

## **Study Protocol and Statistical Analysis Plan**

Sedative Effect of Music Listening on Pain Induced by a Thermal Stimulus (Cold Pressor Test)

14/11/2025

## Protocol 2: Sedative Effect of Music Listening on Pain Induced by a Thermal Stimulus (Cold Pressor Test)

This study aims to evaluate the sedative effect of music listening on pain induced by a thermal stimulus (cold pressor test) in a significant sample of healthy young adults ( $n = 40$ ), by examining the musicological components likely to induce analgesic and anxiolytic sedation.

### I- Objectives

The main objective of this research is to evaluate the sedative effect of music listening on participants' pain and anxiety during the cold pressor test, in order to justify the musical choices used in Protocol 3. This evaluation considers two essential components: cultural contextualization and personal contextualization of the music listening experience.

#### Specific Objectives

- To evaluate the sedative effect of music listening based on transcultural criteria of melodic structure and tempo: This approach aims to determine whether musical sequences selected according to universal musical structures (such as modal scale, intervallic structure, and tempo) can induce analgesic and anxiolytic effects, independently of cultural specificities and individual preferences.
- To analyze the impact of rhythmic stylistic parameters according to cultural contextualization: This objective aims to examine whether musical sequences with rhythmic structures aligned with the participant's cultural musical traditions (particularly Mašriq rhythmic styles) can influence pain and anxiety perception, independently of universal factors and personal preferences.
- To evaluate the influence of the participant's psychomusical profile: The objective is to understand whether music selected according to individual preferences (listening habits and musical tastes) induces an analgesic and anxiolytic sedative effect, independently of universal and cultural musicological criteria.
- To determine the multifactorial effect of music listening: This objective examines whether the sedative effect of music results from a combination of cultural factors (related to musical type) and personal factors (based on individual preferences), and how this interaction may produce an overall therapeutic effect during the cold pressor test.

These objectives aim to deepen the understanding of the musicological and neurocognitive mechanisms underlying the sedative effect of music in healthcare settings and to propose a more precise musical modeling adapted to therapeutic needs in dental care.

## II- Theoretical Framework and Justification of the Cold Pressor Test

In this study, the cold pressor test is used as an experimental method to induce controlled and quantifiable pain in healthy participants, allowing the observation of the sedative effect of music listening on pain perception.

This test consists of immersing one hand in cold water, typically maintained between 0 and 5°C, until a tolerable pain threshold is reached. This type of thermal stimulus, known for producing stable and reproducible pain, provides a valuable experimental model for evaluating therapeutic interventions such as the analgesic effects of music.

The cold pressor test is widely used in psychology and neuroscience research to assess pain responses and to study how interventions (such as meditation, hypnosis, or music listening) modulate pain perception. Previous studies have shown that it activates nociceptive systems and triggers physiological stress responses, making it an ideal model to examine cognitive regulation of pain and stress-reduction interventions.

## III- Population

The study will involve 40 students from Antonine University, aged 18 to 35 years, recruited according to predefined inclusion and exclusion criteria. Participation is voluntary, and all participants will sign an informed consent form prior to the study.

### Exclusion Criteria

- Acute or chronic pain
- History of cardiovascular disorders
- Use of analgesic medication
- Recent wound on either hand
- History of hand fracture
- Dermatological condition affecting the hand
- History of frostbite
- Use of neuropsychiatric medication
- Ability to keep the hand immersed for 4 minutes during the silence phase

## IV- Data Handling and Confidentiality

All collected data will be treated confidentially and anonymized. Physiological and psychophysiological data will be recorded and analyzed strictly for scientific purposes. Each participant will be assigned a code to ensure anonymity. Results will be published only in aggregated form without individual identification.

## V- Procedure

Before the procedure, participants will complete a questionnaire collecting personal information such as age and nationality (to assess cultural influence), as well as psychomusical history, musical expertise, and musical preferences. They will also sign an informed consent form.

Participants will be exposed to pain induced by a thermal stimulus by immersing their hand in a container of ice water maintained at 5°C.

Each participant will undergo a session consisting of seven distinct cold pressor phases, each lasting up to 4 minutes, separated by 15-minute intervals.

Between phases, participants will have access to warm water to restore normal hand temperature and reduce pain sensation.

Participants will alternate hands between phases to minimize adaptation or increased tolerance in a single hand.

Participants may withdraw their hand at any time if the sensation becomes intolerable.

