**A couple of people sitting at a table

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Description

Itch & Ketamine

**Lars Arendt-Nielsen and Silvia Lo Vecchio**

Logo

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# OVERVIEW

The study consists of two sessions:

**1st session**

* *Creams applications:*

Application of 4 creams for 1 hours and 30 minutes in a randomized order. The creams will be applied 30 minutes apart from the previous.

* Ketamine cream
* Amitriptyline cream
* ketamine + amitriptyline cream
* Vehicle cream
* *Pruritogens applications:*

Application of histamine and cowhage will be randomized between sessions. In each session one pruritogen will be applied in all 4 areas.

* *Measurements:*

The following measurements will be performed in each area following pruritogens application:

1. VAS measurements of itch and pain (10 minutes)
2. Superficial blood perfusion (SBP)
3. Touch Pleasantness (TP)
4. Mechanically evoked itch (MEI), intensity approach
5. Mechanically evoked itch, spatial approach
6. Mechanical Pain Thresholds (MPT) use a scale with pinprick numbers
7. Mechanical Pain Sensitivity (MPS), intensity approach (VAS)
8. Mechanical Pain Sensitivity (MPS), spatial approach
9. Cold Detection Thresholds (CDT)
10. Warm Detection Thresholds (WDT)
11. Cold Pain Thresholds (CPT)
12. Heat Pain Thresholds (HPT)
13. Pain to Supra-threshold Heat Stimuli (STHS)

**2nd session**

The second session will be identical to the 1st session, only the pruritogen applied will change. Ex. If histamine is applied in the 1st session, cowhage will be applied in the 2nd session.

# Experimental Procedures

**Touch Pleasantness (TP)**

HOW TO PERFORM THE MEASUREMENT

For the Touch Pleasantness (TP) measurement start from the center of the are of interest (AOI). You will perform 3 measurements, each consisting of three brush strokes. Starting about 1.5 cm outside the area, gently stroke the skin in the same direction 3 times in short succession (approximately 1 s in between) for a length of approximately 3 cm. Remember always to perform the strokes holding the brush perpendicular to the skin surface and with a speed of 3-6 cm/sec (See figure 1A). During the stimulation, please ask the subject to close their eyes or look away and to only focus on their sensation. After the 3 stimulations, you should ask the subject to rate their sensation on the chosen scale. Remember to always check with the subject if their rating corresponds to the felt sensation. Repeat the sequence two more times, each time moving the brush in a different direction (See Figure 1B).

A close-up of a hand holding a yellow brush

Description automatically generated

HOW TO INSTRUCT THE SUBJECT

The stimulation will be performed using this brush (show the brush to the subject). During the stimulation, I will stroke your skin 3 times. While I perform this procedure, I will ask you to look away or alternatively close your eyes so that you can only focus on your perception. After the 3 strokes, you should rate the mean intensity of the sensation you perceived on a scale labeled “very unpleasant” and “very pleasant” at the extremity and “neutral” at the center. You will use the side slider on the scale to choose the rating that corresponds to your feeling, as show in the picture (the picture below will appear on the screen in front of the subject).

A red and black rectangle with a black background

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The all procedure will be repeat two more times, and after each stimulation, I will ask you to rate the itch intensity on the scale. It is important you understand that there is not right or wrong answers, but I ask you to be focused during the entire procedure and to be as honest as possible about what you will feel during each stimulation.

**Mechanically Evoked Itch (MEI): Intensity Approach**

HOW TO PERFORM THE MEASUREMENT

For the Mechanically Evoked Itch (MEI) measurement, using the intensity approach, start from the center of the are of interest (AOI). You will use 3 different von Frey filaments with a force of 1, 1.4 and 2 gr respectively (size 4.08, 4.16, and 4.31, respectively, see figure below). Starting about 0.5 cm outside the area, perform 3 stimulations (a single stimulation is composed of 3 pricks in short succession, approximately 1 s in between) with each filament. Remember always to perform each stimulation holding the von Frey perpendicular to the skin surface and then bend it slightly (try to bend the filament always applying the same force). It is recommended that this assessment is always performed by the same operator. During the stimulation, please ask the subject to close their eyes or look away and to only focus on their sensation. After the 3 stimulations, you should ask the subject to rate the itch sensation on the chosen scale. Remember to always check with the subject if their rating corresponds to the felt sensation. Repeat the sequence two more times, each time applying the filament on a different spot in the area.

