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The effects of cannabidiol on psychosocial stress, situational anxiety and nausea in a virtual reality environment: A protocol for a randomised clinical trial

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ABSTRACT

Introduction: The non-intoxicating plant-derived cannabinoid, *cannabidiol* (CBD), has demonstrated therapeutic potential in many clinical conditions, including epilepsy, anxiety and psychosis. Most successful clinical trials have utilised relatively high (≥ 300 mg) oral doses of CBD. Relatively few studies have investigated the efficacy of lower (<300 mg) oral doses, typical of those available in over-the-counter CBD products.

Methods: We present a protocol for a randomised, double-blind, placebo-controlled, parallel-group clinical trial investigating the effects of a low oral dose (150 mg) of CBD on acute psychosocial stress, situational anxiety, motion sickness and cybersickness in healthy individuals. Participants (n=74) will receive 150 mg of CBD or a matched placebo 90 minutes before completing three virtual reality (VR) challenges (tasks) designed to induce transient stress and motion sickness: (1) a 15-minute "Public Speaking" task; (2) a 5-minute "Walk the Plank" task (above a sheer drop); and (3) a 5-minute "Rollercoaster Ride" task. The primary outcomes will be self-reported stress and nausea measured on 100-mm visual analogue scales. Secondary outcomes will include salivary cortisol concentrations, skin conductance, heart rate, and vomiting episodes (if any). Statistical analyses will test the hypothesis that CBD reduces nausea and attenuates subjective, endocrine, and physiological responses to stress compared to placebo. This study will indicate whether low-dose oral CBD has positive effects in reducing acute psychosocial stress, situational anxiety, motion sickness and cybersickness.

Ethics and dissemination: The University of Sydney Human Research Ethics Committee has granted approval (2023/307, Version 1.5, 28th September 2023). Study findings will be disseminated in a peer-reviewed journal and at academic conferences.

Trial Registration Number: ACTRN12623000872639 (ANZCTR public trials registry, registered 15th August 2023).

Keywords: cannabidiol (CBD), nausea, anxiety, virtual reality.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study will use a robust randomised, double-blind, placebo-controlled design to investigate the effects of a low oral dose (150 mg) of cannabidiol.
- Virtual reality (VR) will be used to administer safe, realistic, and precisely reproducible test paradigms that induce transient stress and nausea in healthy volunteers.
- This study will provide novel insights into the potential efficacy of low doses of CBD to ameliorate stress and nausea, with possible relevance to clinical populations.
- The study only involves a single test session in a laboratory environment and may, therefore, lack ecological validity.

1. INTRODUCTION

Cannabidiol (CBD) is a non-intoxicating constituent of the *Cannabis sativa* plant [1, 2]. It has a good safety and tolerability profile [3-7] and a diverse range of pharmacological targets, including the serotonin receptors (e.g., 5-HT1A), G protein-coupled receptors (e.g., GPR55 and GPR18) and transient receptor potential ion channels (e.g. TRPA1) [8-10]. CBD has demonstrated therapeutic potential in several clinical conditions [4, 5], including anticonvulsant effects in paediatric epilepsy [11-13], anxiolytic effects in anxiety disorders [14, 15], antipsychotic effects in schizophrenia [16, 17] and "anti-addiction" effects in substance use disorders [18, 19]. These clinical benefits are typically observed at relatively high oral doses of CBD (e.g., ~300-1500mg) [4, 5].

In regions such as North America and Europe, CBD is available as both a prescription drug and a 'nutraceutical product' [20]. These nutraceutical or "wellness" products are typically oral formulations (e.g., oils, capsules, gummies) that contain low doses of CBD (i.e., ≤150 mg/day) and are widely available in health food stores and pharmacies [20, 21]. In Australia, regulation of "low-dose CBD products" (containing ≤150 mg/day) was recently eased to allow patients without a prescription access to registered products in pharmacies. However, registration of such products requires approval by Australia's medicines regulator based on demonstrated efficacy and safety [22]. With no products having been registered in the ~3 years since these regulatory changes were enacted (December 2020) [22], questions about whether CBD can demonstrate efficacy at these lower doses have been raised.

Indeed, a recent review found little high-quality evidence to support the efficacy of CBD (in any conditions) at doses ≤200 mg. This review acknowledged, however, that such doses were under-studied [21]. Some promising results have been obtained with public speaking tasks designed to induce "psychosocial stress" in healthy volunteers when 300 mg CBD was administered [23, 24]. Therefore, further research investigating the anxiolytic effects of low to moderate oral doses of CBD is warranted. It should be noted that the oral bioavailability of CBD is limited (~13-19%) [25]; therefore, oral formulations containing constituents that enhance the oral bioavailability of CBD may allow greater efficacy at lower doses; such a formulation will be used in the current study.

Virtual reality (VR) technologies are increasingly being used to investigate psychosocial stress and anxiety in laboratory studies [26, 27]. This approach allows for

minimal resourcing relative to "real-world" studies, customisable and reproducible test paradigms and the accurate monitoring and recording of key outcomes [27, 28]. Furthermore, VR provides the advantage of simulating physiologically-provoking activities without real danger e.g., walking a plank over a sheer drop. However, the use of VR is often accompanied by motion sickness (a pattern of symptoms that arise from exposure to stimuli involving significant visual or physical motion) and cybersickness (a sub-type of motion sickness that arises specifically due to exposure to VR) [29-32]. Interestingly, CBD has shown anti-nausea and anti-emetic effects in preclinical studies involving laboratory animals [33-35] and in human studies when used in combination with Δ^9-tetrahydrocannabinol to treat chemotherapy-induced nausea and vomiting [36]. Accordingly, the current study will investigate CBD's possible anti-nausea effects in participants by exposure to VR scenarios [27].

We have developed a series of three unique VR challenges for the current trial. The "Public Speaking" task was adapted from studies that explored the anxiolytic effects of CBD during public speaking challenges [23, 24, 37] and will allow us to determine the effects of CBD during social threat (i.e., psychosocial stress). The 5-minutes "Walk the Plank" task has been modified from previous VR studies requiring participants to walk along a narrow virtual plank above a precipitous drop, inducing physiological markers of acute anxiety [38-40] (i.e., "situational anxiety"). Finally, the "Rollercoaster Ride" task challenges participants to complete two rounds of a virtual rollercoaster ride and has been modelled from VR rollercoaster ride paradigms that have been reliably used to induce motion sickness and cybersickness in participants [30, 41, 42].

In summary, the current protocol describes a study that aims to investigate the effects of low-dose (150 mg) CBD *versus* placebo on virtual reality (VR)-induced acute psychosocial stress, situational anxiety, acute motion sickness and cybersickness in healthy individuals.

2. METHODS

2.1 Study design

This study is a randomised, double-blind, placebo-controlled, parallel-group, clinical trial comparing the efficacy of low-dose CBD (150 mg) *versus* placebo. The study is known as the CAPSTAN (Cannabidiol for Acute Psychosocial Stress and Nausea) clinical trial. A crossover

design was deemed unsuitable due to the high likelihood of trial-order effects (e.g., habituation to the VR scenarios) [30].

The trial sponsor is the University of Sydney, and the trial site is the Brain and Mind Centre in Sydney, Australia. The study has been approved by the University of Sydney Human Research Ethics Committee (HREC; 2023/307, Version 1.5, 28th September 2023) and registered on the Australian New Zealand Clinical Trials Registry (ACTRN12623000872639, 15th August 2023). The study is financially supported by the Lambert Initiative for Cannabinoid Therapeutics, a philanthropically funded centre for cannabinoid research at the University of Sydney. Recruitment commenced on 9th October 2023 and is anticipated to conclude in mid-2024.

