The Effect of Verbal Instructions on Fear Conditioning

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Abstract

In the context of experimental studies about fear conditioning, verbal instructions were not considered as a possible influencing factor during the past decades. Therefore, they are often not at all or only partly reported in the methodological section of scientific articles. This study focused on the effect of verbal instructions on fear conditioning. A hypothesis was established that presentation of the CS+ should lead to increased startle responsiveness and skin conductance responses in two verbal contingency instructions groups with different degrees of specificity, whereas participants of the no contingency-group were predicted to require more time for a corresponding response. This hypothesis was tested by providing participants with either no, general, or precise instructions about the combination of a conditioned stimulus with an electric shock, and measuring their fear potentiated startle (FPS) and skin conductance response (SCR) during the experiment. In addition, the participants' contingency awareness was assessed afterwards. The results for the FPS indicate that conditioning was more outspoken in the general and precise contingency than in the no contingency instruction, whereas information about the CS-type influenced the SCR in all three contingency groups, but mostly so in the precise contingency group. An additional analysis revealed that anxiety level of participants, as assessed by three questionnaires, did not differ significantly between contingency groups. These results suggest that fear conditioning of the FPS and SCR are influenced by verbal instructions, even though the degree of specificity of the instructions might have a varying influence. Therefore, verbal instructions should be included in the methodological sections of scientific articles.

Keywords: fear conditioning, verbal instructions, fear potentiated startle, skin conductance response

Introduction

Fear conditioning usually refers to the process of developing fear by the repeated pairings of a conditioned stimulus (CS, e.g. a geometrical shape) and an aversive, unconditioned stimulus (US, e.g. electric stimulation) (Mertens, Boddez, Sevenster, Engelhard & De Houwer, 2018), resulting in a conditioned fear response (CR) when confronted with the CS. The conditioned fear response includes behavioural, subjective, and physiological components that can be assessed by measuring behavioural responses (e.g. pressing a button to avoid the US), self-report questionnaires, or physiological responses, such as the fear potentiated startle response or skin conductance response (Mertens et al., 2018). Furthermore, fear conditioning has been found to exist in animals, as well as in humans (Kim & Jung, 2018). This raises the question whether fear conditioning is an automatic process (i.e., requiring minimal awareness, intention, attention and control) and, further, whether verbal instructions have an influence on fear conditioning and should therefore be reported in scientific articles.

For the past several decades, the majority of fear conditioning paradigms was conducted with animals (primarily rodents; Kim & Jung, 2018). This procedure has been helpful in establishing the neurobiological underpinning of fear conditioning, for instance, that the neuropsychological fear conditioning circuit considers the amygdala to play a central role (Kim & Jung, 2018). As laboratory animals do not possess language and thus are never instructed in fear conditioning experiments, possible effects of instructions have typically not been taken into regard for research with humans either. However, for human fear conditioning research, verbal instructions are not only necessary for the course of the research procedure, but also required by the American Psychological Association (APA; 2017). According to the Ethical Principles of Psychologists and Code of Conduct by the American Psychological Association (2017), psychological researchers have to inform their participants about “the purpose of the research, expected duration and procedures”, as well as “reasonable foreseeable factors that may be expected to influence their willingness to participate such as potential risks, discomfort, or adverse effects” (APA, 2017). If not adhered to these rules, any informed consent given by participants is invalid, which can lead to the withdrawal of the ethical approval of one’s research (Bordens & Abbott, 2011). Another factor highlighting the importance of verbal instructions addresses the necessity to inform participants about the process of the experiment in order to enable their participation (World Medical Association, 2018). Therefore, it can be concluded that conducting research without the use of verbal instructions is impossible and unethical in humans. Nevertheless, verbal instructions and their possible impact are often not included in the methodology section of scientific articles about human fear conditioning studies, possibly due to the gradual transition from animal to human studies.

In order to examine whether verbal instructions are included in the methodological part of scientific articles, a small literature review was conducted[1], which included 25 studies about fear conditioning published between 2004 and 2019, with the vast majority of studies being published between 2015 and 2019. Of these 25 studies, only four studies (16%) included specific information about which specific verbal instructions were given at which point during the experimental procedure. In addition, two studies (4%) mention verbal instructions but do not include details about the content or time point of those instructions during the experimental procedure. The remaining 18 studies (80%) do not give any information about verbal instructions. This lack of reporting given to the participants may be problematic, however, as the obtained results may depend on these instructions.

Until now, whether to provide verbal instructions about the CS-US contingency or not during the course of the experiment depends on the set-up of one’s fear-conditioning study and the concept one intends to measure. Potential advantages of including instructions pertain to a

[1]Experimental RCT’s about fear conditioning, published mostly between 2015 and 2019, were selected. Their method sections and appendixes were screened in order to assess whether information about provided verbal instructions was included.

