. Following these instructions, the experiment continued with the same procedure as in

the acquisition phase, except that CS+ and CS- were each shown five times (instead of eight) and the CS+ was only reinforced once after the third trial. We decided to only reinforce the CS+ after three trials to ensure that reversal up to this point was only based on the verbal contingency instructions. After the reversal phase, participants were asked to indicate the contingencies of the previous phase. Finally, they were debriefed and compensated for their participation.

2.5. Data Preprocessing and Analysis

2.5.1. Skin conductance responses (SCRs). SCRs were calculated by subtracting the mean value of a baseline period (2 s before CS onset) from the highest peak during the 1 to 8 s interval post CS onset (Pineles, Orr, & Orr, 2009). Thereafter, skin conductance values were range corrected using the largest response for each participant and square root transformed to normalize the data (Dawson, Schell, Filion, & Berntson, 2007). A minimum response criterion was set at .02 μS.

2.5.2. Fear potentiated startle (FPS). The electromyography signal of the startle response was filtered (28-500 Hz), smoothed (15.9 Hz low-pass filter), and rectified. Startle magnitude was calculated by subtracting the baseline value (time window: 0-20 ms after probe onset) from the highest peak value in the 21 to 150 ms time window after startle probe onset. These values were then T-transformed using each participants' individual mean and standard deviation (Blumenthal et al., 2005).

2.5.3. Data analysis.

2.5.3.1. Data exclusion. The data of six participants was excluded due to incorrect storage of the data (n =4) or problems with the storage of markers in the datafiles (n = 2). These

data were replaced with data of six new participants to maintain our targeted sample size (n = 102; see the Participants section).

2.5.3.2. Planned statistical analyses. The focus of this study was on the number of participants who show successful conditioned fear acquisition. This was defined as a positive difference between the CS+ and CS- at the end of the acquisition phase (i.e., Fear_CS+ - Fear_CS- > 0; see the preregistration file). To reduce the influence of error variance, we averaged responses of the last two acquisition trials to calculate this index. This criterion is commonly used for fear acquisition (e.g., Ahmed & Lovibond, 2015; Atlas et al., 2016; Golkar, Tjaden, & Kindt, 2017; Javanbakht et al., 2016; Klucken et al., 2016; Morriss, Christakou, & van Reekum, 2016) and is straightforward to interpret and implement. Participants who did not meet this criterion were coded as unsuccessful fear acquisition. We tested whether the rate of participants who showed successful fear acquisition differed between the different conditions by conducting a Chi-square test. Because the same focal hypothesis was tested both with SCRs and FPS, an alpha-value of .025 (i.e., 0.5/2) was used (see our preregistration).

In addition to successful acquisition of conditioned fear, we investigated the number of participants who showed successful contingency awareness. Contingency awareness is a common exclusion criterion in fear conditioning research (e.g., Dirikx, Vansteenwegen, Eelen, & Hermans, 2009; Golkar et al., 2017; Mertens et al., 2019; Rowles, Lipp, & Mallan, 2012). We considered participants to be contingency aware if they correctly indicated which CS was followed by the electrical stimulation and which CS was not followed by the electrical stimulation (see Singh et al., 2013). Furthermore, participants had to be “completely certain” or “fairly certain” of their answer for both the CS+ and CS- (to account for guessing). Otherwise,

they were categorized as contingency unaware. The number of contingency aware participants in the three contingency instructions conditions was analyzed using a Chi-square test.

2.5.3.3. Secondary analyses. In additional secondary analyses (see preregistration file), results of SCR and FPS in the acquisition phase were analyzed using a repeated measures ANOVA with factors instructions (between-subjects: precise contingency, general contingency and no contingency), CS type (within-subjects: CS+ and CS-) and trial (within-subjects: 1 to 8). This analysis takes into account all the trials of the acquisition phase, rather than only the last two trials, which allows us to also test the course of learning rather than only the final two trials. Furthermore, the continuous nature of this analysis provides more statistical power than the preregistered categorical analysis.