2.2 Participant Population

2.2.1 Inclusion Criteria

We aim to recruit 74 participants who will be: (a) healthy adults aged between 18 and 50 years; (b) proficient in English and able to provide informed consent; (c) residing in the Greater Sydney region of New South Wales, Australia; and (d) willing to follow the protocol requirements.

2.2.2 Exclusion Criteria

The following exclusion criteria will apply:

a. Self-reported regular use (i.e., more than twice weekly) of:
    i. Cannabinoid-containing products (e.g., cannabis or CBD)
    ii. Psychotropic drugs (prescriptive or illicit) (e.g., cannabis, amphetamines, cocaine, ecstasy (MDMA), LSD (acid), antidepressants, antiepileptics, opioids, benzodiazepines)
    iii. Medication that may affect the stress response (e.g., corticosteroids, beta-blockers)
b. Self-reported history of allergic reaction (e.g., urticaria or anaphylaxis) to cannabis or cannabinoid-containing products.

c. Self-reported history of liver disease, renal disease, epilepsy or heart disease (excluding medically controlled high blood pressure <140/90mmHg).
d. Current (i.e., within the past 2 weeks) otologic (vestibular) disease.
e. A history of repeated episodes of syncope.
f. Pregnant, lactating, or trying to conceive.
g. Self-reported history of drug and/or alcohol dependence (or suspected drug and/or alcohol dependence as determined by the trial physician).
h. A medically diagnosed anxiety disorder (e.g., social anxiety disorder) within the past 12 months.
i. Current suicidal ideation (i.e., a score >0 on Question 9 of the Patient Health Questionnaire) or suspected suicidal ideation as determined by the trial physician.
j. Current depression, anxiety, and stress scores outside the 'healthy range' on the Depression Anxiety Stress Scale-21 [43] (i.e., > moderate scores for depression (>20), anxiety (>14) and stress (>25)).
k. An uncontrolled chronic medical condition (mental or physical).
l. Self-reported high vulnerability to cybersickness or motion sickness.
m. Frequent (i.e., more than weekly) use of VR technologies, which tends to produce desensitisation towards cybersickness [30].
n. Self-reported intense fear of heights.

2.2.3 Recruitment and Retention

Participants will be recruited via social media, word of mouth, printed or online study advertisements and direct emails to individuals who have previously registered their interest in participating in clinical trials with the Lambert Initiative for Cannabinoid Therapeutics. Participation is voluntary, and participants can withdraw at any time. Participants will be reimbursed with a $200 gift voucher as compensation for time and expenses incurred as a result of study participation.

2.3 Treatments

The treatments will be purchased from Avecho Biotechnology Limited (Clayton, Victoria), manufactured (Catalent Pharma Solutions, St. Petersburg, FL), as well as packaged and labelled (Central Pharmacy Logistics, Coburg North, VIC) at GMP-licenced facilities, stored at the Brain and Mind Centre (in a secure, temperature-controlled room), and dispensed by the trial coordinator (who is also a registered pharmacist) and another blinded investigator.

As this trial utilises a non-clinical (healthy) population, a placebo comparator is the most suitable and ethical choice. Accordingly, an acute dose of CBD (as opposed to chronic administration) will be utilised. Indeed, the treatment of many ailments utilises an *ad hoc* treatment regime.

2.3.1 Intervention

The investigational product (Avecho Biotechnology Limited, Victoria, Australia) is an oil-based, soft-gel capsule. Each gel capsule contains 75 mg of pure, synthetic (-)-CBD enantiomer and 75 mg of 'Tocopheryl Phosphate Mixture' (TPM®) in medium chain triglyceride (MCT) oil. TPM® is a proprietary blend of tocopherol phosphates that has been shown to increase the bioavailability of lipid-soluble substances [44, 45]. The capsules will not contain any other cannabinoids or cannabis constituents.

2.3.1a Safety

CBD is generally considered to have a good safety profile [3, 4, 21, 46, 47]. In previous studies, 150 mg CBD caused a very low frequency of mild adverse events that did not differ from placebo [21]. The current study's comprehensive screening and exclusion criteria aims to reduce the likelihood of adverse events.

2.3.1b Dose

A dose of 150 mg of CBD (i.e., two soft-gel capsules) will be administered orally.

2.3.1c Control

The control is a matched placebo. The placebo is identical to the intervention but contains no CBD and will also be administered via oral ingestion.

2.3.2 Randomisation, Allocation Concealment and Blinding

Participants will be randomised to one of two possible treatments in a 1:1 ratio at the beginning of their test day. Specifically, they will be assigned a unique randomisation number that is linked to a treatment via a pre-populated randomisation schedule. The schedule will be generated in seven balanced blocks of 10 and one balanced block of four by an independent statistician using an online random number generator and stored in a password-protected system inaccessible to blinded study personnel (centralised computerised randomisation). The schedule will only be available to the statistician, an independent researcher and the company that will package and label the treatments (Central Pharmacy Logistics, Coburg North, Victoria).

Treatment allocation will be concealed using numbered containers (or 'sachets'). Each 'dose' will be packaged in a separate, opaque, aluminium sachet labelled with a unique randomisation number.

As this is a double-blind study, participants and the remainder of the research team will not be aware of the assigned treatment. In the event of an emergency, the principal investigator or trial physician may request the unblinding of a participant for medical care.

2.4 Eligibility Screening

A Study Flowchart is presented in **Figure 1**. Willing volunteers will consent to and complete a comprehensive online Screening Questionnaire in REDCap™ (~20 minutes). The questionnaire will assess their eligibility to participate. Each volunteer who attempts the Screening Questionnaire will be assigned a unique screening number to anonymise their identifying information. The trial physician will review volunteers' responses and decide on their eligibility for the trial. They will document their decision by completing an Eligibility Declaration form and a prescription for the trial drug (valid only when the volunteer has been randomised).

[INSERT FIGURE 1 HERE]

Figure 1. Study flowchart summarising the screening, enrolment and randomisation of participants in the CAPSTAN trial.

2.5 Enrolment

Eligible participants will be invited to complete a telehealth interview with the trial coordinator. Here, they will receive detailed information about the trial procedures and risks and be informed that their enrolment requires a negative urine drug and pregnancy screen at the start of the experimental procedures (i.e., the "Test Day"). The trial coordinator will invite the volunteer to ask questions and to discuss participation in the trial with the trial doctor, or to take additional time to consider their decision to participate. Once the trial coordinator is confident that the volunteer understands the requirements of the trial, they will request that the participant sign the informed consent form. The trial coordinator will then counter-sign the consent form, collect basic demographic information and book the participant for a Test Day.

2.6 Experimental Procedure

Each participant will complete one Test Day (~3 hours) at the Brain and Mind Centre at the University of Sydney (**Table 1**).