reduced variance in acquisition learning as a result. Furthermore, focusing participants' attention on the contingencies in the task through instructions leads to improved awareness of fear learning, which is useful when the focus of research is on post-acquisition manipulations or phases (Lonsdorf et al., 2017). On the other hand, not providing instructions allows for experience-based associative learning to take place and additionally increases the variance in conditioned responding (Lonsdorf et al., 2017). Lonsdorf and colleagues (2017) thus point out that verbal instructions may have an effect on acquisition learning. This finding is corroborated by other research, which, for example, shows that verbal instructions about the contingency between a CS and an unpleasant US can produce conditioned fear responses towards the CS, despite the absence of actual CS-US pairings (Mertens et al., 2018). Additional support comes from the findings that verbal threat instructions about the CSs lead to faster and more strongly acquired fear (Atlas, Doll, Li, Daw & Phelps, 2016; Field & Storksen-Coulson, 2007; Ugland, Dyson & Field 2013) and the delayed extinction of fear (Mertens & De Houwer, 2017). Even though the different effects of verbal instructions on aspects of fear conditioning are found by several studies with different foci and structures, however, some contradicting findings create ambiguity about the impact of verbal instructions. According to Olsson and Phelps (2004), verbal instructions lead to a lower expression of conditioned fear compared to social observation and stimulus pairings when using masked CSs. In addition, fear that is conditioned to fear-relevant CSs, such as images of spiders and snakes, does not seem to be responsive to instructed extinction, as well as fear conditioned with intense electric stimuli (Luck & Lipp, 2016).

As illustrated above, the effect of verbal instructions in experimental studies including fear conditioning is still controversial and requires clarification. Experimental studies with human participants cannot be conducted without verbal instructions. In addition, the American Psychological Association and the World Medical Association both pose the requirement of verbal instructions in experimental settings with human participants. Therefore, their effect on fear conditioning should be investigated, which is what this experimental study is concerned with. In order to overcome the limitations of previous studies, an explicit focus is put on the effect of verbal instructions by including three different between-subjects conditions with varying degrees of specificity in verbal instructions, namely no contingency, general contingency, and precise contingency. The corresponding hypothesis deals with the question whether verbal instructions have an effect on physiological fear responses typically collected in fear conditioning studies (i.e., skin conductance and the startle response) following presentation of the CS+. More precisely, presentation of the CS+ should lead to increased startle responsiveness and skin conductance responses in both verbal contingency instructions groups, whereas participants of the no contingency-group are predicted to require more time for a corresponding response.

Method

2.1. Participants

Participants were Dutch and International students at Utrecht University fluent in English, and did not participate in prior studies involving electrical stimulation. A total of 108 participants took part in the study and were recruited through convenience sampling by the use of advertisement posters around Utrecht Science Park and social media (e.g. Facebook groups). Six participants were excluded due to equipment and experimenter error. The sample was selected on voluntarily basis and consisted of 69% females and 31% males aged between 18 and 35 (M = 23.25, SD = 3.57). Participation reward consisted of 8€/hour or one participant credit point. Exclusion criteria consisted of various conditions such as pregnancy or current psychiatric problems/diagnoses (see Appendix for more information). Table 1 depicts various demographic information and the average scores for each of the three conditions.

2.2 Material

2.2.1 Conditioned Stimuli (CS)

CSs were two grey geometric shapes (circle, square) of 300 by 300 pixels presented on a white background of a HP EliteDisplay E231 screen with a resolution of 1920 by 1080 pixels. Both CSs were presented during the acquisition and the reversal phase.

2.2.2. Unconditioned Stimulus (UCS)

The UCS was an electric stimulus that was presented six times during the acquisition phase and once during the reversal phase. Shocks were delivered through two lubricated Fukuda standard Ag/AgCl electrodes (1-cm diameter, inter-electrode distance: ~2cm). A wristband with the electrodes administered the shocks by use of a constant current stimulator (DS7A, Digitimer, Hertfordshire, UK). Each participant determined the intensity of the electric stimuli individually in a stepwise work up procedure starting with the lowest intensity of 0.5 (see 2.6.1.). An unpleasant, but not painful intensity of the stimulus was selected for each participant.

2.3. Psychophysiology

2.3.1. Fear potentiated startle (FPS)

FPS was measured using two BioSemi EMG electrodes (0.4 cm diameter) filled with conductive gel (signal gel by Parker). One electrode was placed below the pupil of the left eye and the other one approximately 1cm laterally on the side. Two ground electrodes were placed in the middle of the participant's forehead 1 inch below the hairline (Blumenthal et al., 2005). Additionally, an auditory stimulus in the form of a loud noise delivered by headphones was used to elicit the startle response, which is known to increase in anticipation of a shock (Lonsdorf et al., 2017).

Startle responses were scored automatically by subtracting the mean baseline value (0-20 ms) from the highest peak value in the 20–120 ms time frame following the startle probe onset. T-transformations were then applied to these values using each participant's individual mean and standard deviation (Blumenthal et al., 2005).

2.3.2. Skin Conductance Response (SCR)

SCR was collected using two BioSemi GSR electrodes (0.8 cm diameter) with conductive gel that were attached to the thenar and hypothenar eminences of the left palm and measured using the BioSemi system.

SCRs were calculated by subtracting a mean baseline value (2s preceding CS onset) from the highest response value within a 1- to 7-s interval after CS onset (Pineles, Orr & Orr, 2009). A minimum criterion of 0.01 µS was applied for the SCRs. Values lower than this cut-off were recoded to zero. An additional correction for the inter-personal variability in response rates was conducted by dividing every participant's value by their respective maximum value. In order to normalize the data, a square root transformation was applied to all SCR responses (Dawson, Schell, Filion, & Berntson, 2007).

2.4. Questionnaires

The trait version of the State-Trait Anxiety Inventory (STAI-T; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) was used to assess the participants' general trait anxiety level. The STAI consists of 20 items (e.g.: "I feel secure") and participants were asked to use the rating scale to rate how much the item describes themselves. The scale had a high level of internal consistency, as determined by a Cronbach's alpha of 0.915.