Furthermore, we analyzed results of the reversal phase by comparing the last trial of the acquisition phase to the first trial in the reversal phase using a repeated measures ANOVA with factors instructions (between-subjects: precise contingency, general contingency and no contingency), CS type (within-subjects: CS+ and CS-) and phase (within-subjects: acquisition and reversal).

All analyses were run in SPSS v25, using an alpha-value cut-off of .05, unless otherwise stated. Violations of the sphericity assumption were corrected using Greenhouse-Geisser corrections. Figures were generated in R using the ggplot2 package (Wickham, 2016).

3. Results

3.1. Primary analyses

3.1.1. Successful psychophysiological fear acquisition. Table 2 provides the results regarding successful CS discrimination for both SCR and FPS. Descriptively, successful acquisition rates were higher in the precise contingency instructions and general contingency instructions conditions for SCR and in the general contingency instructions condition for FPS. Statistically, however, we did not find evidence for differences in acquisition rates between the conditions (p-values > .05; see Table 2).

3.1.2. Contingency awareness rates. Table 2 also provides the results for retrospective contingency awareness rates in the different conditions. As expected, the percentage of contingency aware participants in the general contingency instructions condition was significantly higher than in the no contingency instructions condition ($\chi^2(1) = 11.88$, $p = .001$). However, unexpectedly, it was not significantly higher in the precise contingency instructions condition than in the no contingency instructions condition ($\chi^2(1) = 2.51$, $p = .113$). Also, surprisingly, the percentage of contingency aware participants in the general contingency instructions condition was significantly higher than in the precise contingency instructions condition ($\chi^2(1) = 2.51$, $p = .041$).

Table 2. Fear acquisition and contingency awareness rates for the different conditions in the experiment.

	Precise contingency instructions (n = 35)	General contingency instructions (n = 33)	No contingency instructions (n = 34)	Group comparison
% successful SCR discrimination	68.6% (24 out of 35)	63.6% (21 out of 33)	52.9% (18 out of 34)	$\chi^2(2) = 1.86$ (ns)
% successful FPS discrimination	60.0% (21 out of 35)	81.8% (27 out of 33)	67.65% (23 out of 34)	$\chi^2(2) = 3.92$ (ns)
% contingency aware	71.4% (25 out of 35)	90.9% (30 out of 33)	52.9% (18 out of 34)	$\chi^2(2) = 11.87$*

*$p = .003$

3.2. Secondary analyses and effects of reversal instructions

3.2.1. Skin conductance responses.

3.2.1.1. Acquisition phase. The repeated measures ANOVA of the results of the acquisition phase revealed main effects of CS type, $F(1, 99) = 92.00$, $p < .001$, $\eta^2_p = 0.48$, and trial, $F(6.02, 595.67) = 13.20$, $p < .001$, $\eta^2_p = 0.12$. These main effects were qualified by an interaction between CS type and trial, $F(7, 693) = 2.48$, $p = .016$, $\eta^2_p = 0.02$, and, crucially, CS type and condition, $F(2, 99) = 5.52$, $p = .005$, $\eta^2_p = 0.10$. This interaction between CS type and condition was due to stronger differentiation between CS+ and CS- in the precise contingency condition, $F(1, 34) = 49.89$, $p < .001$, $\eta^2_p = 0.60$, and the general contingency condition, $F(1, 32)$

= 49.54, $p < .001$, $\eta^2_p = 0.61$, than in the no contingency condition, $F(1, 33) = 7.56$, $p = .010$, $\eta^2_p = 0.17$ (see Figure 1). No other main or interaction effects were significant, F-values < 1.5, p-values > .15, $\eta^2_p < 0.03$.

3.2.1.2. Reversal phase. The repeated measures ANOVA looking at the effect of the reversal instructions indicated main effects of CS type, $F(1, 99) = 4.82$, $p = .030$, $\eta^2_p = 0.05$, and phase, $F(1, 99) = 12.37$, $p = .001$, $\eta^2_p = 0.11$, and, crucially, an interaction effect between CS type and phase, $F(1, 99) = 17.98$, $p < .001$, $\eta^2_p = 0.15$. This interaction effect was due to lower CS+ SCR values after the reversal instruction (M = 0.37, SD = 0.35) compared to before (M = 0.40, SD = 0.37), whereas this pattern was the reverse for CS- (before reversal instructions: M = 0.19, SD = 0.28; after reversal instructions: M = 0.44, SD = 0.36; see Figure 1). The other main and interaction effects were not significant, F-values < 1.5, p-values > .2, $\eta^2_p < 0.03$.