Table 1. The CAPSTAN trial experimental procedure

Time (minutes) from start	Approximate Duration	Activity
0	10 min	Introduction and pre-trial compliance checks
10	5 min	Randomisation
15	15 min	Pre-treatment measures
30	10 min	CBD or placebo administration and caloric beverage
40	50 min	Rest period 1
90	15 min	Virtual reality orientation
105	15 min	Baseline measures
120	15 min total (2 min) (3 min) (5 min) (5 min)	**Public Speaking task:** Instructions Speech preparation Speech delivery Arithmetic challenge
135	10 min	Rest period 2
145	5 min total (2 min) (3 min)	**Walk the Plank task:** Instructions Walk the Plank task
150	10 min	Rest period 3
160	5 min total (2 min) (3 min)	**Rollercoaster Ride task:** Instructions Rollercoaster ride task
165	10 min	Rest period 4
175	15 min	Study Close
190	-	**End Of Test Day**

2.6.1 Standardisation Procedures

Prior to each onsite Test Day, participants will be instructed to: (a) abstain from alcohol (≥24 h); (b) avoid greater than one standard serving of caffeine at least 2-hours before the Test Day; (c) ensure they are well hydrated; and (d) ensure that they are adequately fed by consuming a meal at least 2 hours before arrival to the Test Day. These factors aim to reduce the likelihood of malaise, gastrointestinal disturbances or heightened anxiety on the Test Day.

2.6.2 Compliance Checks

Participants will complete a urinary drug screen (to identify any recent use of cannabis and other psychoactive substances such as cocaine, ecstasy (MDMA), amphetamines, benzodiazepines and opioids) and, if they are female, a urine pregnancy screen, on arrival at the study site. The trial coordinator will also confirm compliance with the standardisation procedures and if there have been any changes to the participant's health status or medication use since the last contact. Participants who meet these requirements will be randomised; those who do not may be invited to return at another time if suitable.

2.6.3 Experimental Procedures

Following randomisation, participants will be fitted with the Equivital EQ02+ LifeMonitor belt and the VR headset (detailed below). There will then be a 10-minute collection of pre-treatment measures (see *Study Outcomes* below). After this, the VR headset and Equivital EQ02+ LifeMonitor belt will be temporarily removed, the treatment administered, and the participant provided with a standardised caloric beverage (i.e., 500 mL "Up & Go Liquid Breakfast", Sanitarium, Berkeley Vale NSW, Australia) to consume. This beverage aims to potentiate the absorption of CBD in the gastrointestinal tract [6, 48-50] and provide participants with sustenance during the test session. Participants will be provided with a rest period of approximately 50 minutes, during which they will be left alone in a quiet reception area and encouraged to undertake some low-stress reading; this delay is aimed at allowing CBD plasma concentrations to approach a near-maximal level [48]. Following this, participants will be provided with instructions on the three VR tasks and the functionality of the VR hardware during a 15-minute "VR Orientation" session. The VR headset and Equivital EQ02+ LifeMonitor belt will then be re-fitted for a 10-minutes collection of the pre-task measures. The devices will remain fitted to participants until the end of the experimental procedure.

2.6.4 Virtual Reality Tasks

The three tasks developed for this clinical trial are the "Public Speaking" task (for psychosocial stress), the "Walk the Plank" task (for situational anxiety) and the "Rollercoaster

Ride" task (for motion sickness). Although these exact VR tasks have not been previously used in clinical trials or laboratory studies, similar tasks have been reported in the broader scientific literature [27, 30, 38-40, 51].

The 15-minute "Public Speaking" task was modelled on previous studies that found CBD to have anxiolytic effects during public speaking challenges [23, 24, 37] and the Trier Social Stress Test (TSST) [52]. The abovementioned public speaking tasks varied in their methodology and involved either "simulated" public speaking tasks whereby participants delivered a speech in front of a video camera or real-life tasks where participants delivered speeches to a live audience [23, 24, 37]. Conversely, the TSST is a structured stress paradigm involving a 10-minute speech preparation period followed by a 10-minute test period during which participants complete a 5-minute job application speech followed by 5 minutes of mental arithmetic [53]. The TSST reliably induces psychosocial stress (i.e., stress involving the perception of one's worth, competence, or status by others) and results in an acute and reliable cortisol response in most participants [53-55]. In one study, a VR adaption of the TSST elicited similar salivary cortisol and subjective stress responses to a real-life TSST, demonstrating that the two were equivalent [27]. The current study utilises a modified VR version of the TSST involving a panel of virtual judges who withhold all feedback or affirmation (**Figure 2A**).

The task involves a three-minute "speech preparation period" (i.e., "please mentally prepare a speech on what attributes and experience you have that makes you the ideal candidate for your dream role") and a 10-minute "test period" consisting of (a) five-minute speech delivery period (i.e., "please deliver your speech and aim to speak for the full five-minutes") and (b) a five-minute mental arithmetic challenge (i.e., "please calculate 2703 – 13. From the result, please subtract 13 again and state your answer out loud. Repeat this process a total of five times. At the end of this process, please recall all five of your answers aloud").

The five-minute "Walk the Plank" task requires participants to virtually walk across a narrow virtual plank between two skyscrapers. Participants will be advised that they have become stranded on the top of the building and are required to signal for help using the safety beacon placed at the opposite end of the plank. For this task, participants will walk up and down the length of the clinic room with their movement mapped to the virtual plank and guided by virtual boundaries, signifying a safe space (**Figure 2B**). VR studies utilising a similar

challenge provided realistic experiences that induce physiological markers of stress that are consistent with acute anxiety (i.e., "situational anxiety") [38-40].

The five-minute "Rollercoaster Ride" task challenges participants to complete a virtual rollercoaster ride (**Figure 2C**). Participants will remain seated throughout this task and can indicate if they would like to stop the task early. Similar VR tasks have been shown to induce motion sickness in participants [30, 41, 42]. Motion sickness will be explored explicitly during the "Rollercoaster Ride" task, while cybersickness will be explored during all three VR scenarios.

[INSERT FIGURE 2A,2B AND 2C HERE]

Figure 2. (A) The panel of virtual judges from the "Public Speaking" task; (B) The roof of the building and plank from the "Walk the Plank" task; and (C) The view from the seat of the "Rollercoaster Ride" task.

2.6.5 Post Trial Procedures

After completing the final VR task, participants have a 10-minute recovery period. After this, they will be queried on any adverse events (AEs) experienced (i.e., "Have you experienced any unfavourable symptoms?"), What treatment do they think they received (i.e., CBD or placebo), and what are their confidence estimates (i.e., How sure are you of your guess on which treatment you received?"). All participants will be provided with the contact details of the research team to self-report any AEs over the next 24 hours. The trial coordinator or research assistant will record all adverse event reports using a REDCap™ AE report form and communicate to the trial physician if required.

The occurrence of AEs will be discussed weekly with the trial physician. Furthermore, a blinded summary of AE reports will be emailed to the trial physician monthly, and the trial physician will indicate if the rate of AEs is unacceptably high. In the unlikely event of a severe adverse event (SAE), the trial physician and the principal investigator (PI) will be immediately notified, and all SAEs will be reported to the trial sponsor and the HREC within 72 hours.

