

Order of procedure:

Morning 1:

1. Heart rate variability
2. N-back test
3. Blood draw
4. Temporal summation
5. Conditioned pain modulation
6. 6-minute walk test
7. Baseline QST
8. HFS
9. Questionnaires
10. Follow-up QST
11. Flu vaccine

Morning 2:

1. Heart rate variability
2. N-back test
3. 24-hour follow-up QST
4. Blood draw
5. Temporal summation
6. Conditioned pain modulation
7. 6-minute walk test
8. Questionnaires

Morning 1

Introduction

Welcome and thank you for being willing to participate. My name is Gill, and I will be doing the procedure with you today and tomorrow morning.

Consent

Please read the consent form again. I am going to ask you a few questions to make sure you fully understand what you have read and then if you agree to participate. I will need you to sign the form.

[check for queries]

[sign full study consent form]

Eligibility

Before we start, I will need to ask you a few questions to confirm that you are eligible to participate in this study.

[perform suicidality and acute psychosis screening]

Preparation

Now, we will begin the procedure. I will speak from this script throughout the procedure, just so that everything is standardised.

HRV

First, I am going to place these electrodes onto your chest like this *[show picture of ECG placements – remove jewellery near electrodes]*. These electrodes measure your heart rate. I am going to gently exfoliate your skin and then clean your skin with this alcohol swab and then I will stick these electrodes on your chest.

Right, we are going to use this computer during the procedure. Please follow the instructions on the screen.

[put TruCulture in heat block at 37°C and time 15 min]

N-back test

[make sure participant understands the task and assist with practice trial] Okay, now we will do the task for real. It is a bit faster than the practice round. There are also multiple trials. Some trials are like this one (i.e. the practice trial) where you need to remember the letter that was 1 before. But some ask you to remember the letter that was 2 before, and then some ask you to remember the letter that was 3 before. But at the beginning of each trial, it will tell you which order of letters you need to remember. Please keep your hand ready on the spacebar and respond as quickly and accurately as possible. *[make sure participant understands the task before starting]*.

[remove TruCulture from the heat block and check that it's completely defrosted]

Blood draw

Now, I am going to take a small sample of blood from your arm.

[put TruCulture sample back in heat block at 37°C and set timer for 24 hours]

[put unstimulated sample to rest upright for 30 min]

Scale intro

We use this scale in the experiment so it's important that you understand it.

[Show SPARS and read it to the participant. Answer any questions.]

This scale runs from -50, which is no sensation – you don't feel anything – through 0, the exact point at which what you feel transitions to pain, to +50, which is the most intense pain you can imagine. If I give you

a stimulus and it was non-painful, you'll rate it between -50 and 0 *[show on scale]*. A rating closer to -50 means the stimulus was less intense, and closer to 0 means it was more intense and closer to being painful – but not yet painful, because it's still below zero and in the non-painful range of the scale. If the stimulus is painful, then you'll rate it between 0 and +50. A higher positive number means more painful. 0 is the exact point of transition between not painful and painful.

[there are 3 practice rounds]

Practice 1: When you receive a stimulus, first decide if it was painful or non-painful. Then you can work out where in the appropriate range it fell. Click on the scale and press the 'SPACE BAR' when you are happy with your response.

[Researcher to first demonstrate how to submit rating].

Practice 2. Now, you can try. *[show participant the 38mN VFF - as you can see, this filament isn't sharp, and bends when it touches the skin, demonstrate on yourself and then give them a stimulation on their hand]*. Can you rate how that felt using the scale?

Practice 3. *[repeat with 256mN]*. Can you rate how that felt using the scale?

[after each practice round, check in with them to make sure that they understood the scale. E.g. if they rate it close to -50 say something like, "okay, so it was non-painful and really light, like you almost didn't feel it?"].

Preparation for TS and CPM

We will perform 2 different sensory tests at your shoulder and at your lower back. First, I am going to clean your skin and then put a small mark on your shoulder and lower back so that I know where to do the tests.

[shoulder – deltoid insertion – opposite arm to blood draw]

[lower back – L2/3 – 2cm lateral, towards direction of shoulder site]

Temporal summation – 256mN

Now, I am going to introduce you to two of the sensory tests that will perform on your upper arm and lower back. For the first test, I will touch you once with this filament and then need to give me a rating on the scale for how it felt. *[give one stimulation]*.

