**Protocol for the Experimental CPT**

**Master Dissertation:** Physiological indicators in induced pain

**Student:** Ana Luísa Baltazar Bento

**Supervisors:** Doutora Raquel Sebastião, Doutora Susana Brás

# Description and objectives of the study

This study aims to obtain physiological indicators of pain. For this purpose, a Cold Pressor Task (CPT) will be implemented to induce pain. In this task, participants are asked to place their forearm and non-dominant hand in a cold-water tank for a maximum of 2 minutes or until they consider the pain intolerable. At the same time, physiological signals, namely electrocardiogram (ECG), surface electromyography (sEMG), electrodermal activity (EDA) and blood pressure (BP), will be simultaneously collected through minimal invasive techniques and assessing pain through self-report instruments (Numerical Pain Scale - NPS), at 4 distinct moments:

* Before the beginning of the CPT;
* Before the end of the CPT (as well as the maximum pain experienced);
* Immediately after the end of the CPT (as well as the maximum pain experienced);
* 10 minutes after the CPT (as well as the maximum pain experienced);

Along with this experiment, participants will also fill out several questionnaires (regarding health, anxiety and depression and personality, as well as a COVID-19 screening questionnaire).

# Before the study

1. Obtain ethical approval from the Ethics Committee of the University of Aveiro;
2. Check the necessary material (see section 3. Preparation of necessary material);

# Setup requirements

1. System to realize the CPT:

The equipment that will be used to perform the CPT was developed by the student Pedro Santos, from the Integrated Master in Electronic and Telecommunications Engineering, at the Department of Electronics, Telecommunications and Informatics (DETI), University of Aveiro, which is an electronically controlled system to control and preserve the temperature, thus avoiding the contact of the forearm and the hand with ice, and, therefore, being safer. It consists of two electronic equipment that allow to control the temperature of the water in a tank, intending that the water reaches temperatures alternately, with programmable times, 37ºC (without inducing pain) and 7ºC (to induce pain), using a microcontroller, which allows to revert losses in order to keep the water in the tank stabilized at the desired temperature (± 0.5ºC or better). This equipment was also designed with circulating water pump to avoid the heating/cooling of the water.

1. Non-invasive system to collect physiological signals (Biosignalsplux Explorer):

Biosignalsplux Explorer is a 4-channel tool-kit designed to allow wireless and high-quality acquisitions of physiological signals.

1. Electrodes to collect physiological signals;
2. Informed Consents (in Portuguese);
3. Questionnaires (in Portuguese):
   1. SF-36v2: Health questionnaire;
   2. HADS: anxiety and depression hospital scale;
   3. Eysenck’s personality questionnaire (short version with 48 itens);
   4. Instrument for data collection, namely gender, age, mental severe diseases or neurological pathologies, and diseases that causes chronic pain, and to assess self-reported pain using the Numerical Pain Scale;
4. Description of the study (in Portuguese);
5. Desk for support and chair;
6. Material for disinfection and personal protective equipment (PPE), namely NK95 disposable masks, protection visor, along-sleeved water-resistant gown;

# Participants

Participants will be recruited from the local community, among university students, researchers, teachers, and collaborators, under the following inclusion criteria:

1. All gender;
2. Healthy;
3. Age superior to 18 years and inferior to 65 years;
4. Not presenting mental illness neither neurological disorders;
5. Not suffering from any disease which causes of chronic pain;
6. Ability to comprehend and answer the self-report measures;
7. Agree to provide informed consent and participate in this study;

Due to the Cold Pressor Task procedures, participants with a history of cardiovascular disease and Raynaud’s disease seizures, frostbite, arthrosis, cuts, sores or fractures on the hand to be immersed will be excluded.

# Preparation of necessary material

1. Print the required documents before the CPT:
   1. Informed Consent for all participants, detailing this study and explaining the objective of the data collection, the processing of personal data and participants’ rights;
   2. Questionnaires for assessing health conditions, anxiety and depression and personality;
   3. Instrument for data collection (with numerical pain scale);
2. See if the laboratory has the necessary conditions: if it’s clean, if the material is working properly and is available, namely:
   1. System for the CPT and Biosignalsplux Explorer;
   2. Material for disinfection and personal protective equipment (PPE), namely NK95 disposable masks, protection visor, along-sleeved water-resistant gown;
   3. Paper documents – COVID-19 screening questionnaires, Informed Consents, health, anxiety and depression and personality questionnaires and Instrument for data collection;
3. Contact volunteer and scheduling participation

The volunteers who match the inclusion criteria will be contacted by email or/phone to schedule participation. A toll to do so is Doodle.com.