A white and purple hammer

Description automatically generated

HOW TO INSTRUCT THE SUBJECT

The stimulation will be performed using this 3 filaments (show each filament to the subject). During the stimulation, I will prick your skin 3 times with each one. While I perform this procedure, I will ask you to look away or alternatively close your eyes so that you can only focus on your perception. After the 3 prick, you should rate the mean intensity of the itch sensation you perceived on a scale ranging from 0 to 10, where 0 means “no itch” and 10 means “worst imaginable itch”. You will use the side slider on the scale to choose the rating number that corresponds to your feeling, as show in the picture (the picture below will appear on the screen in front of the subject).

A screenshot of a computer

Description automatically generated

The all procedure will be repeat two more times, and after each stimulation, I will ask you to rate the itch intensity on the scale. It is important you understand that there is not right or wrong answers, but I ask you to be focused during the entire procedure and to be as honest as possible about what you will feel during each stimulation.

**Mechanically Evoked Itch (MEI): Spatial Approach**

HOW TO PERFORM THE MEASUREMENT

For the Mechanically Evoked Itch (MEI) measurement, using the spatial approach, use the plastic template showed in figure 1A. The template is formed by 4 vectors located at 45 degrees angles to each other. Each vector is formed by holes located at 0.5 cm intervals along the vector. All vectors will be represented by 8 lines numbered from 1 to 8 as showed in figure 1 A. Firstly, align the center of the plastic template with the center of the are of interest (AOI, Figure 1B). From the previous MEI assessment, the program will automatically select the von Frey filament that evoked the highest itch intensity. This von Frey should be used to carried out this assessment. Starting with the selected filament, you will insert it in the furthest point (from the center) of vector 1 and you will prick in each hole going towards the center of the AOI (the stimulation in each point should take approx. 2 sec). The same pressure should be applied to each stimulated point. After each prick, you will ask if the subject felt a change in sensation from pressure to a ‘different sensation’, ‘unpleasant’, or ‘itch’ sensation and you will mark that point on the vector and move to the adjacent vector. You will repeat the same procedure along the 8 lines. At the end, you will insert in the program the distance between the marked point and the center for each line and the program will automatically calculate the area of MEI.

A diagram of a hexagon with points

Description automatically generated

HOW TO INSTRUCT THE SUBJECT

The Stimulation will be performed using this von Frey (show the filament to the subject) and this plastic template (show the template to the subject). During the stimulation, I will prick your skin in each hole along 8 different lines. While I perform this procedure, I will ask you to look away or alternatively close your eyes so that you can only focus on your perception. After each prick, I will ask you if you felt a change in sensation from pressure to a ‘different sensation’, ‘unpleasant’, or ‘itch’ sensation and you will answer “yes” or “no”.

The all procedure will be repeat along the 8 lines, and after each stimulation, I will ask you if you felt aa change in sensation. It is important you understand that there is not right or wrong answers, but I ask you to be focused during the entire procedure and to be as honest as possible about what you will feel during each stimulation.

**Mechanically Pain Threshold (MPT)**

HOW TO PERFORM THE MEASUREMENT

For the Mechanically Pain Threshold (MPT) measurement you will use a pinprick set. The set consists of 7 stimulators having all the same diameter of 0.6 mm, but different application force: 8, 16, 32, 64, 128, 256, and 512 millinewton (mN), respectively. Starting with the lightest stimulator, apply each stimulator in sequence. the stimulation should be performed at a rate of of 2 sec on, 2 sec off in an ascending order until the first perception of sharpness is reached. Starting with the lightest stimulator, gently press it against the skin and ask the subject if he/she feels a change in sensation from pressure to a ‘different sensation’, ‘unpleasant’, or ‘burning pain’ sensation. Continue with each stimulator in ascending order until the subject answer “yes” to the question. At this point you follow the following diagram (N= no; Y= yes):

8 16 32 64 128 256 512

N N N Y

At this point you select again the previous pin (in this case 32): if the subject answer “No” than you mark the 64 pin as the threshold.

Es:

8 16 32 64 128 256 512

N N N Y

N 64 is the threshold.

If the subjects answer “Yes” then you select the previous pin again (16) and you continue in descending order until the subject does not feel the change in perception.

Es:

8 16 32 64 128 256 512

N N N Y

Y

Y

N 16 is the threshold.