The 20-item Context Sensitivity Index (CSI; Bonanno, Maccallum, Malgaroli & Hou, 2018) was used to measure the participant's ability to recognize the presence and absence of stressful context cues. It consists of two subscales, namely Cue Presence, which represents the participant's sensitivity to the presence of cues, and Cue Absence, which represents sensitivity to the relative absence of cues. The subscale of Cue Presence had a medium level of internal consistency, as determined by a Cronbach's alpha of 0.398, and the subscale of Cue Absence had a high level of internal consistency, as determined by a Cronbach's alpha of 0.677.

The 12-item short version of the Intolerance of Uncertainty Scale (IUS) was developed to measure intolerance of uncertainty. The revised version maintains excellent internal consistency, while also being highly correlated to the original IUS and related measures of anxiety (Carleton, Norton & Asmundson, 2007). In this sample, the questionnaire had a high level of internal consistency, as determined by a Cronbach's alpha of 0.835.

2.5. Manipulation of contingency instructions

Participants in the *no instruction group* received general instructions consisting of "In the following experiment you will repeatedly see one of two different geometric shapes on the computer screen: A square and a circle. You will also sometimes receive an electric shock. First you will hear a short loud noise through the headphones a number of times, thereafter you will see the shapes and feel the shock."

Participants in the *general instructions group* were given the above general instructions with the addition of "You can predict when the shock will be administered by paying close attention to the presentation of the shapes. One of the shapes will SOMETIMES be followed by an electric shock and the other shape will NEVER be followed by an electric shock".

Participants in the *specific instructions group* were given above general instructions with the addition of "You can predict when the shock will be administered by paying close attention to the presentation of the shapes. More precisely, the circle will SOMETIMES be followed by an electric shock and the square will NEVER be followed by an electric shock."

2.6. Procedure

2.6.1. General information and work up procedure

The research study has been approved by the Faculty Ethical Review Board of the Faculty of Social Sciences of Utrecht University. Upon arrival, participants washed their hands and were then given an information sheet, and a declaration of consent form to sign. Following this, participants were asked to complete the three questionnaires. Next, the SCR and FPS electrodes were attached after the participants skin was cleaned with a scrub gel (NuPrep Skin Prep Gel by Weaver and Company). This was followed by the work up procedure to determine the intensity of the electric shock. Participants were reminded to select an intensity that they found unpleasant but

Table 1

Descriptive Statistics of the Demographic Information of the Participants, respectively in the three experimental conditions

Variable	No instructions	General instructions	Specific instructions	Contingency awareness	Total
Number	34	33	35	80	102
Mean age	23,5	23,09	23,14	23,37	23,24
Gender	23 females, 11 males	21 females, 12 males	25 females, 10 males	56 females, 24 males	70females, 32 males
Mean shock pain rating	5,75	5,43	5,5	5,61	5,56
Mean shock intensity	5,45	4,2	4,1	4,45	4,58

Note. Number of Trials in Contingency Awareness Rating: N=8

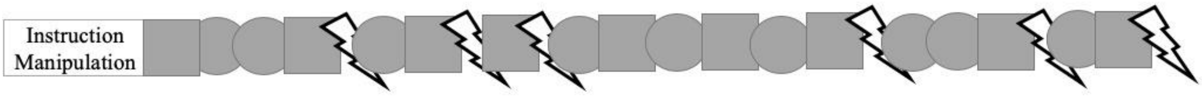
not painful and were asked to rate their discomfort verbally using a scale ranging from zero (not painful) to ten (extremely painful). The shock intensity was gradually increased and was stopped when participants rated the intensity six or higher. Finally, for the startle probe administration, headphones were put on. Participants were then asked to provide their age, their dominant hand and their gender on the screen. Further instructions were provided on the screen that participants were asked to read carefully. Participants received different instructions depending on which group they were assigned to (see 2.5.).

2.6.2. Conditioning phase

Following the contingency instructions, the conditioning phase started with six startle probe trials. The two geometric shapes were presented eight times each (16 in total) in a

Figure 1

Schematic overview of the CS and US presentations in the conditioning phase.



Note. The exact order of the CSs was pseudo-randomized (see Section 2.6.2.).

pseudorandom order (i.e., no more two consecutive trials of each CS type; see Figure 1 for an overview). Additionally, the CS associated with the shock (CS+) was followed by the electric shock six times on a partial reinforcement schedule (75% reinforcement rate). The other geometric shape (CS-) was never reinforced during the conditioning phase. Startle probes were delivered after each CS.

Following the conditioning phase, participants were questioned to determine their contingency awareness between each shape and the electric shock (i.e.: Did you think that the circle[/square] would be followed by the electric shock?). An additional question asked them about the certainty of their answer (i.e.: How sure are you about your answer?) on a scale containing the answer options ‘very sure’, ‘quite sure’, ‘quite unsure’, ‘very unsure’.

2.7. Data analysis

The research design is a combination of between-subjects design concerning the contingency instructions and a within-subjects design regarding the type of condition stimulus and trial number. In between-subjects designs, individual participants get assigned to and participate in one condition of the experiment (instead of completing all experimental conditions), whereas they complete all conditions of an experiment in a within-subjects design (Field, 2005). The independent variables are the type of conditioned stimulus (i.e. CS+ or CS-; within-subjects design), trial number (i.e. one to eight; within-subjects design), and contingency instructions (no instructions, general instructions, precise instructions; between-subjects design). The dependent variables consist of the physiological response of the participant in terms of skin conductance response and fear potentiated startle, and contingency awareness. Therefore, the chosen method for the data analysis is a repeated measure ANOVA (Field, 2005) with CS type and trial number as within-subject factors and contingency instructions group as a between-subjects factor.

