**Impact of Acute Stress Exposure on Reactivity to   
Loss of Control Over Threat**

Michalina Dudziak1,4, Tom Smeets2, Bram Vervliet3,4, and Tom Beckers1,4

1Centre for the Psychology of Learning and Experimental Psychopathology, KU Leuven, Belgium.

2Department of Medical and Clinical Psychology, Tilburg University, the Netherlands.

3Laboratory of Biological Psychology, KU Leuven, Belgium.

4Leuven Brain Institute, KU Leuven, Belgium.

**Author Note:**

Correspondence concerning this article should be addressed to Tom Beckers, Tiensestraat 102 - bus 3712, 3000 Leuven, Belgium.

Email: [tom.beckers@kuleuven.be](mailto:tom.beckers@kuleuven.be)

**Abstract**

Uncontrollable negative events yield increased stress responses compared to situations over which we have control. Previous studies have assessed the impact of uncontrollability of threat on stress reactivity. Less is known about whether and how acute stress exposure influences how we react to uncontrollable threats. Until now, research has primarily focused on investigating the lack of control despite the idea that losing control may cause greater distress and be more clinically relevant. The current study aims to investigate whether acute stress exposure impacts reactivity to a subsequent loss of control over threat. **One hundred twenty-eight** participants will be equally and randomly allocated to a stress or a no-stress group. Participants will undergo an acute stress induction or a non-stressful procedure, followed by a behavioral loss-of-control task. The loss-of-control task is designed to effectively induce control followed by a subsequent loss of control over aversive electrical stimulation. We hypothesize that participants exposed to acute stress will show stronger biological and psychological responses to the loss of control over threat than those in the no-stress group, as expressed in salivary cortisol and salivary alpha-amylase assays, blood pressure measurements, and self-report ratings. Additionally, we will assess biological sex, general perceived stress, and childhood adversity as factors that might moderate the relation between acute stress exposure and reactivity to loss of control. Investigating the sensitizing effect of acute stress on the reaction to a loss of control could offer valuable insights into their role in the development and maintenance of anxiety and stress-related disorders.

*Keywords:* loss of control, cortisol, sAA, perceived stress, acute stress, stress sensitization

**Introduction**

In everyday life, we face numerous stressful situations. How we respond to a stressor depends on its characteristics, such as its duration, severity, predictability, and controllability (Cohodes et al., 2021; De Raedt & Hooley, 2016; Lindau et al., 2016; Mineka & Hendersen, 1985). Controllability over threat was first defined in the learned helplessness literature as an actual or perceived ability to control an aversive event or internal state (Maier & Seligman, 1976). It has been proposed that uncontrollable negative events are perceived as more challenging than controllable negative events (Foa et al., 1992). Specifically, previous research recognized that uncontrollable experiences lead to adverse consequences such as passivity, negative affect, poor cognitive performance, and heightened stress responses (Bollini et al., 2004; Henderson et al., 2012; Maier & Seligman, 1976). Moreover, rodent research indicates that uncontrollability increases vulnerability to future stress, while having control over threats lessens the impact of subsequent stressors (Amat et al., 2010; Lucas et al., 2014; Maier, 2015).

Until now, research has focused on the link between threat (un)controllability and stress reactivity from a unidirectional perspective, looking at the impact of threat (un)controllability on stress responses (Agrigoroaei et al., 2013; Bollini et al., 2004; Isowa et al., 2006; Meine et al., 2020; Müller, 2011). In general, uncontrollable situations induce higher cortisol levels than controllable situations (Dickerson & Kemeny, 2004). However, the significance of perceived and situational (un)controllability and their influence on stress responses differ across studies. For instance, one study has found that the cortisol response after an acute stress task was correlated with the levels of general control beliefs and self-esteem (Pruessner et al., 2005). Other studies reported higher cortisol levels when there was a conflict between the expectation of control and the experience of uncontrollability (Agrigoroaei et al., 2013; Bollini et al., 2004). Specifically, participants with high prior control beliefs showed increased cortisol responses to a low controllability task compared to those with prior lower control beliefs (Agrigoroaei et al., 2013). Lastly, some studies failed to find any effect of uncontrollability on stress reactivity (Isowa et al., 2006; Peters et al., 2003).

While discrepancies emerge regarding the impact of threat (un)controllability on stress responses, even less is known about how exposure to stressful situations might influence the later reaction to uncontrollable threats. A study by Bhanji and colleagues (2016) found that acute stress can impair behavioral persistence when participants face uncontrollable setbacks. However, the perception of control over the setback protected against adverse consequences of prior stress and encouraged persistence. Other research has shown that acute stress exposure can impact subsequent fear learning (Jackson et al., 2006; Merz et al., 2013; Merz & Wolf, 2017; Peyrot et al., 2020; Simon-Kutscher et al., 2019). For instance, a review by Raio and Phelps (2015) stated that exposure to acute stress facilitates cued fear learning, making the fear memory more resistant to later extinction. Furthermore, it has been reported that experiencing acute stress leads to higher cortisol concentrations during subsequent fear conditioning (Merz et al., 2013). A body of work has also focused on how the modulatory effects of stress on fear learning depend on sex (Jackson et al., 2006; Merz & Wolf, 2017). Based on a review by Peyrot and colleagues (2020), exposure to acute stress impacts fear learning differently in men and women. Specifically, in men, acute stress led to heightened fear acquisition on a physiological level (e.g., higher skin conductance responses to threatening cues). In contrast, the impact of stress on fear acquisition in women was inconsistent between studies. For instance, a study by Jackson et al. (2006) showed that exposure to stress inhibited fear learning in women but facilitated it in men. Riggenbach et al. (2019) found that sex differences in fear conditioning after stress are expressed differently in different measures of fear. For example, stress exposure enhanced fear-potentiated startle to fearful stimuli in both men and women, whereas it reduced US-expectancy ratings in men but not in women. Based on the findings discussed above, we speculate that since exposure to stress affects how we acquire fear and respond to threats, it may also influence how we perceive and respond to the uncontrollability of future threats.

The feeling of lacking or losing a sense of control is a feature of many stress-related disorders, including PTSD (Ehlers & Clark, 2000; Hancock & Bryant, 2020). The cognitive model of PTSD (Ehlers & Clark, 2000) proposes that maladaptive appraisals and beliefs related to a traumatic experience (e.g., an ongoing sense of uncontrollability) might generalize to other domains of life, promoting emotional distress and avoidance. As a result, excessive avoidance behavior might prevent engagement with trauma-related cues, thus reducing the capacity to process them and to update maladaptive beliefs (Ehlers & Clark, 2000). Support for this model comes from a study by Hancock and Bryant (2018) in which females with PTSD symptoms who were previously told they lacked control over aversive images exhibited greater avoidance compared to women who were led to believe that they could control the disappearance of those images. Furthermore, research has shown that appraising PTSD symptoms as uncontrollable and reporting negative beliefs about personal control is negatively related to treatment outcomes (Hancock & Bryant, 2018; Livanou et al., 2002). Given that lack of perceived control is a vulnerability factor across anxiety disorders (Gallagher et al., 2014), investigating a sensitizing effect of stress on loss of control could offer valuable insights into their role in the development and maintenance of anxiety and stress-related disorders.

The main aim of the current study is to investigate whether acute stress exposure impacts reactivity to a subsequent loss of control over threat. Previous research on threat (un)controllability has mostly compared a complete lack of control to a situation of continuous control (Bollini et al., 2004; Diener et al., 2009; Havranek et al., 2016; Isowa et al., 2006; Müller, 2011). However, a sudden loss of control can cause even more distress than an enduring lack of control and may, moreover, be more clinically relevant (Hancock & Bryant, 2020; Mineka & Kihlstrom, 1978; Yao et al., 2019). One rodent study found that mice in a loss-of-control group exhibited more pronounced helplessness than mice in a lack-of-control group (Yao et al., 2019). In a human study by Hancock and Bryant (2020), women with PTSD symptoms who experienced a loss of control over an aversive noise showed more avoidance behavior than those without control or not exposed to the noise. This observation suggests that a severe stressor, for example, a traumatic event may increase sensitivity to the negative effects of a loss of control.