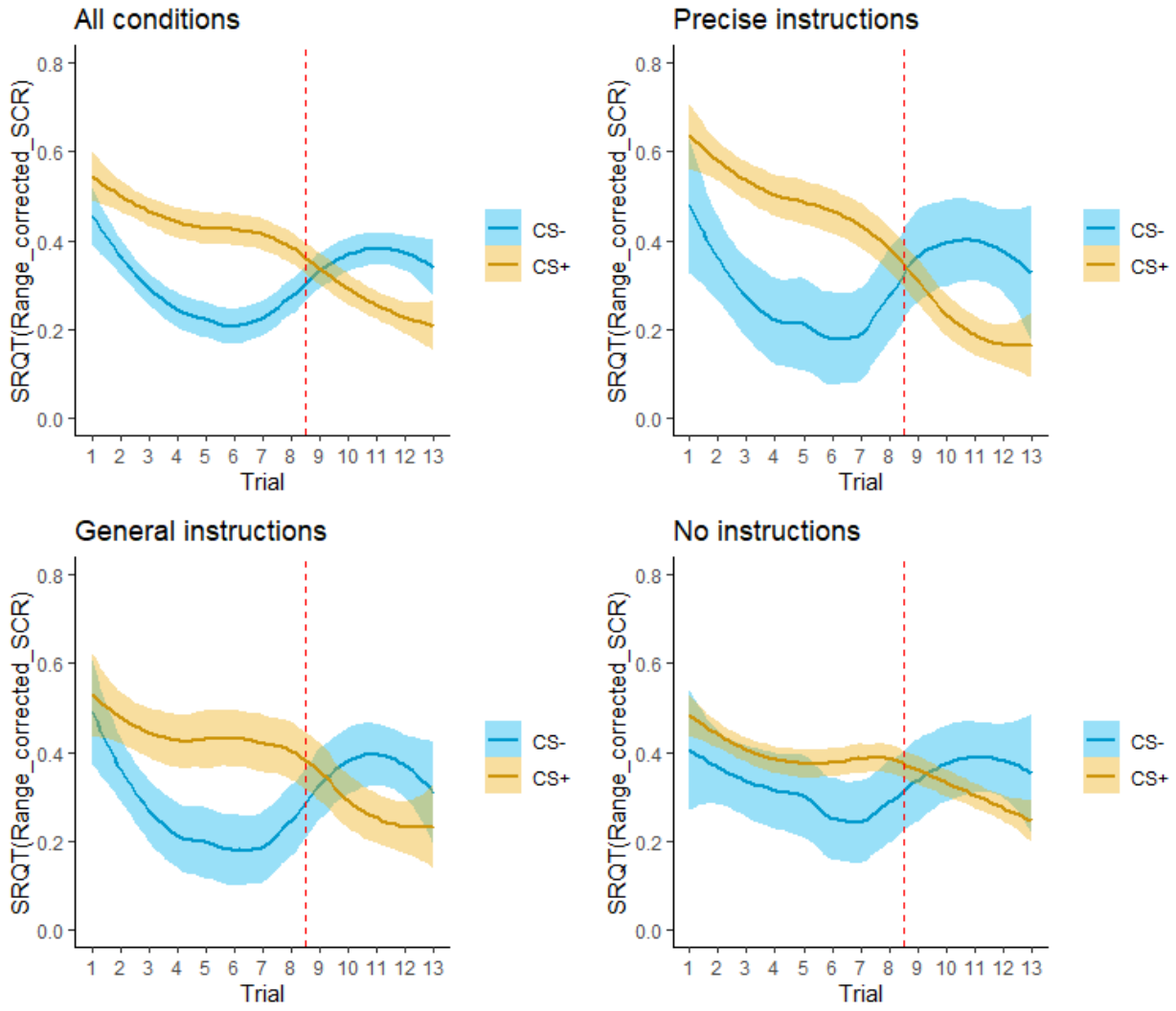

Figure 1. Range corrected and square root transformed skin conductance responses with the whole sample (top left panel) and across the three different conditions (top right and bottom panels). The dashed lines indicate when contingency reversal instructions were given.