## Experimental Phases

- Phase 1: Silence
- Phase 2: Instrumental music with pleasant emotional valence and measured rhythm
- Phase 3: Instrumental music with unpleasant emotional valence and measured rhythm
- Phase 4: Instrumental music with pleasant emotional valence and non-measured rhythm
- Phase 5: Instrumental music with unpleasant emotional valence and non-measured rhythm
- Phase 6: Participant-selected music from a predefined set of relaxing musical sequences
- Phase 7: Silence (to assess potential habituation effects)
- Phase 8: (optional): Participant's own preferred music

## VI- Outcome Measures

- Pain Intensity : Measured using a Visual Analog Scale (VAS) before, during, and after each phase.
- Reaction Time : The time taken for participants to perceive pain will be recorded.
- Pain Tolerance Duration : The duration of hand immersion will be measured using a stopwatch.
- Qualitative Feedback : Participants will provide qualitative feedback on their experience, offering deeper insight into subjective perceptions of music's effect on pain.

## VII- Materials

- Plastic containers ( $28 \times 19 \times 10$  cm)
- Water
- Ice packs ( $10 \times 1$  kg)
- Water thermometer
- Pulse oximeter
- Stopwatch
- Visual analog scale
- Questionnaires for qualitative feedback
- Musical sequences

## VIII- Statistical Analysis Plan (SAP)

This Statistical Analysis Plan (SAP) specifies the statistical methods and procedures that will be used to analyze the data collected in this experimental within-subject study evaluating the analgesic and anxiolytic effects of music listening during a cold pressor test.

The study follows a repeated-measures design in which each participant undergoes multiple experimental conditions (silence and different musical conditions), allowing intra-individual comparisons.

### ➤ Study Design Overview

- Design: Within-subject repeated measures experimental design
- Sample size:  $n = 40$  healthy adults
- Conditions (within-subject factor):
  - Silence (baseline and final control)
  - Structured musical conditions (valence  $\times$  rhythm)
  - Self-selected music condition(s)
- Primary comparison: Differences in pain and anxiety outcomes across experimental conditions

### ➤ Objectives and Hypotheses

#### Primary Objective

To evaluate whether music listening enhances pain tolerance during the cold pressor test compared to silence.

Primary Hypothesis (H1):

Music conditions will significantly reduce pain intensity compared to silence.

### Secondary Objectives

1. Assess the effect of musical structure (valence, rhythm)
2. Evaluate cultural contextualization effects
3. Evaluate individual preference effects
4. Examine interaction effects between musical factors

### ➤ Outcome Measures

#### Primary Outcome

- Pain tolerance: Duration of hand immersion

#### Secondary Outcomes

- Pain intensity (Visual Analog Scale – VAS)
- Reaction time to pain onset
- Qualitative feedback (exploratory analysis)

### ➤ Data Structure

- Within-subject factor: Condition (7–8 levels)
- Repeated measures:  
Measurements taken: during
- Covariates:
  - Age
  - Musical expertise
  - Music preference

### ➤ General Statistical Principles

- Significance level ( $\alpha$ ): 0.05
- Two-tailed tests
- Software: Python
- Data presentation:
  - Mean  $\pm$  SD for normally distributed variables
  - Median (IQR) for non-normal data

## ➤ Assumption Testing

Before parametric analyses:

Normality

- Shapiro–Wilk test
- Q-Q plots

## ➤ Primary Analysis

Effect of music on pain tolerance

- Test: Repeated Measures ANOVA
- Factor: Condition (within-subject)

Model: Pain ~ Condition

Post-hoc comparisons: Bonferroni correction

## ➤ Secondary Analyses

### 1. Musical Structure Effects

- Factors:
  - Valence (pleasant vs unpleasant)
  - Rhythm (measured vs non-measured)
- Test: Two-way repeated measures ANOVA

Model:

Pain ~ Valence × Rhythm

### 2. Individual Preference Effect

- Compare:
  - Self-selected music vs imposed music
- Test: repeated measures ANOVA

### 3. Reaction time to pain

- If normally distributed → ANOVA
- If skewed → non-parametric test (Friedman test)

## ➤ Non-Parametric Analyses

If assumptions are violated:

- Friedman Test (alternative to repeated measures ANOVA)
- Wilcoxon Signed-Rank Test (post-hoc comparisons)

## ➤ Exploratory Analyses

Correlation Analyses

- Between:
  - Music preference scores
  - Pain reduction
- Test:
  - Pearson (normal data)
  - Spearman (non-normal)