To investigate the impact of acute stress on the response to a loss of control over threat, we will make use of a between-subjects design and equally randomize 128 participants (*N* = 64 participants per group) to one of two groups - stress versus no-stress. During the 120-minute session, participants will undergo an acute stress induction or a non-stressful control procedure, followed by a behavioral loss-of-control task. The behavioral loss-of-control task is designed to first effectively induce control followed by a loss of control over aversive electrical stimuli. Throughout the session, we will obtain saliva samples to measure salivary cortisol and salivary alpha-amylase (sAA), record blood pressure, and collect self-report ratings of experienced stress, uncontrollability, and predictions of control. We hypothesize that acute stress will amplify the negative effects of losing control over threats. Specifically, we expect participants in the stress group to show higher biological (cortisol and sAA levels), physiological (blood pressure), and perceived (ratings) stress responses in the loss-of-control task compared to participants from the no-stress group (hypothesis 1; H1). Moreover, we predict that participants exposed to the acute stress procedure will report higher perceived uncontrollability in the loss-of-control task (hypothesis 2; H2) and expect less control after the loss-of-control task (hypothesis 3; H3) than participants who did not experience prior stress. Finally, we expect that participants in the acute stress group will report higher perceived fear in response to threat-predictive cues (CS fear) upon the loss of control than participants in the no-stress group (hypothesis 4; H4).

Several studies have shown that men exhibit higher cortisol levels in response to acute stress than women (Bourke et al., 2012; Carr et al., 2016; Henderson et al., 2012; Kelly et al., 2008). However, it has also been shown that women are more likely to appraise events as threatening and report higher distress when losing control than men (Eisler & Skidmore, 1987; Olff, 2017). Based on these findings, in the current study we will also investigate sex differences in stress responses and their potential impact on differences in responding to a loss of control. Our own unpublished results show that women who experienced a loss of control reported higher perceived stress than men. It is thus plausible that sex differences in responses to acute stress exposure might amplify sex differences in the loss-of-control task. Based on the literature and our unpublished findings, we expect that men in the stress group will show higher salivary cortisol levels, sAA, and blood pressure during the loss-of-control task compared to women in the stress group (hypothesis 5a; H5a). On the other hand, we predict that women in the stress group will report higher perceived stress during the loss-of-control task than men in the stress group (hypothesis 5b; H5b).

In addition to our main hypotheses (H1-H5), we will investigate secondary hypotheses (H6-H8) on how individual differences may shape the relationship between acute stress exposure and reactivity to loss of control. Specifically, we want to assess the role of real-life perceived stress and preexisting childhood adversity on how acute stress exposure shapes responses to a loss of control over threat.

An individual’s daily-life stress might influence their experience of acute stressors (Liston et al., 2009). Stawski et al. (2008) reported that high global perceived stress was associated with greater reactivity to stressors. Another study showed that perceived chronic stress interacted with participant's response to acute stress exposure and, in turn, influenced their cognitive flexibility (Knauft et al., 2021). Lempert and colleagues (2012) also recognized the importance of global perceived stress by showing that individuals with high versus low perceived stress made different choices regarding rewards when faced with acute stress. Moreover, on a biological level, higher levels of perceived stress over the past month were associated with lower cortisol concentrations at baseline in a study by Obasi and colleagues (2017). In summary, this evidence suggests that global perceived stress may influence the impact of acute stress exposure on the reaction to a subsequent loss of control. In the current study, we will evaluate the association between general perceived stress over the past month and acute perceived stress experienced in the acute stress induction procedure and the loss-of-control task. We hypothesize that participants with higher general perceived stress scores, as measured with the Perceived Stress Scale (PSS-10; Cohen & Williamson, 1988), will report higher perceived stress in response to the acute stress induction procedure (hypothesis 6a; H6a), and the loss-of-control task (hypothesis 6b; H6b). Additionally, we hypothesize that perceived stress in response to the lab-induced stressful event will mediate a relationship between the general perceived stress (measured by PSS-10) and the perceived stress reported in the loss-of-control task (hypothesis 7; H7).

According to a meta-analysis, individuals with early-life adversities show a blunted cortisol response to acute social stress (Bunea et al., 2017). The largest difference in cortisol levels between people with and without childhood adversities appears to occur at the peak or in the recovery phase of an acute stress procedure rather than at the baseline. A study by Kuras et al. (2017) found a positive association between sAA reactivity and childhood physical abuse. Healthy adults with low-to-moderate childhood adversity showed increased sAA levels following stress exposure. On the other hand, one study showed that as the severity of childhood maltreatment increased, so did the cortisol level in response to a psychosocial acute stress task (Ouellet-Morin et al., 2019). Another study by LoPilato and colleagues (2019) found that threat exposure in childhood was associated with heightened stress perception in adult females. We will evaluate whether childhood adversity is associated with stress responses in an acute stress task and subsequent responses to a loss of control in healthy adults. Specifically, we hypothesize that participants with higher childhood adversity, as measured with the Childhood Trauma Questionnaire - Short Form (CTQ-SF; Bernstein et al., 2003), will report higher perceived stress in response to the acute stress induction procedure (hypothesis 8a; H8a), and the loss-of-control task (hypothesis 8b; H8b). Due to discrepancies in findings (Raymond et al., 2021), we refrain from making a directional hypothesis regarding the association between childhood adversity and biological stress markers such as cortisol or sAA.

Investigating the impact of acute stress on the reaction to a loss of control over threat will shed more light on the directionality of the relation between threat (un)controllability and stress reactivity. Furthermore, assessing potential modulating factors such as biological sex, general perceived stress, and childhood adversity will enhance our understanding of how acute stress influences loss of control over threats and help extend our laboratory findings into real-world contexts.

**Materials and Methods**

**Transparency and Openness**

The aim, hypotheses, measures, study design, and data analysis plan will be preregistered on the Open Science Framework (OSF) before the start of data collection. The study will be supported by a Doctoral Fellowship of the Research Foundation – Flanders (FWO) [Michalina Dudziak, grant number 11PHG24N] and a KU Leuven Research Grant (C16/19/02) awarded to Tom Beckers and Bram Vervliet. The study protocol was approved by the Ethics Committee Research (EC Research) UZ/KU Leuven (number: S69431).

**Participants**

***Sample Size Justification***

We will recruit 128 participants (*N* = 64 per group, 32 per biological sex) based on an a-priori sample size calculation (power (1 – β) set at 0.80, α = .05, *f* = 0.15). No prior study has examined the impact of lab-induced acute stress on reactivity to loss of control. Therefore, we based our effect size estimate on a study by Bhanji et al. (2016), who found that exposure to lab-induced acute stress decreased persistence in an uncontrollable setback condition compared to a no-stress control condition, *t*(77) = - 1.81, *p* = .037 (one-tailed), *d* = 0.41. Given that effect sizes for between-within interaction effects tend to be smaller than for main effects, we adjusted the effect size from Bhanji et al. (2016) downward, yielding an expected small-to-medium effect size (*f* = 0.15) for the present study. The sample size calculation was conducted for a within-between interaction in a repeated-measures ANOVA, using G\*Power software 3.1 (Faul et al., 2007). The calculation yielded a required sample size of 128 individuals. Excluded participants will be replaced until the predetermined sample size of 128 participants is reached.

***Exclusion and Inclusion Criteria***

We will include healthy adult volunteers (≥ 18 years old) who provide written informed consent to participate in this study. All included individuals must have an adequate command of Dutch or English.

To be eligible for this study, participants **must not**meet any of the following criteria:

• being color blind (due to the use of colorful pictures as stimuli),

• having participated in previous studies on loss of control (to prevent any familiarity with the behavioral loss-of-control task),

• presenting a cardiovascular, endocrine, or neurological disorder,

• presenting current psychopathology (e.g., depression, anxiety disorders),

• having hypertension (baseline blood pressure of 140/90 mmHg or higher),

• being pregnant,

• having been recommended (e.g., by a physician) to stay away from stressful situations,

• having acute and/or chronic pain in the hand and/or forearm,

• presence of an electronic implant (i.e., pacemaker),

• working on night shifts,

• having a repeated history of fainting,

• regularly using drugs and/or smoking more than 5 cigarettes per day,

• taking any medication directly related to cardiac, emotional, or cognitive function or that can influence hormonal levels, such as glucocorticoids or β-blockers (intake of hormonal contraception is allowed).

Participants who meet one or more of the above exclusion criteria will not be eligible for the experiment. Furthermore, participants must follow strict guidelines (see below) to allow reliable salivary cortisol and alpha-amylase (sAA) measurement. These guidelines will be clearly communicated to participants before the testing session. A short questionnaire will be administered at the beginning of the testing session to assess whether participants adhered to the stated guidelines. Only participants who followed the stated guidelines will be included in the study.

1. The day before participation:

• No alcohol consumption in the evening (after 7 pm).

• Not going to sleep after 1 am.

2. On the day of participation:

• No alcohol consumption.

• No food intake at least 2 hours before the participation.

• No beverage consumption at least 2 hours before the participation (except non-sparkling water, which is always allowed).

• No smoking for at least 2 hours before the participation.

• No teeth brushing for at least 2 hours before the participation.

• No chewing gum for at least 2 hours before the participation.

• No strenuous physical activity for at least 2 hours before the participation.

Participants who report feeling unwell during the stress induction procedure will be asked to withdraw from the current study.