3.2.2. Fear potentiated startle.

3.2.2.1. Acquisition. Similar to the results of SCR, the repeated measures ANOVA of the FPS data during the acquisition phase revealed main effects of CS type, $F(1, 99) = 47.84$, $p < .001$, $\eta^2_p = 0.33$, and trial, $F(5.91, 585.09) = 12.42$, $p < .001$, $\eta^2_p = 0.11$. These main effects were qualified by a three-way interaction between CS type, trial, and condition, $F(14, 693) = 1.74$, $p = .044$, $\eta^2_p = 0.03$. Breaking down this interaction, with separated CS by trial repeated measures ANOVA's, only a clear interaction between CS type and trial was observed in the general contingency instruction condition, $F(7, 224) = 2.95$, $p = .006$, $\eta^2_p = 0.08$, whereas no such interaction was observed for the precise contingency instruction or the no contingency instruction condition, F-values < 1, p-values > .6, $\eta^2_p < 0.03$. In all conditions, a significant effect of CS type was observed (p-values < .017), but the effect was more pronounced in the precise contingency ($\eta^2_p = 0.39$) and the general contingency instruction conditions ($\eta^2_p = 0.42$), than in the no contingency instruction condition ($\eta^2_p = 0.16$) (see Figure 2). No other main or interaction effects were significant, F-values < 1.7, p-values > .2, $\eta^2_p < 0.04$.

3.2.2.2. Reversal. As for the SCR results, the repeated measures ANOVA looking at the effect of the reversal instructions for FPS indicated a main effects of phase, $F(1, 99) = 5.54$, $p = .021$, $\eta^2_p = 0.05$, and, crucially, an interaction effect between CS type and phase, $F(1, 99) = 28.70$, $p < .001$, $\eta^2_p = 0.23$. This interaction effect was due to lower CS+ FPS values after the reversal instruction (M = 48.19, SD = 7.95) compared to before (M = 50.87, SD = 9.55), whereas this pattern was the reverse for CS- (before reversal instructions: M = 46.67, SD = 7.82; after reversal instructions: M = 53.32, SD = 9.52; see Figure 2). The other main and interaction effects were not significant, F-values < 2.1, p-values > .13, $\eta^2_p < 0.04$.