Repeat this method for a total of 5 times, always starting with the pin below the threshold you found. The final threshold is the geometric mean of five series of ascending and descending stimuli. If the subjects report a threshold below 8, please mark it as 0, if the subjects does not feel any change in sensation after the pin 512, please report 1024 as the threshold.

The stimulator itself will guarantee that the same pressure is applied to each stimulated point. During the stimulation, please ask the subject to close their eyes or look away and to only focus on their sensation. The final threshold is the geometric mean of five series of ascending and descending stimuli (see figure below).



HOW TO INSTRUCT THE SUBJECT

The stimulation will be performed using this pinprick stimulator (show the stimulator to the subject) and this plastic template (show the template to the subject). During the stimulation, I will prick your skin in each hole along 8 different lines. While I perform this procedure, I will ask you to look away or alternatively close your eyes so that you can only focus on your perception. After each prick, I will ask you if you felt a change in sensation from pressure to a ‘different sensation’, ‘unpleasant’, or ‘burning pain’ sensation and you will answer “yes” or “no”.

The all procedure will be repeat along the 8 lines, and after each stimulation, I will ask you if you felt aa change in sensation. It is important you understand that there is not right or wrong answers, but I ask you to be focused during the entire procedure and to be as honest as possible about what you will feel during each stimulation.

A close-up of a pen

Description automatically generated

**Mechanically Pain Sensitivity (MPS): Intensity Approach**

HOW TO PERFORM THE MEASUREMENT

For the Mechanically Pain Sensitivity (MPS) measurement, using the intensity approach, you will use a pinprick set. The set consists of 7 stimulators having all the same diameter of 0.6 mm, but different application force: 8, 16, 32, 64, 128, 256, and 512 millinewton (mN), respectively (the figure below shows you the image of a pinprick stimulator). Starting from the lightest stimulators, apply each pinprick on the area of interest (AOI) at a rate of 2 sec on, 2 sec off in an ascending order. Remember always to perform each stimulation holding the stimulator perpendicular to the skin surface. The stimulator itself will guarantee that the same pressure is applied to each stimulated point. During the stimulation, please ask the subject to close their eyes or look away and to only focus on their sensation. After each pinprick stimulation, you should ask the subject to rate the itch sensation on the VAS scale ranging from 0 to 10, where 0 means “no pain” and 10 means “worst imaginable pain”. Repeat the stimulation witch each pinprick. The entire sequence will be repeated two times.

A close-up of a pen

Description automatically generated

HOW TO INSTRUCT THE SUBJECT

The stimulation will be performed using a set of 7 stimulators (show the pinprick set to the subject). During the stimulation, I will prick your once with each stimulator. While I perform this procedure, I will ask you to look away or alternatively close your eyes so that you can only focus on your perception. After each prick, you should rate the mean intensity of the itch sensation you perceived on a scale ranging from 0 to 10, where 0 means “no pain” and 10 means “worst imaginable pain”. You will use the slider on the scale to choose the rating that corresponds to your feeling, as show in the picture (the picture below will appear on the screen in front of the subject).

A red and black rectangle with a black background

Description automatically generated

The all procedure will be repeat two times, and after each stimulation, I will ask you to rate the pain intensity on the scale. It is important you understand that there is not right or wrong answers, but I ask you to be focused during the entire procedure and to be as honest as possible about what you will feel during each stimulation.

**Mechanically Pain Sensitivity (MPS): Spatial Approach**

HOW TO PERFORM THE MEASUREMENT

For the Mechanically Pain Sensitivity (MPS) measurement, using the spatial approach, use the plastic template showed in figure 1A. The template is formed by 4 vectors located at 45 degrees angles to each other. Each vector is formed by holes located at 0.5 cm intervals along the vector. All vectors will be represented by 8 lines numbered from 1 to 8 as showed in figure 1 A. Firstly, align the center of the plastic template with the center of the are of interest (AOI, Figure 1B). From the previous Mechanical Pain Threshold (MPT) assessment, the program will automatically calculate the geometric mean and select the stimulator that should be used to carried out this assessment. Starting with the selected stimulator, you will insert it in the furthest point (from the center) of vector 1 and you will prick in each hole going towards the center of the AOI (the stimulation in each point should take approx. 2 sec). The stimulator itself will guarantee that the same pressure is applied to each stimulated point. After each prick, you will ask if the subject felt a change in sensation from pressure to a ‘different sensation’, ‘unpleasant’, or ‘burning pain’ sensation and you will mark that point on the vector and move to the adjacent vector. You will repeat the same procedure along the 8 lines. At the end, you will insert in the program the distance between the marked point and the center for each line and the program will automatically calculate the area of MPS.