Results

3.1 Contingency awareness

A chi-square test of homogeneity was conducted in order to determine the proportion of contingency awareness in each instruction group. Contingency awareness reflects whether participants were able to discriminate between which CS was followed by the US (CS+) and which one was not (CS-). It was assessed by the questions asked before and after contingency reversal (e.g. “Did you think that the circle[/square] would be followed by the electric shock?”). 102 participants received either no instructions, general instructions, or specific instructions (see Table 1). 64.7% of those who received no instructions were contingency aware, compared to 93.9% in the general instructions group and 77.1% of those receiving precise instructions. This was a statistically significant difference in proportions, $p = .014$. Post hoc analysis involved pairwise comparisons using multiple Fisher’s exact tests (2 x 2) with a Bonferroni correction. Statistical significance was accepted at $p < .016667$. There was no significant difference in contingency awareness between the no contingency and the precise contingency group, $p = .297$, and also no significant difference in contingency awareness between the general contingency and the precise contingency group, $p = .085$. However, there was a significant difference in contingency awareness between the no contingency and the general contingency group, $p = .006$. The number of contingency aware participants for each contingency group can be found in Table 2.

3.2 FPS

A three-way mixed ANOVA was run to understand the effects of CS-type, trial and contingency instruction (verbal instruction) on fear potentiated startle response (FPS)[2]. There was a statistically significant three-way interaction between type of CS, trial and contingency instruction, $F(14, 693) = 1.740$, $p = .044$, partial $\eta^2 = .034$. This result demonstrates that the simple two-way within-subjects CS-type*trial interactions are different for the different groups of contingency instruction.

For the follow-up analysis, two-way ANOVAS were run respectively for each contingency group. For the no contingency group, the assumption of sphericity was met, as assessed by Mauchly's test of sphericity ($\chi^{2(27)}$ = 15,875, $p = .956$). There was no statistically significant simple two-way interaction between CS-type and trial for the no contingency group, $F(7, 231) = .738$, $p = .640$, partial $\eta^2 = .022$. However, there was a statistically significant main effect of CS-type, $F(1, 33) = 6.429$, $p = .016$, partial $\eta^2 = .163$. For the general contingency group, the assumption of sphericity was met, as assessed by Mauchly's test of sphericity ($\chi^{2(27)}$ = 32,165, $p = .230$). There was a statistically significant simple two-way interaction between CS-type and trial for the general contingency group, $F(7, 224) = 2.947$, $p = .006$, partial $\eta^2 = .084$. For the precise contingency group, the assumption of sphericity was met, as assessed by Mauchly's test of sphericity ($\chi^{2(27)}$ = 30,913, $p = .279$). There was no statistically significant simple two-way interaction between CS-type and trial for the precise contingency group, $F(7, 238) = .563$, $p = .785$, partial $\eta^2 = .016$. However, there was a statistically significant main effect of CS-type, $F(1, 34) = 21.597$, $p = .000$, partial $\eta^2 = .388$.

Combined, these results demonstrate that conditioning depended on the type of instructions that the participants received. More precisely, conditioning was more outspoken in the general and precise contingency than in the no contingency instruction. The mean fear

[2] A summary of the assumption checks for the FPS analysis can be found in the appendix.

potentiated startle response when no verbal instructions were provided was 49.14 (*SD* = 8.73) for the CS- and 51.51 (*SD* = 8.96) for the CS+. It was 47.4 (*SD* = 8.01) for the CS- and 51.91 (*SD* = 10.33) for the CS+ when general instructions were given; and in the group provided with precise instructions the mean was 42.16 (*SD* = 8.86) for the CS- and 52.43 (*SD* = 8.91) for the CS+ (all depicted in Figure 2).

3.3 SCR

A three-way mixed ANOVA was run to understand the effects of CS-type, trial and contingency instruction (verbal instruction) on skin conductance response (SCR)[3]. There was no statistically significant three-way interaction between CS-type, trial and contingency group, $F(14, 693) = 1.141$, $p = .318$, partial $\eta^2 = .023$. However, a statistically significant two-way interaction between CS-type and condition was found, $F(2, 99) = 5.515$, $p = .005$. This means that the effect of CS-type depended on contingency group.

For the follow-up analysis, a two-way mixed ANOVA was conducted to assess the simple and/or main effects of conditions-type for each instruction group. Since CS-type as a within-subjects factor consisted of two levels, the assumption of sphericity was automatically met for all three contingency groups. For the no contingency group, the main effect of CS-type was statistically significant, $F(1, 33) = 7.563$, $p = .010$, partial $\eta^2 = .186$. For the general contingency group, the simple effect of CS-type was statistically significant, $F(1, 231) = 49.538$, $p = .000$, partial $\eta^2 = .608$. For the precise contingency group, the main effect of CS-type was statistically significant, $F(1, 34) = 49.894$, $p = .000$, partial $\eta^2 = .595$.

Combined, these results demonstrate that CS-type influenced the skin conductance response in all three contingency groups, but mostly so in the precise contingency group ($F(1, 34) = 49.894$). However, the effects in the general contingency group and the precise contingency group were almost similar (partial $\eta^2_{general} = .608$ vs. partial $\eta^2_{precise} = .595$, respectively). The

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[3] A summary of the assumption checks for the SCR analysis can be found in the appendix.