***Randomization Procedure for Conditioned Assignment***

Each participant will be assigned to one of the two groups (stress vs no-stress). The assignment order will be pseudorandomized using the "=RAND()" function in Excel. First, the Excel list will contain four columns including biological sex (*N* = 64 females and 64 males), group (*N* = 64 stress versus 64 no-stress), and counterbalancing conditions (*N* = 2 distributed equally between 128 participants). In the last column, we will enter the formula =RAND(), which will then generate a random decimal number between 0 and 1 for each row. All the columns will be then sorted by the column with the random numbers in ascending order, creating a random and equal assignment of biological sex and counterbalancing condition to each group. Lastly, the column of participant numbers (P01-P128) will be added.

***Counterbalancing and Yoking Procedure***

To avoid stimulus bias, participants from both groups will be pseudorandomly and equally assigned to two counterbalancing conditions. Each condition contains a combination of a picture (office room) and the color of lamp (yellow versus red) paired with electrical stimulation, yielding the following two counterbalancing conditions: (1) office room x yellow lamp CS+; and (2) office room x red lamp CS+. In the second part of the computer task (trials 13-24), participants will receive a duration of electrical stimulation matched to the duration of the electrical stimulus experienced by a participant in the continuous control group of our unpublished study. In that study, participants from the continuous control group could terminate the electrical stimulus throughout the whole task. We will choose four participants (one female and one male from two counterbalancing condition) from the previous continuous control group to match (yoke) the duration of their electrical stimuli to our participants. Matching will be done across biological sex. We will ensure that each group (stress versus no-stress) contains an equal number of each counterbalancing conditions and yoked participants.

**Manipulation**

***Maastricht Acute Stress Test (MAST)***

The MAST procedure (Smeets et al., 2012) will be conducted in stress and no-stress control versions. The stress version of MAST will be used to elicit experiential, biological, and physiological stress responses. This manipulation consists of approximately a 5-minute preparation and a 10-minute acute stress phase (Smeets et al., 2012). In the preparation phase, participants are seated before the computer and provided instructions through an on-screen presentation. Participants are informed that they will be asked to immerse their non-dominant hand (including their wrist) in ice-cold water (0-4°C) for multiple trials. The duration of each trial will never exceed 90 seconds. In between hand immersion trials, participants are instructed to take their hand out of the water and start the mental arithmetic task that includes counting backward starting at 2043 in steps of 17 as fast and accurately as possible. Upon making a mistake, the experimenter provides them with negative feedback (e.g., "You made a mistake", "Count faster") and urges the participant to start over from the number 2043. The experimenter will instruct participants who make few or no errors in the mental arithmetic task to count faster. If necessary, the experimenter will further increase the difficulty of the task by asking participants to count in increments of different numbers, such as 13 or 33. Participants are told to continue counting until the computer signals the subsequent hand immersion trial, which is after a minimum of 45 seconds. Participants are also informed that they will be videotaped for the duration of the task and that these video recordings will be later used to analyze their facial expressions. In practice, no video recordings are collected. Furthermore, the order and duration of the hand immersion and mental arithmetic trials are predetermined for all the participants (see Figure 1). The MAST elicits robust self-reported, biological, and physiological stress responses in humans comparable to those observed in the Trier Social Stress Test (Smeets et al., 2012). In the no-stress version of the task, participants are asked to immerse their non-dominant hand into a tank containing lukewarm water (35-37 °C). In between the hand immersion trials, participants are asked to start counting consecutively and repeatedly from 1 to 25 at their own pace until the computer signals the start of the next trial. The experimenter remains in the room to check participants' compliance with the instructions, but no performance feedback is given during the task. The duration and order of the trials are similar to the one for the stress version (see Figure 1). The no-stress variant of MAST is procedurally similar to the regular MAST but does not elicit stress responses, making it a valid control condition (Smeets et al., 2012).

**Figure 1**

*Order and Duration (in Seconds) of Hand Immersion and Mental Arithmetic Trials in the MAST*

*A diagram of a music chart

Description automatically generated with medium confidence*

*Note.* Adapted from“Introducing the Maastricht Acute Stress Test (MAST): A quick and non-invasive approach to elicit robust autonomic and glucocorticoid stress responses” by T. Smeets, S. Cornelisse, C. W. E. M. Quaedflieg, T. Meyer, M. Jelicic, & H. Merckelbach, 2012, *Psychoneuroendocrinology*, *37*(12), 1998–2008. Copyright 2012 by Elsevier Ltd. Adapted with permission.

***Behavioral Loss-of-Control Task***

To induce a sense of loss of control, we developed a behavioral loss-of-control task. In this task, participants initially view an image of an office room with a lamp that is turned off. In the subsequent image, the lamp lights up in either yellow or red. After 7 seconds, one of these lamp colors (CS+) terminates with the delivery of a mild electrical stimulation (US), whereas the other lamp color (CS-) is never paired with the US. The US lasts for a maximum of 2.8 s. The electrical stimulation is delivered through two electrodes attached to the participant's forearm. Each participant selects the intensity of the stimulation individually before the start of the experiment via a gradual work-up procedure (US calibration procedure) to a level that is "clearly uncomfortable, but not painful". After the calibration procedure, the main task starts. Participants can terminate the electrical stimuli only during the first half of the trials (trials 1-12, including 6 trials with the electrical stimulus). The termination of the stimulus is possible from 300 ms after the onset of electrical stimulation onwards by clicking the correct button out of three buttons presented on the computer screen. At the onset of the electrical stimulus, participants are shown a progress bar that advances in parallel with the duration of the stimulus. Pressing the correct button terminates both the aversive stimulus and the progress bar. The disrupted progress bar visualizes that action (pressing the correct button) has a consequence in terminating the stimulus, to enhance the participants' sense of control. After 12 trials, participants suddenly lose the ability to stop the electrical stimulation (buttons and the progress bar are no longer available on the computer screen). From then on (trials 13-24), the durations of the electrical stimulation are matched trial-by-trial to a participant (female or male) who continued to be able to terminate the electrical stimulus in a previous study. To further increase the perceived feeling of losing control, participants are presented with a picture of the buttons crossed out with a red cross. Each time the CS+ or CS- is presented, participants assess the likelihood of the electrical stimulus's occurrence on a scale from 0 "certainly no electrical stimulus" to 100 "certainly an electrical stimulus". Before every 4 trials (2 CS+ and 2 CS- trials), participants are asked to what extent they expect to have control in the next part of the task (control expectancy) on a scale from 0 "no control" to 100 "complete control". Furthermore, after every 4 trials (2 CS+ and 2 CS- trials), participants rate how stressed they felt on a scale from 0 "not at all stressed" to 100 "very stressed" and how fearful they felt when the lamp color changed to yellow or red on a scale from 0 "not at all fearful" to 100 "very fearful". The general layout of a trial in the loss-of-control task is presented in Figure 2.

**Figure 2**

*Trial Flow of the Behavioral Loss-of-Control Task*

*A diagram of a process

Description automatically generated*

*Note.* CS = Conditioned Stimulus (red versus yellow lamp); US = Unconditioned Stimulus (electrical stimulation).

***Neutral Filler Task***

After the behavioral loss-of-control task, all participants will perform a neutral filler task for 15 minutes. In this task, participants will be asked to circle the correct neutral picture (e.g., a violin, a house, a kettle, a tree) in each row corresponding to the picture at the top of the page. The neutral filler task was compiled from a website [https://tinasblumenwiese.com](https://tinasblumenwiese.com/) that offers similar free materials. This task was chosen because it does not rely on language skills, making it suitable for participants who speak either English or Dutch. The task ensures comparability between different language groups. No data will be analysed from this task.

**Measures**

***Biological and Physiological Stress Measures***

Throughout the experiment, salivary cortisol and alpha-amylase (sAA) will be collected to measure stress-induced increases in cortisol and adrenergic activity in response to the MAST and the loss-of-control task. The biological measures will be obtained with Salivette® synthetic swabs (Sarstedt AG & Co., Nümbrecht, Germany) at specific time points (see Figure 3). The salivettes will be stored in a (-21°C) freezer before shipping to the laboratory (Dresden LabService GmbH) for processing. Furthermore, pulse, systolic (SBP), and diastolic (DBP) blood pressure will be measured at specific time points (see Figure 3) using a fully automated electronic blood pressure monitor (OMRON M2, n.d.), with a band applied around the dominant upper arm.

**Figure 3**

*Timeline of the Study with the Task Durations and Sampling Points of Biological and Physiological Measures*

A diagram of a process

Description automatically generated

***Self-Report Ratings***

**Experiences of the MAST Procedure.** After the MAST (t50), participants will be asked to indicate on a scale from 0 "not at all" to 100 "extremely", how stressful, painful, and unpleasant they experienced the MAST to be, and how relieved they felt when they got to remove their hand from the water for the final time on a scale from 0 "not at all relieved" to 100 "extremely relieved".