Now, I am going to touch you a few times in a row and then I need you to give me rating for the very last one using the scale. I will let you know when the last one is coming, I will say, "this one". *[give 16 stimulations]*.

[repeat on lower back].

Pain pressure threshold

For the second sensory test, we will use this device *[show pressure algometer]*. I am going to push this device against your skin. You will feel some pressure. I want you to tell me when the pressure becomes pain. We are not looking to see how tough you are or how much pain you can handle. We just want you can say 'now'/'yes' when the pressure becomes pain.

[perform PPT twice at deltoid and lumbar each]

Ice bucket

Next, I will need you to put your hand *[opposite hand to blood draw]* into this cold water. We want your whole hand, up until your wrist in the bucket. Once your hand is in the bucket, I need you to tell me when the pain in your hand reaches a 20 on the scale – so moderately painful. Once it reaches 20, I will re-do the sensory test with this device (pressure algometer) on your arm and lower back. Very importantly, please keep your hand in the bucket while I am re-doing the sensory tests. I will tell you when you can remove your hand. Here is a towel to dry and warm up your hand straight afterwards.

Okay, so it's hand in the bucket up until your wrist, tell me when the pain in your hand is 20 on the scale, and keep your hand in the bucket while we redo the tests with this device at your shoulder and lower back.

[perform cold water immersion and PPT]

Now, I want you to tell me when your hand feels normal again. I will then perform the tests again but you won't have to put your hand back in the water.

[re-do PPT at deltoid and lumbar when participant reports hand feeling normal again].

6MWT

[assess baseline HR for 60min]

The object of this task is to walk/run as far as possible for 6 minutes. You will walk/run back and forth in this hallway. While you are walking or running, we want you to maintain a rate of perceived exertion of 13 on this scale, which is 'somewhat hard' *[show scale]*. This scale runs from no exertion; sitting and resting through to maximal exertion. We want you to maintain a rate of perceived exertion of 13, which is somewhat hard. You can walk or run, speed up or slow down, whatever you need to do to maintain a rate of perceived exertion of 13, which is somewhat hard.

At 1 minute: "You are doing well. 1 minute down, 5 minutes to go."

At 2 minutes: "Keep up the good work. 2 minutes down, 4 minutes to go."

At 3 minutes: "You are doing well. You are halfway done. Remember to maintain a perceived exertion of somewhat hard".

At 4 minutes: "Keep up the good work. 2 minutes to go."

At 5 minutes: "You are doing well. Only have 1 minute to go."

With 15 seconds to go: "In a moment I'm going to tell you to stop. When I do, just stop right where you are and I will come to you."

At 6 minutes: "Stop" Remain where you are, please.

[assess HR directly after for 60min]

[assess HR 3min after for 1min]

Marking radial lines and electrode placement

Now I'm going to mark up your arm for the testing procedure.

[mark up using foam template, with A towards the cubital fossa; centre of radial lines approximately 8cm from cubital fossa but NOT on prominent vein or scarred area.]

These are the electrodes we use. This one has some blunt pins. The pins do not penetrate your skin. I will place them on your skin like this, and they will stay there for the whole procedure. *[Strap electrode goes around upper arm. Disc electrode approximately 8cm from cubital fossa.]*

Detection threshold testing

This next test is not about pain, it is about feeling an electrical stimulus. We start at an intensity of zero. I will gradually increase the intensity until you tell me that you can feel it. Please say "yes" if you feel it, even a little bit. It will feel like a very tiny pinprick.

[find threshold for each arm; choose most sensible approximation, write it down, set DS7A to that level and then flick switch to x10].

Test battery intro

Each time we test your skin, we will use 6 different tests. Each test uses a different kind of stimulus: we can

- Touch you lightly with this filament (demo VFF)
- Brush your skin clearly with a brush (demo)

- Press a tiny, blunt-ended metal rod against your skin (demo both)
- Or give you a single electrical stimulus - which I won't do now because we haven't attached the electrodes yet.