2.7 Data Collection

A summary of the data collected during the CAPSTAN trial and time of collection is provided in **Figure 3**

[INSERT FIGURE 3 HERE]

Figure 3. Data collected during the CAPSTAN trial "Test Day.
IPS = instructions for the "Public Speaking" task, **SP** = speech preparation, **SD** = speech delivery, **AC** = arithmetic challenge, **IWP** = instructions for the "Walk the Plank" task, **WPT** = "Walk the Plank" task, **IRR** = instructions for the "Rollercoaster Ride" task, **RRT** = "Rollercoaster Ride" task

2.7.1 Study Outcomes

The primary outcome measures include:

- Self-reported stress ratings on a VAS (*nervous 0-100*)
- Self-reported nausea ratings on a VAS (*nauseous 0-100*)

The secondary outcome measures include:

- Salivary Cortisol
- Heart rate
- Skin Conductance
- Vomiting or near-vomiting episodes
- Self-reported anxiety ratings on VASs include:
    - (*tense 0-100*)
    - (*calm 0-100*)
    - (*excited 0-100*)
    - (*bored 0-100*)

The exploratory (tertiary) outcome measures are:

- Salivary testosterone and progesterone
- Eye-tracking data including:
    - The frequency and duration of eye-closing
    - The frequency and duration of gaze at areas of interest

2.7.2 Visual Analogue Scales (VASs)

VASs will be used to measure the two primary outcomes: self-reported stress (nervousness) and nausea. They will also be used to measure the secondary outcome of self-reported anxiety (as detailed below) **(Figure 4).** These measures will be recorded before drug administration (pre-treatment measurement), at baseline, prior to the delivery of instructions for each VR task, prior to the performance of the task and at completion of each VR task. All VASs will appear on the screen of the VR headset, and participants will use the VR hand controllers to click on the numerical value (0-100) that best describes their current state (**Figure 4**). Using such virtual scales allows seamless execution of self-report without disturbing the participant's sense of immersion within the virtual environment.

For a well-rounded exploration of CBD effects on anxiety, we have utilised VASs that query participant's affect (i.e., the outward expression of an emotion) [56]. Here, we consider 1) the "valence" of an emotion (i.e., its positivity or negativity) and 2) the intensity of the emotion (i.e., whether it is arousing or deactivating). This provides four self-reported anxiety rating VASs, including:

- positively arousing (*excited 0-100*)
- positively deactivating (*calm 0-100*)
- negatively arousing (*tense 0-100*)
- negatively deactivating (*bored 0-100*)

[INSERT FIGURE 4 HERE]

Figure 4. Self-reported Visual Analogue Scales (VASs) as they appear on the virtual reality headset. Values are provided by the participants by clicking on the scales using the hand controllers.

2.7.3 Salivary Cortisol, Testosterone and Progesterone

During the experimental procedure, oral fluid samples will be collected from participants five times using Salivette® collection tubes (Sarstedt AG and Co., Nümbrecht Germany). These samples will be collected immediately before drug administration, before

the first VR task, and after each VR task. Participants will be advised to open the Salivette® tube, place the pad in their mouth for two minutes or until soaked with saliva, then return the pad to the tube and firmly seal it. The samples will be centrifuged for 2 minutes at 1,000 x g and analysed simultaneously for cortisol (a secondary outcome) and testosterone and progesterone (exploratory outcomes) using an in-house developed mass spectrometry (MS) method [57, 58].

2.7.4 Heart Rate

Heart rate will be measured for 10 minutes immediately before drug administration (baseline measurement), for 10 minutes at baseline and continuously throughout all three VR tasks using the EQ02+ LifeMonitor and belt, as described in previous studies [59, 60] (Equivital Ltd, Cambridge, United Kingdom; https://www.adinstruments.com/partners/equivital). This is a wireless, medical-grade monitoring system that records a range of physiological measures (such as electrocardiogram, breathing rate, tri-axial acceleration, galvanic skin response, skin temperature) using a wearable vest and permits live data streaming and download using the LabChart software.

2.7.5 Skin Conductance

Skin conductance will be measured for 10 minutes immediately before drug administration (baseline measurement), for 10 minutes at baseline and continuously throughout all three VR tasks.

Skin conductance of the forehead will be measured using the Equivital GSR Sensor connected to the EQ02+ LifeMonitor and belt (as described above). Skin conductance of the fingers will be measured using MLT117F/10 GSR finger electrodes connected to a FE116 GSR Amp with a PLCF1 front-end interface used with PowerLab C software (ADInstruments, Oxford United Kingdom), a system used in previous studies [61, 62]. Research suggests that forehead skin conductance may correlate with motion sickness, while finger skin conductance correlates with acute stress [30, 63]. Therefore, both will be measured in the current study.

2.7.6 Vomiting Episodes

The Test Day source document will record all vomiting episodes throughout the experimental procedure.

2.7.6 Eye-tracking

Eye tracking data will be collected continuously throughout the VR tasks using the VR headset. These measures will provide highly precise, objective data by which to examine participant's compliance (frequency and duration of eye closing) and engagement and response (frequency and duration of gaze at areas of interest, that is, the panel of judges in the “Public Speaking” task or the plank in the “Walk the Plank” task) during the VR challenges [64]. Gazing at pre-defined points of interest (e.g., the judges in the "Public Speaking" task or the plank in the "Walk the Plank" task) is based on the "Eye-Mind Hypothesis" that describes the tendency for people to direct their gaze towards what they are thinking about [64-66]. Both eye closing and gaze away from points of interest may correlate to fear or avoidance behaviours [67].

2.9 Data Management

Participant information collected for this trial will be securely stored and treated as confidential. Participants' identifying information will be anonymised using unique codes: initially, a screening number and, later, a randomisation number. The key to these codes will be securely stored in password-protected files inaccessible from the internet. Clinical trial data will be collected and managed using the Research Electronic Data Capture (REDCap™) web-based system, a secure, online program supported by the University of Sydney; access is password-protected and will only be available to approved research staff. Hard copies of patient data will be securely stored in locked filing cabinets at the study site. Only the study investigators have access to the participant data. All trial data will be stored securely for at least 15 years. The findings of this clinical trial will be disseminated via conferences, publications, and media, as applicable. Participants will be informed of the results of the study at the conclusion of the trial. No participants will be identified in any report or publication of this study or its results.

2.10 Data and Safety Monitoring

Data monitoring will occur monthly for all new participant entries logged into the REDCap system; a designated team member will adhere to a standardised process of data review, raising queries and locking forms once the review has been completed. Safety monitoring of AEs will be managed according to sponsor and HREC requirements. As this is a small, single-centre, low risk, clinical trial utilising an acute, low-dose CBD, an independent data safety monitoring committee will not be formed. However, an independent 'Expert Group' has been formed and will be consulted by the research team and HREC in the unlikely instance of a severe adverse event. The Expert Group comprises three research physicians with extensive experience with CBD clinical trials. The decision to terminate the trial lies with the principal investigator based on safety data and recruitment targets.

2.11 Roles and Responsibilities

The study investigators have led the design of this study and are responsible for the management and conduct of this clinical trial. The study investigators will conduct the analysis and will make all publication-related decisions.

2.12 Statistical Methods

2.5.1 Sample Size Estimation

The target sample size was determined *a priori* using power calculation software (G*Power Version 3.1.9.6, University of Kiel, Germany). In an earlier investigation, Linares *et al.* (2019) found that 150 mg CBD decreased subjective ratings of anxiety on the Visual Analogue Mood Scale (VAMS) during a simulated public speaking test, albeit non-significantly (Placebo: 18.6±15.9 mm, n=15; CBD: 7.6±15.5 mm; n=15; Cohen's *d*=0.70) [23].

Using an equivalent effect size (Cohen's *d*=0.70), a power (1-ß) of 0.8 and a two-sided α=0.05, we estimate that n=68 participants will be required to detect a significant effect of CBD on anxiety. Clinical trials conducted by the Lambert Initiative for Cannabinoid Therapeutics have indicated participant retention of ≥90% [48, 68]. Therefore, the n=74 will be recruited to account for attrition.