Figure 2

Means of Fear Potentiated Startle for No Contingency, General Contingency, and Precise Contingency

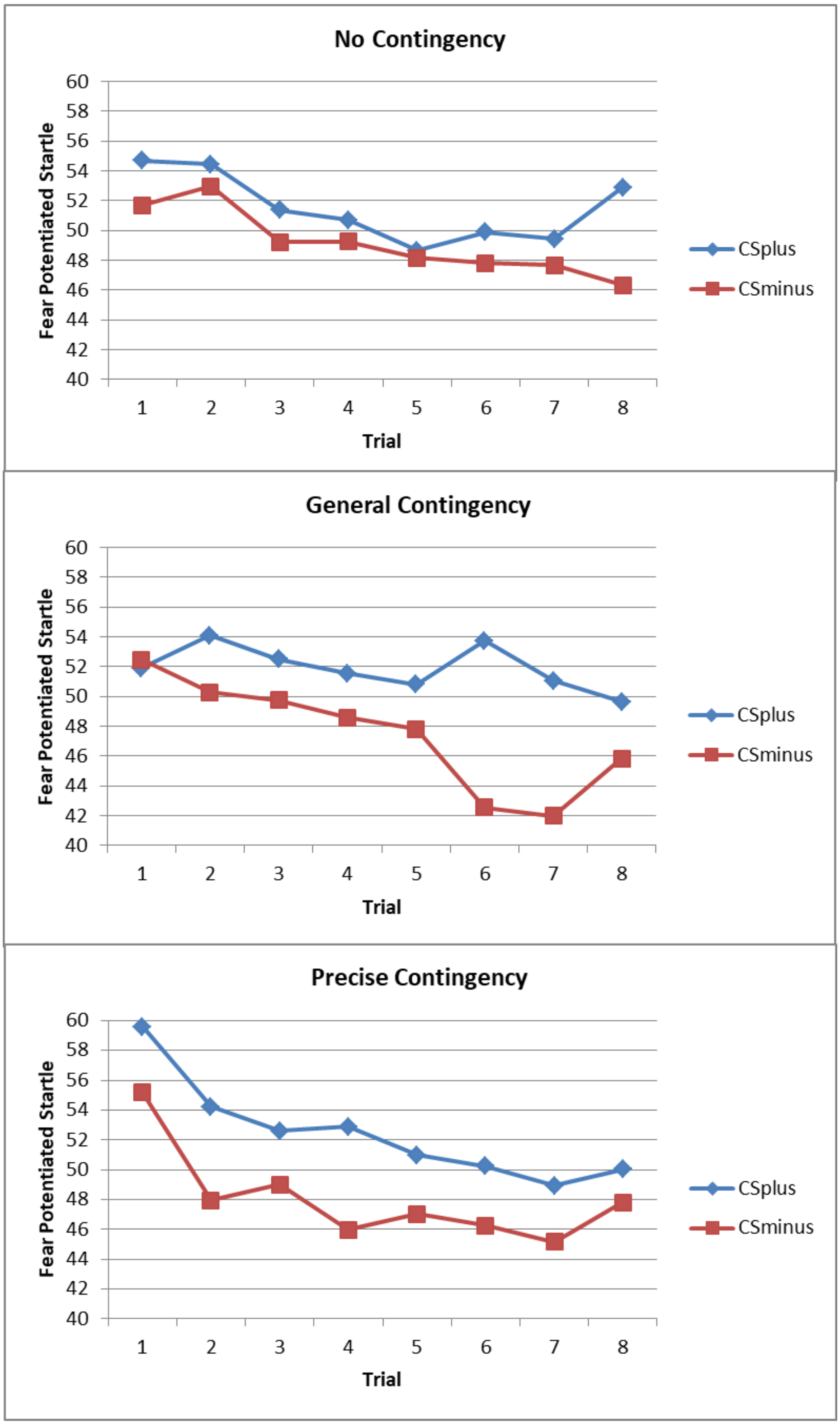

Figure 3

Means of Skin Conductance Response for No Contingency, General Contingency, and Precise Contingency