**CS Fear.** The fear elicited by the conditioned stimuli (CSs) will be assessed after each block of four trials (2 CS+, 2 CS-) of the loss-of-control task. Participants will rate how fearful they felt when the lamp color (CSs) changed to yellow or red on a scale from 0 "not at all fearful" to 100 "very fearful".

**Perceived Controllability.** At the end of the behavioral loss-of-control task (t75), participants will be asked to determine to what extent they had control over the task at the beginning and the end of the task on a scale from 0 "no control" to 100 "complete control". After the MAST procedure, participants will also be asked “To what extent did you feel in control during the water task? (on a scale from 0 "no control” to 100 "complete control").

**Perceived Stress Throughout the Experiment.** Participants will be asked to indicate how stressed they are on a scale from 0 ("not at all stressed") to 100 ("very stressed") at baseline (t30), after the MAST procedure (t50), right before the loss-of-control task (t60), after the loss-of-control task (t75) and after the neutral filler task (t95).

**Control Expectancy Throughout the Experiment.** Participants will be asked to rate to what extent they expect to have control in the next part of the experiment on a scale from 0 ("no control") to 100 ("full control"). These ratings will be collected at t10, t40, t50, t75, t95.

***Questionnaires***

**Perceived Stress Scale**(PSS-10; Cohen & Williamson, 1988; Cohen et al., 1983; Lee, 2012). The PSS-10 is a self-report scale assessing individuals' perceived stress levels over the past month (e.g., "In the last month, how often have you felt nervous and stressed?"). The questionnaire consists of 10 items assessed on a 4-point Likert scale ranging from 0 ("never") to 4 ("very often"). A higher score on the scale indicates a higher stress level. The original version includes 14 items, but the 10-item version showed improved scale reliability (Cohen & Williamson, 1988). A review by Lee (2012) reported that Cronbach's alpha across 19 studies exceeded the standard threshold of .70 and varied between .74–.91. The test-retest reliability was satisfactory for a 4-week interval (ICC = .72 - .88; Wongpakaran & Wongpakaran, 2010). A confirmatory factor analysis suggests that a 2-factor model (Perceived helplessness, Lack of self-efficacy) best describes the relationship between the items (Taylor, 2015). This study will use the original English version of the questionnaire and a translated Dutch version that has not been independently validated.

**Childhood Trauma Questionnaire - Short Form** (CTQ-SF; Bernstein et al., 1994, 2003). The CTQ-SF scale is a retrospective self-report measure used to assess five types of childhood adversity, namely physical abuse, sexual abuse, emotional abuse, physical neglect, and emotional neglect. It contains 28 items on a 5-point Likert scale from 1 ("never true") to 5 ("very often true"). The standard Cronbach's alpha above .70 reflects good internal consistency of the scale (He et al., 2019). However, it has been repeatedly reported that the physical neglect subscale has poorer internal consistency (Georgieva et al., 2021; Peng et al., 2023). Based on a confirmatory factor analysis, a 5-factor structure best explains the questionnaire (Georgieva et al., 2021). This study will use the original English version of the questionnaire and a translated Dutch version that is used by our faculty.

**General Self-Efficacy Scale** (GSE; Schwarzer & Jerusalem, 1995; Teeuw et al., 1994). The GSE is a 10-item scale that measures a general sense of perceived self-efficacy, which can be interpreted as global confidence in coping abilities in challenging or novel situations. Responses are recorded on a 4-point scale ranging from “not at all true” to “exactly true”. The questionnaire has been translated into 33 languages, each with good psychometric properties (Scholz et al., 2002). Confirmatory factor analysis supported a unidimensional structure of the GSE scale (Scholz et al., 2002). This study will use the validated English and Dutch versions of the questionnaire.

***Deception Assessment***

Prior to the debriefing, participants will be asked a few open- and closed-ended questions to assess their experiences of the deceptive tasks and how believable they found the deception used in the study. Some questions will vary depending on whether participants were in a stress or no-stress condition. Participants from the stress group will be asked the following questions: (1) “What was the purpose of video recording during the cold water task?”, (2) “How personal did you take the negative feedback from the experimenter during the cold water task?” (on a scale from 0 “not at all personal” to 100 “extremely personal”), and (3) “To what extent did you feel that you were failing the water task?” (on a scale from 0 "not at all" to 100 "extremely"). Participants from both groups will be asked these questions: (5) “Do you think the first task (water task) influenced your performance on the computer task?” (“yes/no”); (6a) (if the answer is “yes”) “How did it influence your performance?”; (6b) (if the answer is “no”) “Why do you think that?”.

**Procedure**

Participants will be able to able to sign up for the study through the Experiment Management System platform of the KU Leuven Faculty of Psychology and Educational Sciences. All experimental sessions will take place between 12:00 and 19:00 to reduce the impact of diurnal cortisol variation (Bos et al., 2014). Each testing session will last approximately 120 minutes. The timeline of the session, detailing the duration of tasks and the collection of various measures, is shown in Figure 3. Before the testing session, participants will be reminded to follow specific guidelines the day before and on the day of testing to avoid confounding effects of the cortisol awakening response (see "Exclusion and Inclusion Criteria" section). Participating females will be asked to recall the start date of their last menstruation, specify their menstrual cycle length, and indicate their use of oral contraceptives (yes/no/prefer not to say). These measures will be collected to account for their potential influence on stress responses. Upon arrival in the lab, participants will be reminded of the study objectives and measures. Then, they will be asked to give their informed consent to participate in the current study. After completing the listed documents, participants will fill out some demographic questions (age, gender, biological sex) and the questionnaires (PSS-10, CTQ-SF, GSE). Once the questionnaires are completed, the experimenter will measure blood pressure and collect a baseline saliva sample (t30). Following the baseline measurement, the stimulation electrode bar will be attached to the dominant forearm of a participant, and the experimenter will explain the US calibration procedure. In the US calibration procedure, participants will be individually given a low, nearly undetectable stimulation (1 mA) of a maximum duration of 2.8 s, which will gradually increase until the participant declares that the stimulation fits the description of being "clearly uncomfortable but not painful". Every time the pulse is delivered, participants will be asked to rate it on a scale from 0 to 10, where 0 means " I feel nothing" and 10 means "This is the maximum level I can tolerate". Once the stimulus level is chosen as "clearly uncomfortable but not painful", participants will be asked whether they want to try one stimulus higher with the possibility of always returning to the previous, lower stimulation. Each stimulus will be clearly announced before application. During the experiment, the intensity and length of the electrical stimulation will never exceed the intensity level chosen by participants during the US calibration phase. Following the US calibration procedure, participants will be introduced to the MAST instructions in either the stress or no-stress variant of the task. Participants assigned to the stress version of the MAST will be asked to sign a bogus informed consent form for the videotaping procedure. Saliva samples will be collected at the end of the MAST task (t50), along with the blood pressure measurement. Immediately after the task is finished, participants will report on a scale from 0 "not at all" to 100 "extremely" how stressful, painful, and unpleasant they experienced the MAST procedure. They will also assess their level of relief upon the completion of this task and how much in control they felt during the task. Subsequently, the experimenter will explain the behavioral loss-of-control task orally. Instructions will also be presented on the computer screen at the start of the task. Right before the start of the task, the experimenter will collect another saliva sample and measure the participant's blood pressure (t60). The task completion will be followed by the saliva sample collection and the blood pressure measurement (t75). The electrical stimulation bar will be detached from the forearm, and participants will be asked to perform a neutral filler task. During the filler task, two saliva samples will be taken at t85 and t95, along with the blood pressure measurements. After the last measurement, participants will be asked to inform the experimenter of any adverse events that may have occurred during the study. Then, participants will answer a series of questions to assess whether they believed in the deceptive elements of the study. Finally, they will be debriefed and thanked for their participation. In the debriefing, participants will receive an oral explanation about how stressful events can potentially sensitize us to the effects of losing control over threats. The experimenter will also explain the deceptive elements of the study, namely: (1) bogus videotaping in the stress protocol (MAST), (2) the predetermined order and duration of trials in the stress protocol (MAST), (3) negative feedback and a distant approach of the experimenter during the stress protocol (MAST), and (4) an unannounced loss of control in the computer task. Participants who underwent the stressful version of MAST will be reassured that the mental arithmetic task was intentionally designed to be extremely challenging and that the focus was solely on assessing their biological and perceived stress levels, not their arithmetic abilities. Participants will be able to ask additional questions and share their thoughts or concerns that might have arisen from the experimental procedures. After the debriefing, participants will receive referral information about healthcare professionals and specific places that offer support in case it is needed. Throughout the experiment, perceived stress and control prediction ratings will be collected at time points specified in the "Self-Report Ratings" section.