2.5.2 Statistical Analysis Plan

The primary and secondary outcomes and eye-openness will be analysed using generalized linear mixed models (GLMM), while eye gaze will be analysed using a mixed-effects multinomial logistic regression. Treatment, Time and the Treatment × Time interaction will be included as fixed effects, and the participant will be included as a random effect, with other covartiates included as appropriate to improve goodness of fit (e.g., Sex, Age, Time of day). To refine the models, we will use corrected Akaike Information Criterion [69]. We will calculate Δm between models and exclude models with Δm > 2 as having substantially less support [70]. No covariance structure will be specified (unstructured). To identify the best distribution and link for the GLMM models, the data type, residual plots, Shapiro-Wilk normality test, Levene's test for Homogeneity of Variance, and Pearson's dispersion test will be used. Type III Wald chi-square tests will be used to generate main effects p-values. A priori planned uncorrected pairwise comparisons will be performed to compare:

- subjective ratings of stress on a stress VAS (*nervous 0-100*) across treatments at (Timepoint t=125 (SD), Figure 3)
- subjective ratings of stress on a stress VAS (*nervous 0-100*) across treatments at (Timepoint t=147 (WPT), Figure 3)
- subjective ratings of nausea on a nausea VAS (*nauseous 0-100*) across treatments at (Timepoint t=165, Figure 3)
- subjective ratings of nausea on a nausea VAS (*nauseous 0-100*) across treatments at (Timepoint t=175, Figure 3)

as these are the primary outcome measures.

Dunn-Šidák corrected pairwise comparisons will be performed where additional significant main and interaction effects are present. Statistical significance will be accepted as $p<0.05$. The statistical analysis plan will be finalised before the last participant Test Day and will be available on request.

3. CONCLUSION

The focus of the current study is a non-clinical (i.e., "healthy") population confronted with acute stressors such as delivering a speech (i.e., psychosocial stress), being exposed to

heights (i.e., situational anxiety) or challenging vestibular stimulation (i.e., motion sickness and cybersickness) using VR. This is deemed noteworthy considering the popularity of over-the-counter, low-dose CBD "nutraceutical" or "wellness" products in many regions [20] and the lack of clinical trials investigating the efficacy of such products. This study will provide important practical insights that may inform the optimal use of low-dose CBD products that are accessed without a prescription. Findings from this study will help illuminate the efficacy of low-dose CBD in the four indications of interest and may provide insights into clinical populations that could benefit from low-dose CBD treatment.

4. STATEMENTS AND DECLARATIONS

Ethics and dissemination: The study has been approved by the University of Sydney Human Research Ethics Committee (HREC; 2023/307, Version 1.5, 28th September 2023). The findings of this clinical trial will be disseminated via conferences, publications, and media, as applicable. Participants will be informed of the study's results at the trial's conclusion. No participants will be identified in any report or publication of this study or its results.

Author Contributions

I.S.M, D.M, H.M, M.B.P, N.S.L, R.F and Z.B were involved in the conception and design of the research project. Z.B drafted the manuscript, and all authors were involved in critically revising it. I.S.M is the principal investigator who has overall responsibility for the design, conduct and decision to submit for publication. M.B.P is the study statistician, N.S.L will provide medical oversight, H.M and R.F created the virtual reality challenges in collaboration with I.S.M and Z.B. Z.B is the trial coordinator responsible for collecting trial data. All authors have read and approved the final manuscript.

Funding Statement

This work was supported by The Lambert Initiative for Cannabinoid Therapeutics, a philanthropically funded centre for medicinal cannabis research at The University of Sydney.

Competing Interests

Author I.S.M. acts as a consultant to Kinoxis Therapeutics and has received honoraria from Janssen. He has also served as an expert witness in various medicolegal cases involving

cannabis and cannabinoids. I.S.M. hold patents on cannabinoid therapies (PCT/AU2018/05089 and PCT/AU2019/050554) and has received consulting fees from the Medicinal Cannabis Industry Australia (MCIA). D.M. has also received consulting fees from MCIA. Z.B, H.M N.S.L, R.F and M.B.P have no disclosures to report.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Patient consent for publication

Not applicable.

Trial Sponsor

The University of Sydney, Sydney NSW Australia.

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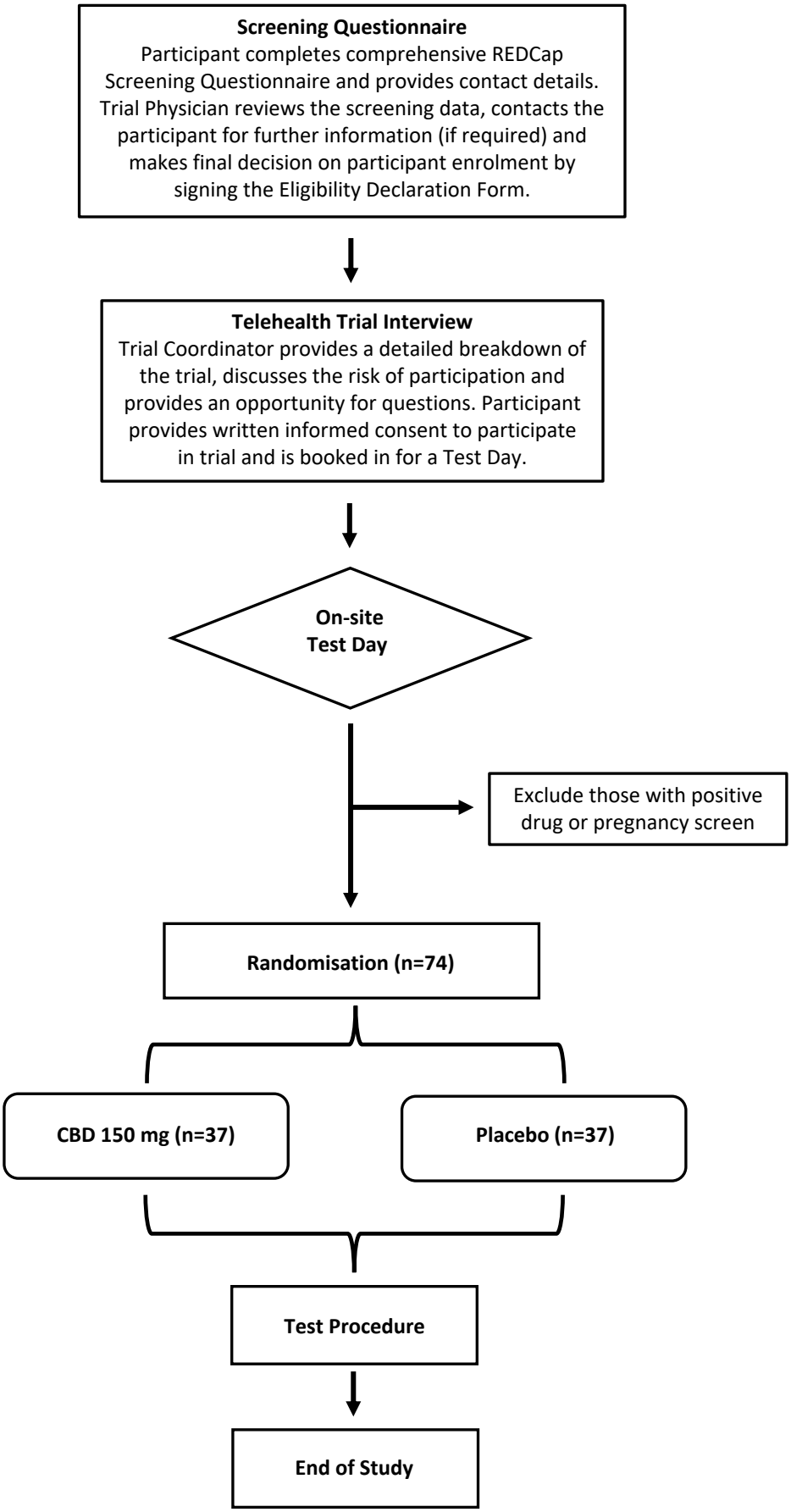