# Procedure in the day of the experiment

On the day of the experiment, send the participants a SMS to confirm and emphasis to use the SMS number phone to apply any orientation to the laboratory room and to advertise when arriving at the facilities.

# Before the participant arrives (1 hour earlier)

1. Turn on the system for CPT (adjust water temperature);
2. Verify the availability of the necessary paper documents;
3. Check the participant schedule and his name;

# When the participant arrives, but before the CPT

1. Wear a KN95 disposable mask;
2. Participant reception at the entrance of IEETA: Confirm the participant name and the participant ID on participant sheet, and the willing to participate in the study;
3. Check participant’s temperature and, while the researcher sanitizes their hands, invite the participant to sanitize their hands. Fill out the COVID-19 screening questionnaire (symptoms and previous presence at high risk countries);
4. If the participant’s temperature is below 38º and he/she passes the COVID-19 screening questionnaire invite him/her to follow you. Otherwise, the experiment can not continue, and he/she can leave the facilities. If the participant’s temperature is above 38º, recommend him/her to contact the “Linha Saúde 24”.
5. Invite the participant to enter the CPT room and to seat at the desk. While the researcher sanitizes their hands, invite the participant to sanitize their hands again;
6. Ask the participant to sign the COVID-19 screening questionnaire (with a sanitized pen) and to turn into silence its mobile phone (to not interfere with the CPT);
7. Explain to the participant the experiment purposes and procedures, if necessary, show the participant the description of the experiment (in portuguese): he/she will remain seated, in a comfortable position, for 10 minutes for baseline collection. After this time, the forearm and non-dominant hand will be sanitized, and the participant will be asked to place the forearm and non-dominant hand in the tank with water at 37ºC of temperature for about 2 minutes. At the end, the participant will report its pain using the NPS. Therefore, the participant will be asked to perform the CPT, placing the forearm and non-dominant hand in the tank with water at 7ºC of temperature for a maximum of 2 minutes or until they consider the pain intolerable. The pain will be self-reported before the end of the CPT and immediately after the end of the CPT (as well as the maximum pain experienced); Afterwards, the participant will be asked to place again the place the forearm and non-dominant hand in the tank with water at 37ºC of temperature for about 2 minutes. And then, the participant will remain seated, in a comfortable position, for 10 minutes. 10 minutes after the end of the CPT, participant will be asked to self-reported the pain and the maximum pain experienced (to dampen the susceptibility to memory bias);
8. Explain to the participant that him/her can refuse to participate at this study and that he/she can decline participation in the study, at any time, during the CPT, and provide him/her the Informed Consent;
9. If the participant doesn’t agree with Informed Consent, he/she can leave and his participation finish here. Else, the researcher continuous the protocol with the participant. Collect the Informed Consent;
10. Start filling the Instrument for data collection: write down date and session time, participant ID, age, gender, the existence of a diagnose of mental severe disease or neurological pathologies and the existence of any disease that causes chronic pain;
11. Confirm if the participant had filled out, previously but in the day of participation in the experiment, the health, the anxiety and depression and the personality questionnaires. If not, request the participant to fill out the questionnaires in paper format;
12. After questionnaires completion, sanitize hands and prepare to applying electrodes to the participant, explaining him/her that the electrodes will be placed to collect ECG, sEMG and EDA, at the following locations:
    1. ECG - 1 lead (2 electrodes placed at the chest – left and right - and a reference electrode placed at the abdomen area);
    2. EDA (2 electrodes placed at the index and ring fingers of the dominant hand);
    3. sEMG (2 electrodes placed at the triceps muscle of the immersed hand, 2 electrodes placed at the upper trapezius muscle and a ground/reference electrode placed in a bone region);
13. As BP will be measure while pain will be self-reported, place the cuff at the bare upper arm of the dominant hand;
14. Connect the cables to the Biosignalsplux Explorer hub, turn it on and verify data communication and collection;