You can feel that the sensations evoked by the different tests can be quite distinct in nature. We will ask you to report what you feel on the scale. Do not try to rate the different tests relative to one another. Each time we test you with a new modality, don't try to compare it to the previous modality. Just consider each test in isolation, and start afresh with the scale. We are interested in your experience of each stimulation modality separately. Remember that -50 means no sensation at all; 0 is the exact point at which what you feel transitions to pain; +50 is the most intense pain you can imagine. Please stay with these reference points during the whole experiment! I will ask you to close your eyes when we test your skin, but in between the test runs you can open your eyes and give a rating.

Orientation

Now I'll run you through the test battery so you get a chance to practice giving ratings for each stimulus modality. When we start the experiment, you'll need to give your rating within about 5 seconds, but for now we have more time.

[perform full test battery on arm]

Baseline testing

[perform full test battery on arm]

Explanation of HFS

The intense electrical stimulation is the part of the procedure that most people find moderately painful. The stimulation takes one minute in total, but it is split up into 5 trains. Each train lasts one second, and then you get a 9-second break. So you'll have one second of stimulation, then 9 seconds' break, one second of stimulation, 9 seconds' break - and so on.

I will count you through the trains. As a safety precaution, I will keep my finger on the safety switch so that if you decide you want to pull out of the study you can say 'STOP' and I will immediately flick the switch down to deactivate the stimulator. I'll be ready in case you need me to stop it. If you pull out, we won't be able to continue with the study. As I say, there are 5 trains, and each one lasts one second before you get 9 seconds' break - so just count yourself through. Please give rating on the scale for each train.

HFS

[Start stopwatch] at first train. Note clock time of HFS onto participation record.

Questionnaires

Now, you will need to answer a few questions on the screen.

30min: Surface area

35min: Magnitude

40min: HRV

45min: Surface area

50min: Magnitude

60min: Surface area

65min: Magnitude

Surface area testing 128mN VFF

Now, I will also use this *[show 128mN VFF]* and I am going to give you a stimulation here *[show distal of electrode]* and here *[show right next to electrode]* and I want you to tell me if there is an obvious increase in sensitivity.

Does it feel different if I touch you here *[distal]...* and here *[adjacent to electrode]*?
[if no, repeat from proximal to electrode]

[if there is a difference, map the area]. Okay, now I am going to give you stimulations up each line and I want you to tell me when you feel a distinct increase in sensitivity please say "now".

Flu vaccine

Last, we will give the vaccine and then we are finished for today.

Morning 2

Welcome back

[check in with participant for any side effects of the flu vaccine]. Welcome back, how are you feeling today? Did you notice any side effects from the flu vaccine?

Preparation

Today will be very similar to yesterday but without the electrical stimulation. Therefore, today's session will be a lot shorter and only last about 45 min – 1 hour.

Check HFS site

How does the skin on your forearm feel today? *[note down what the participant says]*. Do you remember this scale from yesterday? *[show the SPARS and briefly explain it again. Be sure that the participant remembers and understands it again]*. Just like yesterday, I am going to use these tools to stimulate your skin and you'll need to give me a rating for how each stimulation feels using this scale.

[both pinpricks, brush, and 36 mN VFF].

HRV

Same as yesterday, I am going to place these electrodes onto your chest like this *[show picture of ECG placements – remove jewellery near electrodes]*. These electrodes measure your heart rate. I am going to gently exfoliate your skin and then clean your skin with this alcohol swab and then I will stick these electrodes on your chest.

Right, we are going to use this computer during the procedure. Please follow the instructions on the screen.

N-back test

[make sure participant remembers and understands the task and assist with practice trial] Remember, there are also multiple trials. Some trials are like this one (i.e. the practice trial) where you need to remember the letter that was 1 before. But some ask you to remember the letter that was 2 before, and then some ask you to remember the letter that was 3 before. But at the beginning of each trial, it will tell you which order of letters you need to remember. Please keep your hand ready on the spacebar and respond as quickly and accurately as possible. *[make sure participant understands the task before starting]*.

Blood draw

Now, I am going to take a small sample of blood from your arm.

[put unstimulated sample to rest upright for 30 min]

Scale intro

[check that they remember how to use the scale. Go through it quickly again to remind them].

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[perform cold water immersion and PPT]

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Questionnaires

End procedure

[compensate, ask participant to sign receipt for compensation].