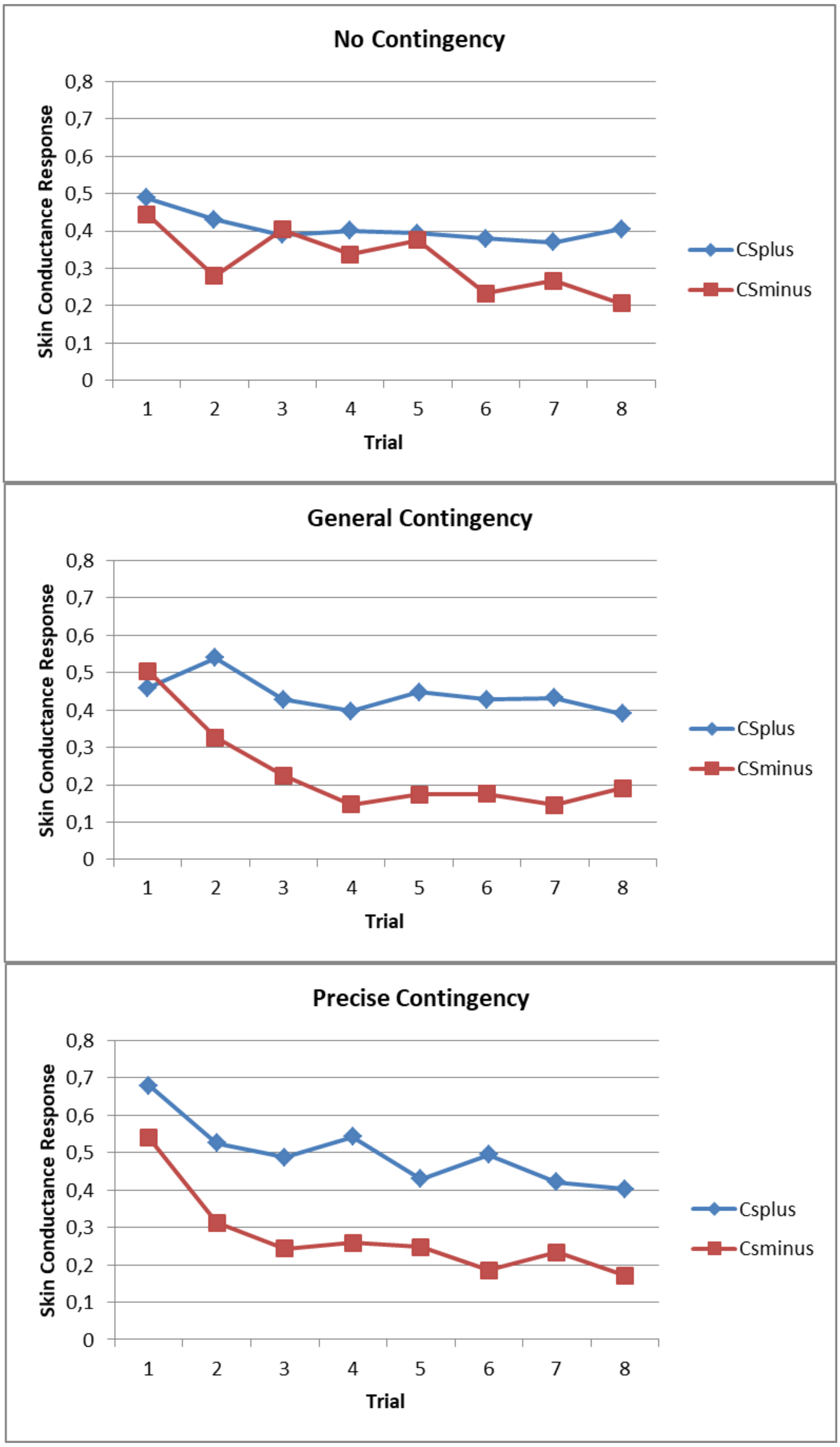

Table 2

Mean ratings of shock pain, shock intensity, STAI, CSI, IUS, and number of contingency aware individuals in each contingency group respectively and in total

Variable	No Contingency	General Contingency	Precise Contingency	Contingency Awareness	Total
STAI mean score	40,17	41,54	42,22	40,91	41,31
CSI mean scores	$52{,}61^1$; $49{,}64^2$	$52{,}03^1$; $49{,}33^2$	$50{,}82^1$; $48{,}85^2$	$52{,}05^1$; $49{,}78^2$	$51{,}82^1$; $48{,}79^2$
IUS mean score	30,14	30	30,31	29,23	30,15
Contingency awareness	22	31	27	/	80

Note. STAI = State-Trait Anxiety Inventory; CSI = Context Sensitivity Index (x^1 = score of cue presence, x^2 = score of cue absence); IUS = Intolerance of Uncertainty Scale; Number of Trials in Contingency Awareness Rating: N=8

mean skin conductance response when no verbal instructions were provided was 0.3186 (*SD* = 0.3188) for the CS- and 0.4075 (*SD* = 0.3514) for the CS+; it was 0.2361 (*SD* = 0.2722) for the CS- and 0.4402 (*SD* = 0.3394) for the CS+ when general instructions were given; and in the group provided with precise instructions the mean was 0.2737 (*SD* = 0.2863) for the CS- and 0.4976 (*SD* = 0.3379) for the CS+ (all depicted in Figure 3).

3.4 STAI, CSI, IUS

A one-way ANOVA was conducted to determine if differences in anxiety, context sensitivity, and intolerance uncertainty exist between different contingency groups. There was homogeneity of variances, as assessed by Levene’s test of homogeneity of variances ($p > .05$).

The mean differences between the different contingency groups were not statistically significant, $F(2, 99) = .015$, $p = .985$. A summary of the mean scores of each questionnaire for each contingency group respectively can be found in Table 2.

Discussion

The current study investigated whether verbal instructions have an effect on conditioned fear responses. A hypothesis was posed that verbal instructions have an effect on the FPS and on the SCR, which would then be elevated during the presentation of the CS+. Conditioned fear response was measured by FPS and SCR. In addition, participants were questioned to determine their contingency awareness and their certainty regarding their fear conditioned response after the conditioning phase. The results demonstrated that for the FPS, conditioning was more outspoken in the general and precise contingency instruction than in the no contingency instruction. Furthermore, the findings suggested that the effect of the CS on the SCR depended on the specific contingency group. More precisely, information about the CS-type influenced the SCR for all three contingency groups, but the largest effect could be observed in the precise contingency group. However, effect sizes of the general and of the precise contingency groups were similar. In addition, participants were mostly contingency-aware in the general instructions group, followed by the precise contingency group. Further, the results of an additional one-way ANOVA showed that anxiety level of participants, as assessed by the STAI, CSI, and IUS, did not differ significantly between contingency groups.