# During the CPT

1. Invite the participant to seat at the chair for the CPT in a comfortable position;
2. Ask if there any more doubts about the experiment. If so answer him/her. If there are not any doubts, inform the participant that the experiment will start and invite him/her to use a protection visor. You should now don your along-sleeved water-resistant gown and protection visor.
3. Tigger the button of the Handheld Switch for begging of the baseline annotation and manual acquisition start;
4. After 10 minutes, trigger the button of the Handheld Switch for ending of the baseline annotation;
5. Sanitize the forearm and non-dominant hand of the participant;
6. Ask the participant to place the forearm and non-dominant hand in the tank with water at 37ºC and trigger the button of the Handheld Switch;
7. After 2 minutes, ask the participant to remove the forearm and non-dominant hand from the tank, trigger the button of the Handheld Switch and request the participant to self-report its pain. Write the self-reported value in the Instrument for data collection. Afterwards, measure the BP of the participant and write it in the Instrument for data collection;
8. Ask the participant to place he/she forearm and non-dominant hand in the tank with water at 7ºC of temperature for a maximum of 2 minutes or until they consider the pain intolerable. At the same time, trigger the Handheld Switch for begging of the CPT annotation;
9. At almost the end of the 2 minutes, or when the participant started expressing that the pain is intolerable, ask the participant to self-report its pain and the maximum pain experienced, and write both in the Instrument for data collection;
10. After 2 minutes, or when the participant decided to conclude the task, ask the participant to remove the forearm and non-dominant hand from the tank and trigger the button of the Handheld Switch for ending of the CPT annotation. Request the participant to self-report its pain and the maximum pain experienced, and write both in the Instrument for data collection. Afterwards, measure the BP of the participant and write it in the Instrument for data collection;
11. Ask the participant to place the forearm and non-dominant hand in the tank with water at 37ºC and trigger the button of the Handheld Switch;
12. After 2 minutes, trigger the button of the Handheld Switch and ask the participant to remain seated, in a comfortable position, for 10 minutes;
13. 10 minutes after the end of the CPT, trigger the button of the Handheld Switch and request the participant to self-reported the pain and the maximum pain experienced, and write both in the Instrument for data collection. Afterwards, measure the BP of the participant and write it in the Instrument for data collection;
14. The data collection ends at this point; Explain to the participant that the experiment was concluded;
15. Turn off the Biosignalsplux Explorer hub and disconnect the cables;
16. Sanitize your hands;
17. Remove the electrodes from the participant and clean the participant’s skin where the electrodes where placed, discard the electrodes and place the hub, the cables, the blood pressure cuff and used pens in the box “material to be disinfected”;
18. Ask if there any more doubts about the experiment. If so answer him/her;
19. Place the participant’s protection visor and yours in the box “material to be disinfected”;
20. Sanitize your hands and invite the participant to sanitize theirs;
21. Acknowledge the participant cooperation and invite him/her to leave the facilities;

# After the participant leaves

1. Archive the Informed Consent, the Instrument for data collection and, if filled out in paper format, the anxiety and depression, the personality questionnaires;
2. If not during the experiment, open the windows for room ventilation and turn on the floor standing fan at the door;
3. Close both the tanks and disinfect the exterior of both;
4. Proceed to the disinfection of the cables, of the Biosignalsplux Explorer hub and of the blood pressure cuff. Place them in the box “disinfected material”. Also disinfect the box “material to be disinfected”;
5. Proceed to the disinfection of the chair and desks;
6. Save the participants files in a secure and password protected folder, under your password protected area;
7. Turn off the floor standing fan and proceed to its disinfection. Place it again outside the room.
8. After room ventilation, if the data collection continues, then prepare the lab for the next row of participants. Doff your along-sleeved water-resistant gown and hang it until the next CPT. Restart on **6.1. Before the participant arrives (30 minutes earlier)**;
9. Else if is the last participant, drain the water from both tanks, store the Biosignalsplux Explorer hub, cables, electrodes and blood pressure cuff and conveniently discard the along-sleeved water-resistant gown;
10. Arrange the lab room, the desks, chairs, etc. Close the door;