Screening Questionnaire
Participant completes comprehensive REDCap Screening Questionnaire and provides contact details. Trial Physician reviews the screening data, contacts the participant for further information (if required) and makes final decision on participant enrolment by signing the Eligibility Declaration Form.
Telehealth Trial Interview
Trial Coordinator provides a detailed breakdown of the trial, discusses the risk of participation and provides an opportunity for questions. Participant provides written informed consent to participate in trial and is booked in for a Test Day.
On-site Test Day
Exclude those with positive drug or pregnancy screen
Randomisation (n=74)
CBD 150 mg (n=37)
Placebo (n=37)
Test Procedure
End of Study

Figure 2. (A) The panel of virtual judges from the "Public Speaking" task

492x318mm (72 x 72 DPI)

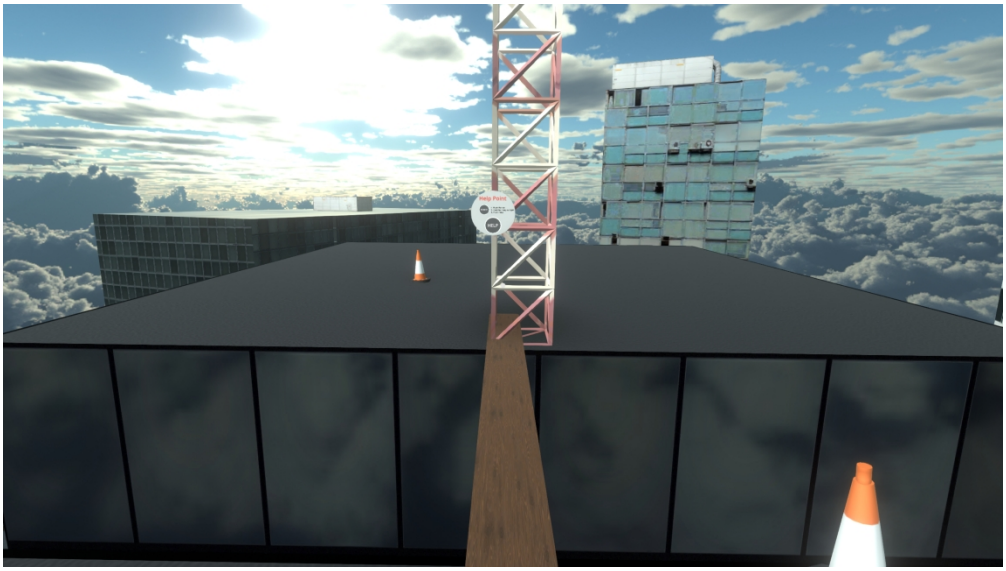
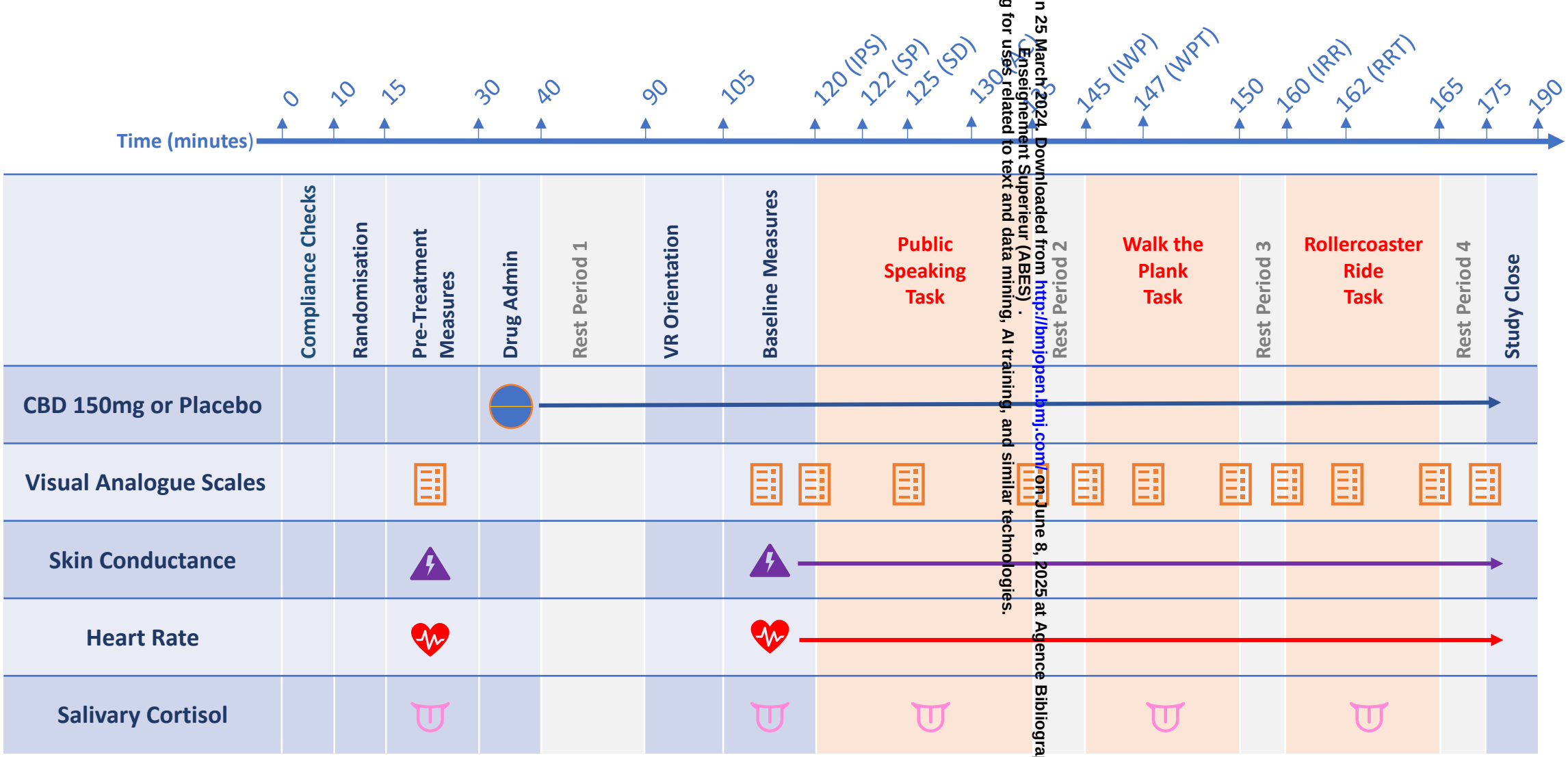


Figure 2. (B) The roof of the building and plank from the "Walk the Plank" task

1711x962mm (38 x 38 DPI)

Figure 2. (C) The view from the seat of the "Rollercoaster Ride" task.