**Expected Duration of the Study**

Each participant will be invited to the lab once for a testing session lasting approximately 120 minutes. Based on our previous experience, we can test a maximum of 9 participants per week. However, the number of tested participants per week depends on lab access, participant availabilities, and available manpower. Data collection might be delayed due to the equal sex inclusion criterion (32 participants per biological sex per group). The expected duration of this experiment is about 12 months.

**Data Analysis**

***Statistical Analysis Plan***

To assess potential group differences in stress responses to the loss of control (H1), separate analyses will be conducted on salivary cortisol (H1a), sAA (H1b), blood pressure (H1c), and perceived stress (H1d). To answer H1a, we plan to conduct repeated-measures Analyses of Variance (rmANOVA) on salivary cortisol with group (stress versus no-stress) and sex-at-birth (female versus male) as between-subjects factors, and timepoints (t85, t95; see Figure 3) as a within-subjects factor. To analyze sAA, systolic and diastolic blood pressure (H1b, H1c), three separate rmANOVAs with group (stress versus no-stress) and sex-at-birth (females versus males) as between-subjects factors, and timepoints (t60, t75; see Figure 3) as a within-subjects factor will be conducted. To investigate potential group differences in perceived stress (H1d), another rmANOVA with group (stress versus no-stress) and sex-at-birth (females versus males) as between-subjects factors, and timepoints (t60, t75) as a within-subjects factor will be performed. We will explore potential differences in perceived controllability in the loss-of-control task (H2) by conducting a one-way ANOVA with group (stress versus no-stress) as an independent variable and perceived controllability as a dependent variable. To assess whether control expectations during the loss-of-control task are influenced by the prior experience of stress versus no-stress (H3), we will perform a one-way ANOVA with group (stress versus no-stress) as an independent variable and control expectancy prediction at t75 as a dependent variable. We will analyze differences in self-reported fear upon losing control over threat by conducting a rmANOVA on CS-fear ratings with group (stress versus no-stress) as a between-subjects factor, and block (6) and cue (CS + versus CS-) as within-subjects factors. We expect either a statistically significant main effect of group, group x block, or group x block x cue interaction. In the follow-up analyses, we expect to see significant group differences in responses to CS fear between blocks 4 and 6, as this is the moment where participants will experience loss of control. We do not expect significant group differences at the beginning of the task (blocks 1 to 3). Potential sex differences in stress responses to the loss-of-control task (H5a, H5b) will be analyzed in the same statistical tests as H1a-d, separately for each stress marker. To analyze the strength and direction of the association between general perceived stress experienced over the past month and (a) acute perceived stress experienced in the MAST procedure (H6a) and (b) the loss-of-control task (H6b), we will conduct two Pearson or Spearman correlation analyses. Subsequently, to investigate H7, we will conduct a mediation analysis with perceived stress in response to the MAST (t50) as a mediator variable, general perceived stress (PSS-10) as a predictor variable, and perceived stress in the loss-of-control task (t75) as an outcome variable. Lastly, to address H8a-b, we will perform two Pearson or Spearman correlation analyses to assess the relationship between childhood adversity and (a) acute perceived stress experienced in the MAST procedure (H8a) and (b) perceived stress in response to the loss-of-control task (H8a).  In all analyses, a p-value below the threshold of .05 will be a cause to reject the null hypothesis. To gauge the relative support for the null hypothesis in case of non-significant effects, Bayesian analyses will be conducted as a complement to the frequentist analyses for hypotheses H1-5. The Holm correction will be applied to account for multiple comparisons in the follow-up rmANOVAs. Partial eta-squared (ηp2) will be reported as a measure of effect size for the ANOVA analyses. In case of conducting non-parametric tests as alternatives to ANOVAs, Rank-Biserial Correlation (r) will be used to measure the effect size for the Mann-Whitney U test, while Kendall's Coefficient of Concordance (Kendall's W) will be used to measure the effect size for the Friedman test. Depending on the choice of the correlation analyses, Spearman's rank correlation coefficient (ρ) or the Pearson correlation coefficient (r) will indicate the strength and direction of the relationship between two variables.

In exploratory analyses, we will investigate the potential relationship between general perceived self-efficacy (as measured by General Self-Efficacy Scale; Schwarzer & Jerusalem, 1995) and control expectancy.

***Data Preparation***

**Calculations.** The perceived controllability score will be calculated by subtracting the perceived controllability rating at the end of the loss-of-control task (scale 0-100) from the perceived controllability rating at the beginning of the task (scale 0-100). A higher score will indicate a higher decrease in perceived controllability over threat, reflecting a greater experience of uncontrollability.

**Assumption Violations.** Greenhouse-Geisser and Huynh-Feldt corrections will be applied in case of violations of sphericity in rmANOVAs. If the normality assumption is violated, the Mann-Whitney U test will be used as a non-parametric alternative to ANOVA, while the Friedman test will serve as a non-parametric alternative to rmANOVA. We will conduct a Pearson's correlation test if the data used in the correlation analyses are normally distributed. Spearman's correlation will be calculated if the data do not meet the normality assumption.

**Transformations.** Log-transformation will be performed to account for the skewness of sAA and salivary cortisol values.

***Data Exclusions***

Data that meet the following criteria will be excluded from the analysis: (a) participants older than 45 years old; (b) participants with missing blood pressure, sAA, or salivary cortisol data at time points essential for evaluating exclusion criteria or testing the main hypotheses (H1-5); (c) participants with a baseline salivary cortisol or sAA of more than 3 SD above the mean will be excluded from the analyses; (d) participants in the stress group who prematurely retract their hand from the cold water 3 or more times during the HIT trials will be excluded from the current study; (e) to ensure that participants gain a sense of control in the initial part of the loss-of-control task, participants must terminate the electrical impulse with a correct button press at least two times during the first 6 CS+ trials. Participants who fail to terminate the electrical stimulus at least two times will be excluded from the analyses. Data of excluded participants will be replaced with new data until the sample size of 128 participants is reached.

***Quality Checks***

We will conduct a manipulation check to ascertain that the stressful version of MAST successfully induces biological, physiological, and perceived stress compared to the control version. We will test whether participants from the stress group show higher noradrenergic activity (higher sAA, blood pressure) at t50 and increased HPA axis activity (higher salivary cortisol) at t60 compared to participants from the no-stress group. We will also test whether participants from the stress group experience the MAST procedure as more stressful, painful, and unpleasant than participants from the no-stress group. The MAST-related stress ratings (sAA, blood pressure, salivary cortisol, and self-reported stress ratings) will be analyzed separately using univariate ANOVA with Group as an independent variable and sAA level at t50, blood pressure measurement at t50, salivary cortisol level at t60, perceived stress, pain and unpleasantness of MAST as the dependent variable.

In addition to the manipulation check for MAST, we will examine whether the distribution of female participants over menstrual cycle phases differs between the stress and no-stress groups. To do so, we will conduct a chi-square test of independence. If the test yields a non-significant result (*p* > .05), we will conclude that the distribution of female participants over menstrual cycle phase is not significantly different across groups, suggesting it is unlikely to confound our results. However, if a significant difference is found (*p* ≤ .05), we will adjust for the menstrual cycle phase in our analyses to account for potential confounding effects.

Lastly, we will evaluate the believability of the deception through questions asked to participants before the debriefing (for specific questions see “Deception Assessment”). These questions will be analyzed in exploratory analyses.

**References**

Agrigoroaei, S., Polito, M., Lee, A., Kranz-Graham, E., Seeman, T., & Lachman, M. E. (2013). Cortisol response to challenge involving low controllability: The role of control beliefs and age. *Biological Psychology*, *93*(1). https://doi.org/10.1016/j.biopsycho.2013.01.003

Amat, J., Aleksejev, R. M., Paul, E., Watkins, L. R., & Maier, S. F. (2010). Behavioral control over shock blocks behavioral and neurochemical effects of later social defeat. *Neuroscience*, *165*(4), 1031–1038. https://doi.org/10.1016/j.neuroscience.2009.11.005

Bernstein, D. P., Fink, L., Handelsman, L., Foote, J., Lovejoy, M., Wenzel, K., Sapareto, E., & Ruggiero, J. (1994). Initial reliability and validity of a new retrospective measure of child abuse and neglect. *American Journal of Psychiatry*, *151*(8). https://doi.org/10.1176/ajp.151.8.1132

Bernstein, D. P., Stein, J. A., Newcomb, M. D., Walker, E., Pogge, D., Ahluvalia, T., Stokes, J., Handelsman, L., Medrano, M., Desmond, D., & Zule, W. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse and Neglect*, *27*(2). https://doi.org/10.1016/S0145-2134(02)00541-0

Bhanji, J. P., Kim, E. S., & Delgado, M. R. (2016). Perceived control alters the effect of acute stress on persistence. *Journal of Experimental Psychology: General*, *145*(3). https://doi.org/10.1037/xge0000137