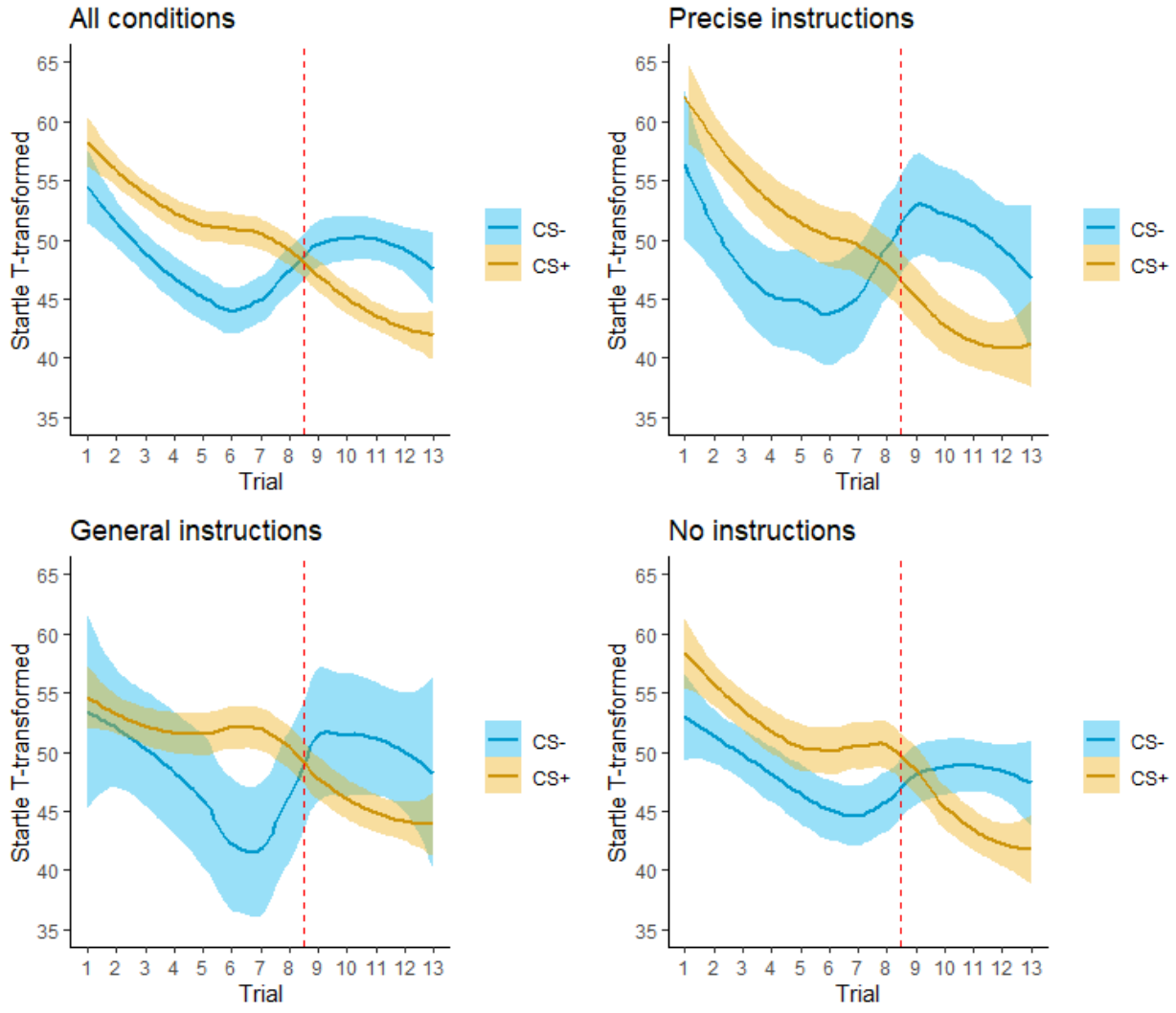

Figure 2. T-transformed startle responses throughout the experiment with the whole sample (top left panel) and across the three different conditions (top right and bottom panels). The dashed lines indicate when the contingency reversal instructions were given.

4. Discussion

In this study, we investigated the effects of contingency instructions prior to conditioning and contingency reversal instructions after conditioning on conditioned psychophysiological responses (SCR and FPS). Contingency instructions before an acquisition phase affected the rate of contingency aware participants, particularly when participants were instructed to discover the contingencies themselves. Using a dichotomic criterion of successful conditioning, we did not observe significant effects of contingency instructions on successful fear acquisition rates for SCR and FPS, although numerically the results were in the expected direction. Secondary continuous analyses on the trial-by-trial data in the acquisition phase did, however, reveal a significant effect of contingency instructions for SCR and FPS, indicating that precise and general contingency instructions resulted in more pronounced differential conditioning compared to no contingency instructions (see Figures 1 and 2). Finally, reversal instructions following the fear acquisition phase reversed conditioned responses with both SCR and FPS. Collectively, these results provide empirical evidence that contingency instructions influence conditioned fear responses.

We believe that our results have important implications for the human fear conditioning field. First, researchers should clearly indicate which instructions they gave participants in all phases of the experiment either in the main paper or in a supplemental file to the main paper. Such information is crucial for a full evaluation of the results and the replication of published research. Currently, a substantial part of the literature fails to report this important methodological aspect (i.e., 39% in 2018, see the Introduction). Such unreported variation in

methodological details can complicate the interpretation and replication of research findings, as the results may hinge on the verbal instructions provided to participants.