FPS

The results for FPS suggest that general verbal instructions influence acquisition learning of fear. Even though the degree of precision of verbal instructions did not seem to make a significant difference for the effect on the FPS, this observation partly supports the findings of previous research (Lonsdorf et al., 2017; Mertens et al., 2018). Additionally, the observation that the FPS for the CS+ and the CS- were approximately equal in the general contingency group, but were higher for the CS+ in the precise contingency group, could confirm the suggestion that verbal instructions about the CSs increase acquisition rate and acquisition strength (Atlas et al., 2016; Field & Storksen-Coulson, 2007; Ugland et al., 2013). However, it should be kept in mind that the obtained data about the precise contingency was non-significant. A possible explanation for the finding that general, but not precise instructions influenced the FPS might consist of the field of application for FPS. Currently, it is debated whether FPS can be considered to measure automatic affective learning (Blair, Schafe, Bauer, Rodrigues & LeDoux, 2001; Lipp & Purkis, 2005), or whether it is sensitive to verbal instructions (Mertens & De Houwer, 2017). As the FPS was suggested to be sensitive to verbal instructions only recently, there is still uncertainty about the effects of varying specificity of verbal instructions. Therefore, the effect of general, but not precise instructions on FPS might also depend on the FPS's characteristics.

SCR

Results obtained for the effect on the SCR were in line with the hypothesis. Additionally, with greater specificity of the verbal contingency, the SCR increased in response to the CS+. These observations support the results of Lonsdorf and others (2017) and Mertens and collegaues (2018) that verbal instructions have an effect on fear acquisition. Further, the presence of a higher SCR during presentation of the CS+ from onwards the first trial in the precise contingency suggests that precise instructions about the CSs lead to faster and more strongly acquired fear, as already proposed (Atlas et al., 2016; Field & Storksen-Coulson, 2007; Ugland et al., 2013).

To include or to not include instructions

These findings suggest that verbal instructions are an important part of fear acquisition. However, previous research suggests that not providing instructions enables experience-based associative learning and, further, increases the variance in conditioned responding (Lonsdorf et al., 2017). Therefore, not providing instructions might be beneficial when the objective of a study consists of gathering information about experience-based associative learning or an increased variance in conditioned responding. However, the resulting data should be interpreted with caution in such cases because it is simply not possible to conduct human research without verbal instructions. Further, Olsson and Phelps (2004) state that verbal instructions lead to a lower expression of conditioned fear compared to vicarious learning and the use of masked CSs. As in this study, verbal instructions were not compared with masked stimuli and/or vicarious learning. Consequently, no conclusions for these types of stimuli can be drawn. However, the present study shows that verbal instructions have a significant effect on fear conditioning, even though it is possible that this effect might be lower than the effect of other acquisition methods. Luck and Lipp (2016) propose that fear-relevant CSs do not seem to be responsive to instructed extinction, as well as fear conditioned extinction with intense electric stimuli. This finding might be partly explained from an evolutionary perspective stating that biological preparedness for some stimuli (e.g. heights, stimuli associated with pain) exists (Seligman, 1971).

Strengths & Limitations

Strengths of this study included the measurement of conditioned fear responses via two measurement methods, namely FPS and SCR. Thereby, the fear potentiated response was reliably measured. Next to automatic/implicit reactions, questioning participants regarding their contingency awareness further enabled to collect information about their conscious cognitive implementation of the verbal instructions. Additionally, an explicit focus was put on the effect of verbal instructions by including three different conditions with varying degrees of specificity in verbal instructions.

There are several limitations to this study that should be acknowledged. A first limitation consists of the non-evolutionary significance of stimuli. Further, many participants were not native English speakers which might have led to unnoticed misunderstandings when filling out questionnaires or during the work-up procedure. Furthermore, the findings do not allow drawing conclusions about the effect of verbal instructions on extinction, which could be a concern for future research.

Additional topics for future research could be a replication of the findings including an examination of the underlying mechanism causing the non-evolutionary significance of stimuli. Additionally, methods to assess the language proficiency of participants could be included before the experimental procedure.

Conclusion

In short, it can be concluded that fear conditioning of the startle reflex and skin conductance response are dependent on verbal instructions, even though the degree of specificity of the instructions might have a varying influence. In addition, verbal instructions should be included in the methodological sections of scientific articles, as they influence a study’s results.

References

American Psychological Association. 8.02 Informed Consent to Research [website article]. Retrieved 5th of November, 2019, from https://www.apa.org/ethics/code/index

Atlas, L. Y., Doll, B. B., Li, J., Daw, N. D., & Phelps, E. A. (2016). Instructed knowledge shapes feedback- driven aversive learning in striatum and orbitofrontal cortex, but not the amygdala. *ELife 2016;* 5:e15192. https://doi.org/10.7554/eLife.15192

Blair, H.T., Schafe, G.E., Bauer, E.P., Rodrigues, S.M., LeDoux, J.E. (2001). Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learning & Memory, 8*(5), 229-242. http://dx.doi.org/10.1101/lm.30901

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & van Boxtel,A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology, 42*(1), 1–15. https://doi-org.proxy.library.uu.nl/10.1111/j.1469-8986.2005.00271.x

Bonanno, G. A., Maccallum, F., Malgaroli, M., & Hou, W. K. (2018). The Context Sensitivity Index (CSI): Measuring the ability to identify the presence and absence of stressor context cues. *Assessment, 27*(2), 261-273. https://doi-org.proxy.library.uu.nl/10.1177/1073191118820131

Bordens, K. S., & Abbott, B. B. (2011). *Research design and methods: A process approach* (9th ed.). New York, NY: McGraw-Hill Education.