Bollini, A. M., Walker, E. F., Hamann, S., & Kestler, L. (2004). The influence of perceived control and locus of control on the cortisol and subjective responses to stress. *Biological Psychology*, *67*(3). https://doi.org/10.1016/j.biopsycho.2003.11.002

Bos, M. G. N., Schuijer, J., Lodestijn, F., Beckers, T., & Kindt, M. (2014). Stress enhances reconsolidation of declarative memory. *Psychoneuroendocrinology*, *46*. https://doi.org/10.1016/j.psyneuen.2014.04.011

Bourke, C. H., Harrell, C. S., & Neigh, G. N. (2012). Stress-induced sex differences: Adaptations mediated by the glucocorticoid receptor. In *Hormones and Behavior* (Vol. 62, Issue 3). https://doi.org/10.1016/j.yhbeh.2012.02.024

Bunea, I. M., Szentágotai-Tǎtar, A., & Miu, A. C. (2017). Early-life adversity and cortisol response to social stress: A meta-analysis. *Translational Psychiatry*, *7*(12). https://doi.org/10.1038/s41398-017-0032-3

Carr, A. R., Scully, A., Webb, M., & Felmingham, K. L. (2016). Gender differences in salivary alpha-amylase and attentional bias towards negative facial expressions following acute stress induction. *Cognition and Emotion*, *30*(2). <https://doi.org/10.1080/02699931.2014.999748>

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, *24*(4). <https://doi.org/10.2307/2136404>

Cohen, S., & Williamson, G. (1988). Perceived stress in a probability sample of the United States. In S. Spacapan & S. Oskamp (Eds.), *The social psychology of health* (pp. 31-67). Sage.

Cohodes, E. M., Kitt, E. R., Baskin-Sommers, A., & Gee, D. G. (2021). Influences of early-life stress on frontolimbic circuitry: Harnessing a dimensional approach to elucidate the effects of heterogeneity in stress exposure. In *Developmental Psychobiology* (Vol. 63, Issue 2, pp. 153–172). John Wiley and Sons Inc. https://doi.org/10.1002/dev.21969

De Raedt, R., & Hooley, J. M. (2016). The role of expectancy and proactive control in stress regulation: A neurocognitive framework for regulation expectation. In *Clinical Psychology Review* (Vol. 45). https://doi.org/10.1016/j.cpr.2016.03.005

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. In *Psychological Bulletin* (Vol. 130, Issue 3). https://doi.org/10.1037/0033-2909.130.3.355

Diener, C., Kuehner, C., Brusniak, W., Struve, M., & Flor, H. (2009). Effects of stressor controllability on psychophysiological, cognitive and behavioural responses in patients with major depression and dysthymia. *Psychological Medicine*, *39*(1), 77–86. https://doi.org/10.1017/S0033291708003437

Ehlers, A., & Clark, D. M. (2000). A cognitive model of posttraumatic stress disorder. *Behaviour Research and Therapy*, *38*(4). https://doi.org/10.1016/S0005-7967(99)00123-0

Eisler, R. M., & Skidmore, J. R. (1987). Masculine Gender Role Stress: Scale Development and Component Factors in the Appraisal of Stressful Situations. *Behavior Modification*, *11*(2). https://doi.org/10.1177/01454455870112001

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G\*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2). https://doi.org/10.3758/BF03193146

Foa, E. B., Zinbarg, R., & Rothbaum, B. O. (1992). Uncontrollability and unpredictability in post-traumatic stress disorder: An animal model. *Psychological Bulletin*, *112*(2). https://doi.org/10.1037/0033-2909.112.2.218

Gallagher, M. W., Bentley, K. H., & Barlow, D. H. (2014). Perceived Control and Vulnerability to Anxiety Disorders: A Meta-analytic Review. *Cognitive Therapy and Research*, *38*(6). https://doi.org/10.1007/s10608-014-9624-x

Georgieva, S., Tomas, J. M., & Navarro-Pérez, J. J. (2021). Systematic review and critical appraisal of Childhood Trauma Questionnaire — Short Form (CTQ-SF). *Child Abuse and Neglect*, *120*. https://doi.org/10.1016/j.chiabu.2021.105223

Hancock, L., & Bryant, R. A. (2018). Perceived control and avoidance in posttraumatic stress. *European Journal of Psychotraumatology*, *9*(1). https://doi.org/10.1080/20008198.2018.1468708

Hancock, L., & Bryant, R. A. (2020). Posttraumatic stress, stressor controllability, and avoidance. *Behaviour Research and Therapy*, *128*. https://doi.org/10.1016/j.brat.2020.103591

Havranek, M. M., Bolliger, B., Roos, S., Pryce, C. R., Quednow, B. B., & Seifritz, E. (2016). Uncontrollable and unpredictable stress interacts with subclinical depression and anxiety scores in determining anxiety response. *Stress*, *19*(1), 53–62. https://doi.org/10.3109/10253890.2015.1117449

He, J., Zhong, X., Gao, Y., Xiong, G., & Yao, S. (2019). Psychometric properties of the Chinese version of the Childhood Trauma Questionnaire-Short Form (CTQ-SF) among undergraduates and depressive patients. *Child Abuse and Neglect*, *91*. https://doi.org/10.1016/j.chiabu.2019.03.009

Henderson, R. K., Snyder, H. R., Gupta, T., & Banich, M. T. (2012). When does stress help or harm?The effects of stress controllability and subjective stress response on Stroop performance. *Frontiers in Psychology*, *3*(JUN). https://doi.org/10.3389/fpsyg.2012.00179

Isowa, T., Ohira, H., & Murashima, S. (2006). Immune, endocrine and cardiovascular responses to controllable and uncontrollable acute stress. *Biological Psychology*, *71*(2). https://doi.org/10.1016/j.biopsycho.2005.04.002

Jackson, E. D., Payne, J. D., Nadel, L., & Jacobs, W. J. (2006). Stress differentially modulates fear conditioning in healthy men and women. *Biological Psychiatry*, *59*(6). https://doi.org/10.1016/j.biopsych.2005.08.002

Kelly, M. M., Tyrka, A. R., Anderson, G. M., Price, L. H., & Carpenter, L. L. (2008). Sex differences in emotional and physiological responses to the Trier Social Stress Test. *Journal of Behavior Therapy and Experimental Psychiatry*, *39*(1). https://doi.org/10.1016/j.jbtep.2007.02.003

Knauft, K., Waldron, A., Mathur, M., & Kalia, V. (2021). Perceived chronic stress influences the effect of acute stress on cognitive flexibility. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-03101-5

Kuras, Y. I., McInnis, C. M., Thoma, M. V., Chen, X., Hanlin, L., Gianferante, D., & Rohleder, N. (2017). Increased alpha-amylase response to an acute psychosocial stress challenge in healthy adults with childhood adversity. *Developmental Psychobiology*, *59*(1). https://doi.org/10.1002/dev.21470

Lee, E. H. (2012). Review of the psychometric evidence of the perceived stress scale. In *Asian Nursing Research* (Vol. 6, Issue 4). https://doi.org/10.1016/j.anr.2012.08.004

Lempert, K. M., Porcelli, A. J., Delgado, M. R., & Tricomi, E. (2012). Individual differences in delay discounting under acute stress: The role of trait perceived stress. *Frontiers in Psychology*, *3*(JUL). https://doi.org/10.3389/fpsyg.2012.00251

Lindau, M., Almkvist, O., & Mohammed, A. H. (2016). Effects of Stress on Learning and Memory. In *Stress: Concepts, Cognition, Emotion, and Behavior: Handbook of Stress*. https://doi.org/10.1016/B978-0-12-800951-2.00018-2

Liston, C., McEwen, B. S., & Casey, B. J. (2009). Psychosocial stress reversibly disrupts prefrontal processing and attentional control. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(3). https://doi.org/10.1073/pnas.0807041106

Livanou, M., Baosglu, M., Marks, I. M., De Silva, P., Noshirvani, H., Lovell, K., & Thrasher, S. (2002). Beliefs, sense of control and treatment outcome in post-traumatic stress disorder. *Psychological Medicine*, *32*(1). https://doi.org/10.1017/s0033291701004767

LoPilato, A. M., Addington, J., Bearden, C. E., Cadenhead, K. S., Cannon, T. D., Cornblatt, B. A., Mathalon, D. H., McGlashan, T. H., Perkins, D. O., Tsuang, M. T., Woods, S. W., & Walker, E. F. (2019). Stress perception following childhood adversity: Unique associations with adversity type and sex. *Development and Psychopathology*, *32*(1). https://doi.org/10.1017/S0954579419000130

Lucas, M., Ilin, Y., Anunu, R., Kehat, O., Xu, L., Desmedt, A., & Richter-Levin, G. (2014). Long-term effects of controllability or the lack of it on coping abilities and stress resilience in the rat. *Stress*, *17*(5). https://doi.org/10.3109/10253890.2014.930430