1711x962mm (38 x 38 DPI)



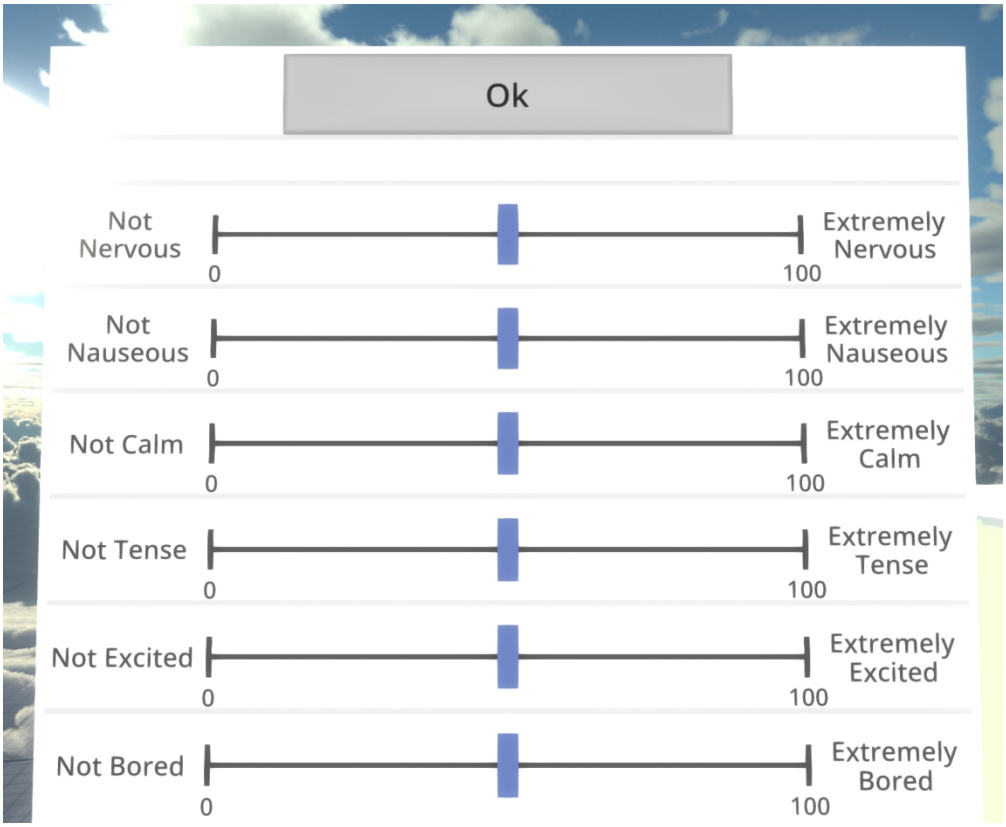


Figure 4. Self-reported Visual Analogue Scales (VASs) as they appear on the virtual reality headset. Values are provided by the participants by clicking on the scales using the hand controllers.

451x369mm (96 x 96 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Manuscript Page and Notes
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	Yes, ANZCTR public trials registry, ACTRN12623000872639. Registered 15 August 2023.
Protocol version	3	Date and version identifier	2, 6 and 25
Funding	4	Sources and types of financial, material, and other support	6, 25
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,25
	5b	Name and contact information for the trial sponsor	6
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	25
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	23
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5-6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA, intervention is a single acute dose of active or placebo with no ongoing involvement of participants.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA, intervention is a single acute dose of active or placebo with no ongoing involvement of participants.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12-13

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	19
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10 (Figure1)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	23
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	22
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7,17
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	22-23
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	23-24
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	23-24
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	23-24
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	23
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22-23
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	2, 6, 25
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	6
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	25
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	17
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	23
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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The effects of cannabidiol on psychosocial stress, situational anxiety and nausea in a virtual reality environment: A protocol for a single-centre randomised clinical trial

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The effects of cannabidiol on psychosocial stress, situational anxiety and nausea in a virtual reality environment: A protocol for a single-centre randomised clinical trial

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ABSTRACT

Introduction: The non-intoxicating plant-derived cannabinoid, *cannabidiol* (CBD), has demonstrated therapeutic potential in a number of clinical conditions. Most successful clinical trials have utilised relatively high (≥ 300 mg) oral doses of CBD. Relatively few studies have investigated the efficacy of lower (<300 mg) oral doses, typical of those available in over-the-counter CBD products.

Methods: We present a protocol for a randomised, double-blind, placebo-controlled, parallel-group clinical trial investigating the effects of a low oral dose (150 mg) of CBD on acute psychosocial stress, situational anxiety, motion sickness and cybersickness in healthy individuals. Participants (n=74) will receive 150 mg of CBD or a matched placebo 90 minutes before completing three virtual reality (VR) challenges (tasks) designed to induce transient stress and motion sickness: (1) a 15-minute "Public Speaking" task; (2) a 5-minute "Walk the Plank" task (above a sheer drop); and (3) a 5-minute "Rollercoaster Ride" task. The primary outcomes will be self-reported stress and nausea measured on 100-mm visual analogue scales. Secondary outcomes will include salivary cortisol concentrations, skin conductance, heart rate, and vomiting episodes (if any). Statistical analyses will test the hypothesis that CBD reduces nausea and attenuates subjective, endocrine, and physiological responses to stress compared to placebo. This study will indicate whether low-dose oral CBD has positive effects in reducing acute psychosocial stress, situational anxiety, motion sickness and cybersickness.

Ethics and dissemination: The University of Sydney Human Research Ethics Committee has granted approval (2023/307, Version 1.6, 16th February 2024). Study findings will be disseminated in a peer-reviewed journal and at academic conferences.

Trial Registration Number: ACTRN12623000872639 (ANZCTR public trials registry, registered 15th August 2023).

Keywords: cannabidiol (CBD), nausea, anxiety, virtual reality.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study will use a rigorous randomised, double-blind, placebo-controlled design to investigate the effects of a low oral dose (150 mg) of cannabidiol.
- The dose of cannabidiol provided (i.e., 150 mg) will be comparable to those available over-the-counter in many countries.
- Virtual reality (VR) will be used to administer safe, realistic, and precisely reproducible test paradigms that induce transient stress and nausea in volunteers.
- Blood samples will not be obtained to verify plasma cannabidiol concentrations as venepuncture has the potential to induce extraneous stress.

1. INTRODUCTION

Cannabidiol (CBD) is a non-intoxicating constituent of the *Cannabis sativa* plant [1, 2]. It has a good safety and tolerability profile [3-7] and a diverse range of pharmacological targets, including the serotonin receptors (e.g., 5-HT1A), G protein-coupled receptors (e.g., GPR55 and GPR18) and transient receptor potential ion channels (e.g. TRPA1) [8-10]. CBD has demonstrated therapeutic potential in several clinical conditions [4, 5], including anticonvulsant effects in paediatric epilepsy [11-13], anxiolytic effects in anxiety disorders [14, 15], antipsychotic effects in schizophrenia [16, 17] and "anti-addiction" effects in substance use disorders [18, 19]. These clinical benefits are typically observed at relatively high oral doses of CBD (e.g., ~300-1500mg) [4, 5].

In regions such as North America and Europe, CBD is available as both a prescription drug and a 'nutraceutical product' [20]. These nutraceutical or "wellness" products are typically oral formulations (e.g., oils, capsules, gummies) that contain low doses of CBD (i.e., ≤150 mg/day) and are widely available in health food stores and pharmacies [20, 21]. In Australia, regulation of "low-dose CBD products" (containing ≤150 mg/day) was recently eased to allow patients without a prescription access to registered products in pharmacies. However, registration of such products requires approval by Australia's medicines regulator based on demonstrated efficacy and safety [22]. With no products having been registered in the ~3 years since these regulatory changes were enacted (December 2020) [22], questions about whether CBD can demonstrate efficacy at these lower doses have been raised.