A second implication of our results is that verbal instructions should be considered in the design of fear conditioning studies. Precise or general verbal contingency instructions can strengthen both psychophysiological conditioning and contingency awareness rates. Hence, such instructions could be used to obtain more robust conditioning. Notably, this has been done in studies that rely on fear acquisition to examine individual differences in extinction learning or interventions to target acquired fear (Leer, Engelhard, Altink, & van den Hout, 2013; Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013), and this practice is supported by the current findings. On the other hand, some research questions may necessitate uninstructed learning of the contingencies. In this case, attention should be devoted to avoiding any references in the instructions to the contingencies in the task, as this may affect the learning of the contingencies. Additionally, given the common practice of excluding participants based on unsuccessful fear acquisition (Lonsdorf et al., 2019) or lack of contingency awareness (Mertens et al., 2019), verbal contingency instructions may also affect the final constellation (and hence statistical power and representativeness) of the sample. Finally, not only in the initial fear acquisition phase, but also in subsequent phases of conditioning experiment (e.g., extinction phase, generalization phase, return of fear), the effects of verbal instructions should be considered. That is, references to the contingencies in the instructions (e.g., “think back to the contingencies in the previous phase”) may influence the results in this phase as well (e.g., stronger return of fear). Hence, researchers should also clarify in their papers which instructions were given in other phases of fear conditioning experiments.

In addition to reporting practices and study design considerations, our results highlight the importance of carefully considering data analysis strategies in the fear conditioning literature. Particularly, our primary analyses on the psychophysiological measures did not support our prediction (i.e., percentages of successful fear acquisition did not differ between the three different conditions). However, trial-by-trial analyses across the whole acquisition phases provided clear evidence for an effect of contingency instructions. Hence, it may be good practice to consider several alternatives for the data-analyses and, ideally, to include such alternatives in the preregistration of the study. Such a 'multiverse' of analyses can provide a better idea of the conditions under which a result holds (Steegen, Tuerlinckx, Gelman, & Vanpaemel, 2016).

With regard to theoretical implications, the results of this study provide further support for models arguing for the involvement of controlled reasoning processes in human learning. That is, there is ongoing debate about the processes that underly human (fear) conditioning. Some authors have proposed that human fear conditioning, and in particular conditioning of psychophysiological measures, occurs largely automatically (i.e., without effort and outside of voluntary control) and without awareness (LeDoux & Pine, 2016). These models also predict that verbal instructions would only have a minimal impact in fear conditioning (Olsson & Phelps, 2007). In contrast, other models have argued that human (fear) conditioning requires propositional reasoning and can also be influenced by verbal instructions (Lovibond, 2011; Mertens & Engelhard, 2020; Mitchell, De Houwer, & Lovibond, 2009). Our results lend more support to the latter class of models by demonstrating the clear impact of verbal contingency instructions on fear conditioning, including psychophysiological measures of fear.

A number of relevant limitations of this work can be noted. First, we did not measure all types of conditioned responses that can be collected in fear conditioning research such as self-reported US expectancy or valence ratings, avoidance behaviors, or other types of psychophysiological responses such a heart rate or functional brain imaging. Despite not providing direct evidence for the effects of instructions on these other types of conditioned responses, there are no a priori reasons to presume that our results and recommendations are not relevant for these outcome measures as well. Indeed, there is evidence that instructions can influence these other types of conditioned responses as well (see Mertens et al., 2018). Another limitation is that we only considered the effects of instructions in one specific version of the fear conditioning paradigm (i.e., using geometrical shapes as CSs and an electrical shock as the US, without trial-by-trial subjective ratings, in a healthy student sample, and using a 75% reinforcement schedule). It is conceivable that the effects of verbal contingency instructions are more or less outspoken when using different parameters (e.g., using 100% reinforcement) or relying on different populations. The interaction between such parameters and the effects of verbal instructions need to be further investigated.

In conclusion, the results of the present study highlight that human fear conditioning can be substantially influenced by verbal instructions provided to participants. As such, scientific reports on human fear conditioning should pay attention to and report the instructions that are provided to the participants.

Material and data availability

The materials, data files and data analysis scripts related to this experiment will be made available through the Open Science Framework (https://osf.io/7j56p/).

Conflict of interest

The authors declare no conflicts of interest with respect to the authorship or the publication of this article.

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