Maier, S. F. (2015). Behavioral control blunts reactions to contemporaneous and future adverse events: Medial prefrontal cortex plasticity and a corticostriatal network. In *Neurobiology of Stress* (Vol. 1, Issue 1). https://doi.org/10.1016/j.ynstr.2014.09.003

Maier, S. F., & Seligman, M. E. (1976). Learned helplessness: Theory and evidence. *Journal of Experimental Psychology: General*, *105*(1). https://doi.org/10.1037/0096-3445.105.1.3

Meine, L. E., Schüler, K., Richter-Levin, G., Scholz, V., & Wessa, M. (2020). A translational paradigm to study the effects of uncontrollable stress in humans. *International Journal of Molecular Sciences*, *21*(17). https://doi.org/10.3390/ijms21176010

Merz, C. J., & Wolf, O. T. (2017). Sex differences in stress effects on emotional learning. In *Journal of Neuroscience Research* (Vol. 95, Issues 1–2). https://doi.org/10.1002/jnr.23811

Merz, C. J., Wolf, O. T., Schweckendiek, J., Klucken, T., Vaitl, D., & Stark, R. (2013). Stress differentially affects fear conditioning in men and women. *Psychoneuroendocrinology*, *38*(11). https://doi.org/10.1016/j.psyneuen.2013.05.015

Mineka, S., & Hendersen, R. W. (1985). Controllability and predictability in acquired motivation. In *Annual review of psychology* (Vol. 36). https://doi.org/10.1146/annurev.ps.36.020185.002431

Mineka, S., & Kihlstrom, J. F. (1978). Unpredictable and uncontrollable events: A new perspective on experimental neurosis. *Journal of Abnormal Psychology*, *87*(2). https://doi.org/10.1037/0021-843X.87.2.256

Müller, M. J. (2011). Helplessness and perceived pain intensity: Relations to cortisol concentrations after electrocutaneous stimulation in healthy young men. *BioPsychoSocial Medicine*, *5*. https://doi.org/10.1186/1751-0759-5-8

Obasi, E. M., Shirtcliff, E. A., Cavanagh, L., Ratliff, K. L., Pittman, D. M., & Brooks, J. J. (2017). Hypothalamic-Pituitary-Adrenal Reactivity to Acute Stress: an Investigation into the Roles of Perceived Stress and Family Resources. *Prevention Science*, *18*(8). https://doi.org/10.1007/s11121-017-0759-3

Olff, M. (2017). Sex and gender differences in post-traumatic stress disorder: an update. *European Journal of Psychotraumatology*, *8*(sup4). <https://doi.org/10.1080/20008198.2017.1351204>

OMRON M2. (n.d.). Healthcare Europe BV. Hoofddorp, The Netherlands.

Ouellet-Morin, I., Robitaille, M. P., Langevin, S., Cantave, C., Brendgen, M., & Lupien, S. J. (2019). Enduring effect of childhood maltreatment on cortisol and heart rate responses to stress: The moderating role of severity of experiences. *Development and Psychopathology*, *31*(2). https://doi.org/10.1017/S0954579418000123

Peng, C., Cheng, J., Rong, F., Wang, Y., & Yu, Y. (2023). Psychometric properties and normative data of the childhood trauma questionnaire-short form in Chinese adolescents. *Frontiers in Psychology*, *14*. https://doi.org/10.3389/fpsyg.2023.1130683

Peters, M. L., Godaert, G. L. R., Ballieux, R. E., & Heijnen, C. J. (2003). Moderation of physiological stress responses by personality traits and daily hassles: Less flexibility of immune system responses. *Biological Psychology*, *65*(1). https://doi.org/10.1016/S0301-0511(03)00096-6

Peyrot, C., Brouillard, A., Morand-Beaulieu, S., & Marin, M. F. (2020). A review on how stress modulates fear conditioning: Let’s not forget the role of sex and sex hormones. *Behaviour Research and Therapy*, *129*. https://doi.org/10.1016/j.brat.2020.103615

Pruessner, J. C., Baldwin, M. W., Dedovic, K., Renwick, R., Mahani, N. K., Lord, C., Meaney, M., & Lupien, S. (2005). Self-esteem, locus of control, hippocampal volume, and cortisol regulation in young and old adulthood. *NeuroImage*, *28*(4). <https://doi.org/10.1016/j.neuroimage.2005.06.014>

Raio, C. M., & Phelps, E. A. (2015). The influence of acute stress on the regulation of Conditioned Fear. *Neurobiology of Stress*, *1*, 134–146. https://doi.org/10.1016/j.ynstr.2014.11.004

Raymond, C., Marin, M. F., Wolosianski, V., Journault, A. A., Longpré, C., Leclaire, S., Cernik, R., Juster, R. P., & Lupien, S. J. (2021). Early childhood adversity and HPA axis activity in adulthood:The importance of considering minimal age at exposure. *Psychoneuroendocrinology*, *124*. https://doi.org/10.1016/j.psyneuen.2020.105042

Riggenbach, M. R., Weiser, J. N., Mosley, B. E., Hipskind, J. J., Wireman, L. E., Hess, K. L., Duffy, T. J., Handel, J. K., Kaschalk, M. G., Reneau, K. E., Rorabaugh, B. R., Norrholm, S. D., Jovanovic, T., & Zoladz, P. R. (2019). Immediate pre-learning stress enhances baseline startle response and fear acquisition in a fear-potentiated startle paradigm. *Behavioural Brain Research*, *371*. <https://doi.org/10.1016/j.bbr.2019.111980>

Scholz, U., Gutiérrez Doña, B., Sud, S., & Schwarzer, R. (2002). Is general self-efficacy a universal construct?1. *European Journal of Psychological Assessment*, *18*(3), 242–251. https://doi.org/10.1027//1015-5759.18.3.242

**Schwarzer, R., & Jerusalem, M. (1995).** Generalized Self-Efficacy scale. In J. Weinman, S. Wright, & M. Johnston (Eds.), Measures in health psychology: A user’s portfolio. Causal and control beliefs (pp. 35-37). NFER-NELSON.

Simon-Kutscher, K., Wanke, N., Hiller, C., & Schwabe, L. (2019). Fear Without Context: Acute Stress Modulates the Balance of Cue-Dependent and Contextual Fear Learning. *Psychological Science*, *30*(8), 1123–1135. https://doi.org/10.1177/0956797619852027

Smeets, T., Cornelisse, S., Quaedflieg, C. W. E. M., Meyer, T., Jelicic, M., & Merckelbach, H. (2012). Introducing the Maastricht Acute Stress Test (MAST): A quick and non-invasive approach to elicit robust autonomic and glucocorticoid stress responses. *Psychoneuroendocrinology*, *37*(12). https://doi.org/10.1016/j.psyneuen.2012.04.012

Stawski, R. S., Sliwinski, M. J., Almeida, D. M., & Smyth, J. M. (2008). Reported Exposure and Emotional Reactivity to Daily Stressors: The Roles of Adult Age and Global Perceived Stress. *Psychology and Aging*, *23*(1). <https://doi.org/10.1037/0882-7974.23.1.52>

Taylor, J. M. (2015). Psychometric analysis of the ten-item perceived stress scale. *Psychological Assessment*, *27*(1). <https://doi.org/10.1037/a0038100>

Teeuw, B., Schwarzer, R., & Jerusalem, M. (1994). Dutch adaptation of the General Self-Efficacy Scale. Freie Universität.