A diagram of a hexagon with points

Description automatically generated

HOW TO INSTRUCT THE SUBJECT

The stimulation will be performed using this pinprick stimulator (show the stimulator to the subject) and this plastic template (show the template to the subject). During the stimulation, I will prick your skin in each hole along 8 different lines. While I perform this procedure, I will ask you to look away or alternatively close your eyes so that you can only focus on your perception. After each prick, I will ask you if you felt a change in sensation from pressure to a ‘different sensation’, ‘unpleasant’, or ‘burning pain’ sensation and you will answer “yes” or “no”.

The all procedure will be repeat along the 8 lines, and after each stimulation, I will ask you if you felt aa change in sensation. It is important you understand that there is not right or wrong answers, but I ask you to be focused during the entire procedure and to be as honest as possible about what you will feel during each stimulation.

**Thermal Sensitivity**

HOW TO PERFORM THE MEASUREMENT

The tests of thermal sensations will be conducted using a PATHWAY ATS (Medoc Ltd, Israel) thermal sensory testing device. A thermode stimulator of 3x3 cm will be placed on the treated/placebo areas and kept in place by means of Velcro tape. During the first 8 seconds, the temperature increases or decreases 1°C per second from a starting temperature of 32°C until the subject identify a specific threshold and presses a button that will terminate the measurement and return the temperature to baseline at a rate of 5°C /s. A cut-off temperature of 52°C will be used.

The specific thresholds are the following:

- a perception of cold or a decrease in temperature for the Cold Detection Threshold (CDT),

- a perception of warm or an increase in temperature for the Warm Detection Threshold (WDT),

- a pain sensation induced by cold for the Cold Pain Threshold (CPT),

- a pain sensation induced by heat for the Heat Pain Threshold (HPT).

Each threshold was detected three times, and an arithmetic mean was calculated (see below picture).

A diagram of a diagram

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HOW TO INSTRUCT THE SUBJECT

The stimulation will be performed using this thermode (show the thermode to the subject). During the stimulation, the thermode will be placed on the treated/placebo areas and attached with Velcro tape. During the first 8 seconds, the temperature increases or decreases 1°C per second from a starting temperature of 32°C until you identify a specific threshold and press a button that will terminate the measurement. While I perform this procedure, I will ask you to look away or alternatively close your eyes so that you can only focus on your perception.

The threshold will be measures 3 times for a total of 12 measurements. It is important you understand that there is not right or wrong answers, but I ask you to be focused during the entire procedure and to be as honest as possible about what you will feel during each stimulation.

**Pain to Supra-threshold Heat Stimuli (STHS)**

HOW TO PERFORM THE MEASUREMENT

The tests of Pain to Supra-threshold Heat Stimuli (STHS) will be conducted using a PATHWAY ATS (Medoc Ltd, Israel) thermal sensory testing device. A thermode stimulator of 3x3 cm will be placed on the treated/placebo areas and kept in place by means of Velcro tape. Starting with a temperature of 32°C, the probe will increase the temperature until it reaches 50°C for 3 sec and then return to the initial temperature with a rate of 5°C /sec (see figure below).

A blue line on a black background

Description automatically generated

At the end, the subjects will rate the pain he/she perceived during the stimulation on a scale ranging from 0 to 10, where 0 means “no itch” and 10 means “worst imaginable itch”. The same stimulation will be performed twice, and the average will be calculated.

HOW TO INSTRUCT THE SUBJECT

The stimulation will be performed using this thermode (show the thermode to the subject). During the stimulation, the thermode will be placed on the treated/placebo areas and attached with Velcro tape. Starting with a temperature of 32°C, the probe will increase the temperature for 3 sec and then return to the initial temperature. At the end of each stimulation, you will report the pain you felt on a scale ranging from 0 to 10, where 0 means “no itch” and 10 means “worst imaginable itch”. You will use the side slider on the scale to choose the rating number that corresponds to your feeling, as show in the picture (the picture below will appear on the screen in front of the subject).

**A white rectangular object with blue and black dots

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