Laser Speckle Contrast Imaging (LSCI) Study Plan

Overview of Experimental Design

1. **Baseline Measurement**
   Record baseline LSCI data from the skin region of interest before any stimulation.
2. **Stimulation of Tissue**
   Apply one or more of the following methods to elicit a change in local skin perfusion:
   - **Heating pad** (localized warming)
   - **Cooling pad** (localized cooling)
   - **Warming ointment** (e.g., capsaicin-based)
   - **Other ideas** (e.g., postural changes, exercise, or reactive hyperemia tests)
3. **Measurement Shortly After Stimulation**
   Acquire LSCI data immediately or shortly after application of the stimulus to capture the early phase of the physiological response.
4. **Concluding Measurement**
   Perform a final LSCI measurement after the stimulus has been in place for a suitable duration or has been removed.

Detailed Protocol

Baseline Measurement

1. **Preparation**
   - Ensure the participant is comfortably seated or lying down.
   - Acclimate the participant to room temperature conditions for 5–10 minutes.
   - Clean the skin area to be imaged if using an ointment later.
2. **Baseline LSCI Recording**
   - Record a short video (e.g., 30–60 seconds) of speckle patterns from the region of interest.
   - Note environmental conditions: temperature, humidity, lighting consistency.

Heating Pad Stimulation

1. **Application of Heating Pad**
    - Place a heating pad on/around the measurement area.
    - Set a consistent, comfortable temperature (e.g., 38–42 °C).
2. **Immediate Post-Stimulation Measurement**
    - Within 1–2 minutes of applying the heating pad, start another LSCI recording (e.g., 30–60 seconds).
3. **Ongoing Stimulation**
    - Keep the heating pad on for a predefined duration (e.g., 5–10 minutes).
    - Optionally, perform intermittent LSCI measurements (e.g., every 2–3 minutes).
4. **Concluding Measurement**
    - After the heating pad has been in place for your set duration (or immediately after removing it), record a final LSCI measurement.
5. **Expected Reaction**
    - **Increased perfusion** due to vasodilation caused by warming.
    - Speckle contrast analysis should show a decrease in contrast over time (indicative of higher flow).

Cooling Pad Stimulation

1. **Removal of Heating Pad** (if applicable) and Rest
    - Allow the tissue to return to near-baseline for 5–10 minutes.
2. **Application of Cooling Pad**
    - Place a cooling pad (e.g., 15–20 °C) on the same or a different skin area.
3. **Immediate Post-Stimulation Measurement**
    - Within 1–2 minutes of placing the cooling pad, record an LSCI measurement (30–60 seconds).
4. **Ongoing Stimulation**
    - Keep the cooling pad in place for 5–10 minutes, taking intermittent measurements.
5. **Concluding Measurement**

- After the cooling pad has been applied for the set duration, record a final LSCI measurement.

6. **Expected Reaction**
   - **Decreased perfusion** due to vasoconstriction under cold.
   - Speckle contrast should increase (indicating lower flow).

Warming Ointment Stimulation (Optional Example)

1. **Application of Ointment**
   - Apply a thin layer of a warming ointment to the skin.
   - Massage gently to ensure even coverage.
2. **Immediate Post-Stimulation Measurement**
   - Record an LSCI measurement about 1–2 minutes after application.
3. **Ongoing Monitoring**
   - Depending on the ointment, vasodilatory effects may develop over 5–15 minutes. Measure at intervals (e.g., 5, 10, 15 minutes post-application).
4. **Concluding Measurement**
   - Take a final LSCI measurement after the vasodilatory effect peaks.
5. **Expected Reaction**
   - **Increased perfusion** through chemical vasodilation.
   - Speckle contrast should decrease (indicating increased blood flow).

Summary of Expected Changes

Stimulation	**Mechanism**	**Expected LSCI Reaction**
Heating Pad/Ointment	Vasodilation	Decreased speckle contrast (higher flow)
Cooling Pad	Vasoconstriction	Increased speckle contrast (lower flow)

Practical Considerations

1. **Standardization**
    - Maintain consistent room temperature, humidity, and lighting.
    - Perform baseline measurements consistently.
2. **Data Analysis**
    - Use the same region of interest (ROI) for each measurement.
    - Compare speckle contrast or perfusion indices across baseline, stimulation, and concluding measurements.
3. **Repetition and Controls**
    - Repeat each condition on different participants.

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