Wongpakaran, N., & Wongpakaran, T. (2010). The Thai version of the PSS-10: An Investigation of its psychometric properties. *BioPsychoSocial Medicine*, *4*. https://doi.org/10.1186/1751-0759-4-6

Yao, L., Li, Y., Qian, Z., Wu, M., Yang, H., Chen, N., Qiao, Y., Wei, C., Zheng, Q., Han, J., Tian, Y., Liu, Z., & Ren, W. (2019). Loss of control over mild aversive events produces significant helplessness in mice. *Behavioural Brain Research*, *376*. <https://doi.org/10.1016/j.bbr.2019.112173>

**Study Design Table**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Research questions** | **Hypotheses** | **Sampling plan** | **Analysis plan** | **Rationale for deciding the sensitivity of the test for confirming or disconfirming the hypothesis** | **Interpretation given different outcomes** | **Theory that could be shown wrong by the outcomes** |
| Does exposure to acute stress amplify perceived, biological and physiological stress responses to the subsequent loss of control? | **H1:** We expect participants in the stress group to show higher biological (cortisol and sAA levels), physiological (blood pressure), and perceived (ratings) stress responses in the loss-of-control task compared to participants from the no-stress group. | One hundred twenty-eight participants (*N* = 64 participants per group, 32 women and 32 men in each group) will be recruited through the Experiment Management System of the KU Leuven Faculty of Psychology and Educational Sciences. | **(salivary cortisol)** A rmANOVA on salivary cortisol with group (stress versus no-stress) and sex-at-birth (female versus male) as between-subjects factors, and timepoint (t85, t95) as a within-subjects factor.  **(sAA and blood pressure)** Three separate rmANOVAs on sAA, systolic blood pressure and diastolic blood pressure with group (stress versus no-stress) and sex-at-birth (female versus male) as between-subjects factors, and timepoint (t60, t75) as a within-subjects factor.  **(perceived stress)**  A rmANOVA on perceived stress with group (stress versus no-stress) and sex-at-birth (female versus male) as between-subjects factors, and timepoint (t60, t75) as a within-subjects factor. | To the best of our knowledge, there has been no published work specifically investigating the impact of acute stress exposure on the subsequent loss of control over threat in humans. Therefore, we based our sample size calculation on the effect size of a study by Bhanji and colleagues (2016). In their study, Bhanji and colleagues (2016) found that exposure to lab-induced acute stress decreased persistence in an uncontrollable setback condition compared to non-stressed participants who also experienced uncontrollable setback, *t*(77) = - 1.81, *p* = .037 (one-tailed), *d* = 0.41. Given the relatively novel nature of this study, a small to moderate effect size of *f* = 0.15 appears reasonable to be detected. Based on the G\*Power 3.1 (Faul et al., 2007) calculation, the sample size of 128 participants will yield 80% power to detect this effect size at a significance level of .05. | If participants from the stress group show statistically significant: (a) increase in salivary cortisol between timepoints t85 and t95, (b) increase in sAA, systolic and diastolic blood pressure between timepoints t60 and t75 and (c) increase in perceived stress rating from before (t60) to after the loss-of-control task (t75) compared to participants from the no-stress group, H1 will be corroborated.  Hypothesis H1 will be partly supported if participants from the stress group compared to no-stress group show higher responses on some stress markers (e.g., sAA and salivary cortisol), but not all of them (e.g., perceived stress).  No significant differences between the stress and no-stress groups on listed stress markers would suggest the absence of a meaningful effect supporting hypothesis H1.  If participants from the no-stress group show higher stress markers in response to the loss-of-control task compared to participants from the stress group, hypothesis H1 will be disconfirmed.  In addition, we will conduct Bayesian analyses for the hypotheses H1-5 to complement the frequentist analyses presented in this table. | This study might provide preliminary evidence or absence of evidence for stress-induced sensitization in humans. Up until now the idea that acute or chronic stressors might sensitize responses to further challenging situations was mainly tested in rodents. |
| Does exposure to acute stress amplify the perceived feeling of uncontrollability upon a subsequent loss of control? | **H2:**We predict that participants exposed to the acute stress procedure will report higher perceived uncontrollability in the loss-of-control task than participants that were not exposed to prior stress. | A one-way ANOVA with group (stress versus no-stress) as an independent variable and the perceived controllability score in the loss-of-control task as a dependent variable. | If participants from the stress group report statistically higher perceived uncontrollability in the loss-of-control task than participants from the no-stress group, hypothesis H2 will be corroborated.  No significant differences between the stress and no-stress groups on perceived uncontrollability would suggest the absence of a meaningful effect supporting hypothesis H2.  In case participants from the no-stress group show higher perceived uncontrollability than participants from the stress group, hypothesis H2 will be disconfirmed. |
| Does exposure to acute stress change individuals’ predictions of control after the experience of losing control over threat? | **H3:**We hypothesize that participants exposed to the acute stress procedure will predict less control after the loss-of-control task compared to participants that did not previously experience stress. | A one-way ANOVA with group (stress versus no-stress) as independent variable and control expectancy prediction at t75 as dependent variable. | Hypothesis H3 will be corroborated if participants exposed to the acute stress report statistically significant less control after the loss-of-control task compared to participants who were not exposed to prior stress.  There would be no sufficient evidence to support this hypothesis if no statistically significant group differences are found.  In case participants from the no-stress group report less control predictions than participants from the stress group then the hypothesis H3 will be disconfirmed. |
| Does exposure to stress amplify perceived fear responses towards threatening cues in the loss of control situation? | **H4:** We expect that participants in the acute stress group will report higher perceived fear in response to threat-predictive cues (CS fear) upon the loss of control than participants in the no-stress group. | A rmANOVA on CS fear ratings with group (stress versus no-stress) as a between-subjects factor, and block in the computer task (6) and cue (CS + versus CS-) as within-subjects factors. | The H4 will be supported if participants exposed to stress report higher perceived fear after losing control than participants in the no-stress condition.  In case of not statistically significant group differences, the evidence will not be sufficient to support claims of hypothesis H4.  Hypothesis H4 will be disconfirmed if participants in the no-stress condition report higher perceived fear after losing control than participants in the stress condition. |
| Does the impact of acute stress exposure on a subsequent loss-of-control situation differ between female and male participants? | **H5a:** We expect that male participants in the stress group will show higher salivary cortisol levels, sAA, and blood pressure during the loss-of-control task compared to female participants in the stress group.  **H5b:**We predict that female participants in the stress group will report higher perceived stress during the loss-of-control task than male participants in the stress group. | Potential sex differences in stress responses to the loss-of-control task (H5a, H5b) will be analyzed in the same statistical tests as H1 separately for each stress marker. | Hypothesis H5a will be supported if males in the stress group show higher salivary cortisol levels, sAA, and blood pressure during the loss-of-control task compared to females in the stress group.  Hypothesis H5b will be supported if females in the stress group report higher perceived stress during the loss-of-control task than males in the stress group.  Hypotheses H5a and H5b will be disconfirmed if the results reveal statistically significant patterns in the opposite direction for each hypothesis.  No statistically significant differences between males and females would mean that the there is insufficient evidence to support hypotheses H5a and H5b. |
| Is general perceived stress associated with perceived stress reported in the acute stress task and loss-of-control task?  Does perceived stress during the acute stress task affect how general perceived stress impacts stress responses in a later loss of control situation? | We hypothesize that participants with higher general perceived stress scores, as measured with the Perceived Stress Scale (PSS-10; Cohen & Williamson, 1988), will report higher perceived stress in response to the acute stress induction procedure (**H6a**), and the loss-of-control task (**H6b**).  **H7:** We hypothesize that perceived stress in response to the lab-induced stressful event will mediate a relationship between the general perceived stress (measured by PSS-10) and the perceived stress reported in the loss-of-control task. | Two Pearson or Spearman correlation analyses between the general perceived stress experienced over the past month and (a) acute perceived stress experienced in the stress induction procedure (H6a), and (b) the loss-of-control task (H6b).  To analyse hypothesis H7, we will perform a mediation analysis with the perceived stress in response to the MAST (t50) as a mediator variable, general perceived stress (PSS-10) as a predictor variable, and perceived stress in the loss-of-control task (t75) as an outcome variable. | Statistically significant positive correlations (*r* > 0 ) between general perceived stress in the one hand and perceived stress reported in the acute stress task and in the loss-of-control task will corroborate hypotheses H6a and H6b.  Statistically significant correlation of *r* < 0 between the listed variables will disconfirm hypotheses H6a and H6b.  Hypothesis H7 will be supported if: (a) general perceived stress significantly affects perceived stress is the acute stress task (path a) ; (b) perceived stress in the acute stress task significantly affects perceived stress in the loss-of-control task (path b); **and** (c) if the indirect effect (path a × path b) is statistically significant **or** (d) after accounting for perceived stress in the acute stress task, the direct effect of general perceived stress on the perceived stress in the loss-of-control task is reduced or nonsignificant.  Hypothesis H7 will be disconfirmed if: (a) general perceived stress does not significantly affect perceived stress in the acute stress task; (b) perceived stress in the acutely stressful situation does not significantly affect perceived stress in the loss-of-control task; **or** (c) if the indirect effect is not significant. |
| Is childhood adversity associated with the level of perceived stress experienced (a) in response to the MAST and (b) in response to the loss of control situation? | **H8a:** We hypothesize that participants with higher childhood adversity, measured by the Childhood Trauma Questionnaire - Short Form (CTQ-SF; Bernstein et al., 2003), will also show higher perceived stress ratings in relation to the acute stress induction (MAST).  **H8b:** We hypothesize that participants with higher childhood adversity, measured by the Childhood Trauma Questionnaire - Short Form (CTQ-SF; Bernstein et al., 2003), will also show higher perceived stress ratings in relation to the loss-of-control task. | Two Pearson or Spearman correlation analyses to assess the relationship between childhood adversity and perceived stress in response to the MAST (t50) and perceived stress in the loss-of-control task (t75). | In order to assess if hypotheses H8a and H8b were supported or not we will follow the same approach proposed for hypotheses H6a and H6b. |