Carleton, R.N., Norton, M.A.P.J., Asmundson, G.J.G (2007). Fearing the unknown: A short version of the Intolerance of Uncertainty Scale. *Journal of Anxiety Disorders, 21*(1), 105-117. https://doi.org/10.1016/j.janxdis.2006.03.014

Dawson, M. E., Schell, A. M., Filion, D. L., & Berntson, G. G. (2017). The electrodermal system. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Cambridge handbooks in psychology. Handbook of psychophysiology* (p. 217–243). Cambridge University Press.

Field, A. (2005). *Discovering Statistics using SPSS* (2nd ed.). London: SAGE.

Field, A. & Storksen-Coulson, H. (2007). The interaction of pathways to fear in childhood anxiety: A preliminary study. *Behaviour Research and Therapy, 45*(12), 3051-3059. https://doi.org/10.1016/j.brat.2007.09.001

Kim, J.J., Jung, M.W. (2018). Fear paradigms: The times they are a-changin'. *Current Opinion in Behavioral Sciences, 24*, 38-43. https://doi.org/10.1016/j.cobeha.2018.02.007

Lipp, O.V., Purkis, H.M. (2005). No support for dual process accounts of human affective learning in simple Pavlovian conditioning. *Cognition & Emotion, 19*(2), 269-282. http://dx.doi.org/10.1080/02699930441000319

Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., Merz, C. J. (2017). Don't fear ' fear conditioning ': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. *Neuroscience & Biobehavioral Reviews, 77*, 247-285. https://doi.org/10.1016/j.neubiorev.2017.02.026

Luck, C. C., & Lipp, O. V. (2016). Instructed extinction in human fear conditioning: History recent developments, and future directions. *Australian Journal of Psychology, 68*(3), 209-227. https://doi.org/10.1111/ajpy.12135

Mertens, G., & De Houwer, J. (2017). Can threat information bias fear learning? Some tentative results and methodological considerations. *Journal of Experimental Psychopathology, 8*(4), 390-412. https://doi.org/10.5127/jep.060616

Mertens, G., Boddez, Y., Sevenster, D., Engelhard, I. M., & De Houwer, J. (2018). A review on the effects of verbal instructions in human fear conditioning: Empirical findings, theoretical considerations, and future directions. *Biological Psychology, 137*, 49-64. https://doi.org/10.1016/j.biopsycho.2018.07.002

Olsson, A., & Phelps, E. A. (2004). Learned fear of "unseen" faces after pavlovian, observational, and instructed fear. *Psychological Science, 15*(12), 822-828.

https://doi.org/10.1111/j.0956-7976.2004.00762.x

Pineles, S. L., Orr, M. R., & Orr, S. P. (2009). An alternative scoring method for skin conductance responding in a differential fear conditioning paradigmwith a long-duration conditioned stimulus. *Psychophysiology*, *46*(5), 984–995. https://doi-org.proxy.library.uu.nl/10.1111/j.1469-8986.2009.00852.x

Seligman, M. E. (1971). Phobias and preparedness. *Behavior Therapy*,*2*(3), 307-320. https://doi.org/10.1016/S0005-7894(71)80064-3

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Ugland, C. C. O., Dyson, B. J., & Field, A. P. (2013). An ERP study of the interaction between verbal information and conditioning pathways to fear. *Biological Psychology,* *92*(1), 69-81. https://doi.org/10.1016/j.biopsycho.2012.02.003

World Medical Association (2018, July 9). WMA Declaration of Helsinki - Ethical Principles for Medical Research involving Human Subjects. Retrieved from https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

Appendix

Exclusion criteria

Exclusion criteria consisted of current psychiatric, neurological, or other medical problems/diagnoses (e.g. epilepsy or heart disease), pregnancy, prior participation in studies involving electrical stimulation, the use of medication that influences attention, responsiveness, memory or concentration, and having an electronic implant (e.g. a pacemaker).

FPS

FPS scores were normally distributed in most cases, as assessed by Shapiro-Wilk's test ($p > .05$). Because of the central limit theorem, however, the three-way mixed ANOVA is robust against a violation. Homogeneity of variances, as assessed by Levene's test for equality of variances ($p > .05$), was present except for the variables T_CSmin1, T_CSmin8, and T_CSplus6. However, group sizes of the different contingency groups were approximately equal ($N_{no\_instruction} = 34$; $N_{general\_instruction} = 33$; $N_{specfic\_instruction} = 35$), so the three-way mixed ANOVA was robust against this type of violation. For the three-way interaction effect, Mauchly's test of sphericity indicated that the assumption of sphericity was met, $\chi^{2(27)} = 23{,}174$, $p = .676$.

SCR

SCR scores were not normally distributed in most cases, as assessed by Shapiro-Wilk's test ($p < .05$). Because of the square root transformation that took place before the actual data analysis, a normal distribution of the SCR data can be assumed nonetheless. In addition, the three-way mixed ANOVA is robust against a violation because of the central limit theorem. Homogeneity of variances, as assessed by Levene's test for equality of variances ($p > .05$), was present except for the variables SQRT_CSmin4 and SQRT_CSmin7. However, group sizes of the different contingency groups were approximately equal ($N_{no\_instruction} = 34$; $N_{general\_instruction} = 33$; $N_{specific\_instruction} = 35$), so the three-way mixed ANOVA was robust against this type of violation.

The assumption of sphericity was met, as assessed by Mauchly's test of sphericity, $\chi^{2(27)} = 34{,}571$, $p = .150$.