Indeed, a recent review found little high-quality evidence to support the efficacy of CBD (in any conditions) at doses ≤200 mg. This review acknowledged, however, that such doses were under-studied [21]. Some promising results have been obtained with public speaking tasks designed to induce "psychosocial stress" in healthy volunteers when 300 mg CBD was administered [23, 24]. Therefore, further research investigating the anxiolytic effects of low to moderate oral doses of CBD is warranted. It should be noted that the oral bioavailability of CBD is limited (~13-19%) but may be enhanced by certain lipid-rich formulations or by administration with fatty foods [6, 25]. The current study will utilise a proprietary blend of tocopherol phosphates (so called 'Tocopheryl Phosphate Mixture' (TPM®)), which has been shown to increase the bioavailability of lipid-soluble substances [26, 27].

Virtual reality (VR) technologies are increasingly being used to investigate psychosocial stress and anxiety in laboratory studies [28, 29]. This approach allows for minimal resourcing relative to "real-world" studies, customisable and reproducible test paradigms and the accurate monitoring and recording of key outcomes [29, 30]. Furthermore, VR provides the advantage of simulating physiologically-provoking activities without real danger e.g., walking a plank over a sheer drop. However, the use of VR is often accompanied by motion sickness (a pattern of symptoms that arise from exposure to stimuli involving significant visual or physical motion) and cybersickness (a sub-type of motion sickness that arises specifically due to exposure to VR) [31-34]. CBD has shown anti-nausea and anti-emetic effects in preclinical studies involving laboratory animals [35-37]. Interestingly, two of these studies demonstrated that CBD administered intraperitoneally at low doses (2.5 - 10 mg/kg) but not higher doses (25 and 40 mg/kg) reduced toxin-induced vomiting in house musk shrews [35, 36]. CBD also reduced vomiting in human studies when used in combination with Δ^9-tetrahydrocannabinol to treat chemotherapy-induced nausea and vomiting [38]. Accordingly, the current study will investigate CBD's possible anti-nausea effects in participants by exposure to VR scenarios [29].

We have developed a series of three unique VR challenges for the current trial. The "Public Speaking" task was adapted from studies that explored the anxiolytic effects of CBD during public speaking challenges [23, 24, 39] and will allow us to determine the effects of CBD during social threat (i.e., psychosocial stress). The 5-minutes "Walk the Plank" task has been modified from previous VR studies requiring participants to walk along a narrow virtual plank above a precipitous drop, inducing physiological markers of acute anxiety [40-42] (i.e., "situational anxiety"). Finally, the "Rollercoaster Ride" task challenges participants to complete two rounds of a virtual rollercoaster ride and has been modelled from VR rollercoaster ride paradigms that have been reliably used to induce motion sickness and cybersickness in participants [32, 43, 44].

In summary, the current protocol describes a study that aims to investigate the effects of low-dose (150 mg) CBD *versus* placebo on virtual reality (VR)-induced acute psychosocial stress, situational anxiety, acute motion sickness and cybersickness in healthy individuals.

2. METHODS

2.1 Study design

This study is a randomised, double-blind, placebo-controlled, parallel-group, clinical trial comparing the efficacy of low-dose CBD (150 mg) *versus* placebo. The study is known as the CAPSTAN (Cannabidiol for Acute Psychosocial Stress and Nausea) clinical trial. A crossover design was deemed unsuitable due to the high likelihood of trial-order effects (e.g., habituation to the VR scenarios) [32].

The trial sponsor is the University of Sydney, and the trial site is the Brain and Mind Centre in Sydney, Australia. The study has been approved by the University of Sydney Human Research Ethics Committee (HREC; 2023/307, Version 1.6, 16th February 2024) and registered on the Australian New Zealand Clinical Trials Registry (ACTRN12623000872639, 15th August 2023). The study is financially supported by the Lambert Initiative for Cannabinoid Therapeutics, a philanthropically funded centre for cannabinoid research at the University of Sydney. Recruitment commenced on 9th October 2023 and is anticipated to conclude in mid-2024.

2.2 Participant Population

2.2.1 Inclusion Criteria

We aim to recruit 74 participants who will be: (a) healthy adults aged between 18 and 50 years; (b) proficient in English and able to provide informed consent; (c) residing in the Greater Sydney region of New South Wales, Australia; and (d) willing to follow the protocol requirements.

2.2.2 Exclusion Criteria

The following exclusion criteria will apply:

a. Self-reported regular use (i.e., more than twice weekly) of:
    i. Cannabinoid-containing products (e.g., cannabis or CBD)

ii. Psychotropic drugs (prescriptive or illicit) (e.g., cannabis, amphetamines, cocaine, ecstasy (MDMA), LSD (acid), antidepressants, antiepileptics, opioids, benzodiazepines)

iii. Medication that may affect the stress response (e.g., corticosteroids, beta-blockers)

b. Self-reported history of allergic reaction (e.g., urticaria or anaphylaxis) to cannabis or cannabinoid-containing products.

c. Self-reported history of liver disease, renal disease, epilepsy or heart disease (medically controlled high blood pressure <140/90mmHg is acceptable).

d. Current (i.e., within the past 2 weeks) otologic (vestibular) disease.

e. A history of repeated episodes of syncope.

f. Pregnant, lactating, or trying to conceive.

g. Self-reported history of drug and/or alcohol dependence (or suspected drug and/or alcohol dependence as determined by the trial physician).

h. A medically diagnosed anxiety disorder (e.g., social anxiety disorder) within the past 12 months.

i. Current suicidal ideation (i.e., a score >0 on Question 9 of the Patient Health Questionnaire) or suspected suicidal ideation as determined by the trial physician.

j. Current depression, anxiety, and stress scores outside the 'healthy range' on the Depression Anxiety Stress Scale-21 [45] (i.e., > moderate scores for depression (>20), anxiety (>14) and stress (>25)).

k. A chronic medical condition (mental or physical) that is uncontrolled i.e., has been either newly diagnosed, or previously diagnosed and remains symptomatic.

l. Self-reported high vulnerability to cybersickness or motion sickness.

m. Frequent (i.e., more than weekly) use of VR technologies, which tends to produce desensitisation towards cybersickness [32].

n. Self-reported intense fear of heights.

2.2.3 Recruitment and Retention

Participants will be recruited via social media, word of mouth, printed or online study advertisements and direct emails to individuals who have previously registered their

interest in participating in clinical trials with the Lambert Initiative for Cannabinoid Therapeutics. Participation is voluntary, and participants can withdraw at any time. Participants will be reimbursed with a $200 gift voucher as compensation for time and expenses incurred as a result of study participation.

2.3 Treatments

The treatments will be purchased from Avecho Biotechnology Limited (Clayton, Victoria), manufactured (Catalent Pharma Solutions, St. Petersburg, FL), as well as packaged and labelled (Central Pharmacy Logistics, Coburg North, VIC) at GMP-licenced facilities, stored at the Brain and Mind Centre (in a secure, temperature-controlled room), and dispensed by the trial coordinator (who is also a registered pharmacist) and another blinded investigator.

As this trial utilises a non-clinical (healthy) population, a placebo comparator is the most suitable and ethical choice. Accordingly, an acute dose of CBD (as opposed to chronic administration) will be utilised. Indeed, the treatment of many ailments utilises an *ad hoc* treatment regime.

2.3.1 Intervention

The investigational product (Avecho Biotechnology Limited, Victoria, Australia) is an oil-based, soft-gel capsule. Each gel capsule contains 75 mg of pure, synthetic (-)-CBD enantiomer and 75 mg of TPM® in medium chain triglyceride (MCT) oil. The capsules do not contain any other cannabinoids or cannabis constituents.

2.3.1a Safety

CBD is generally considered to have a good safety profile [3, 4, 21, 46, 47]. In previous studies, 150 mg CBD caused a very low frequency of mild adverse events that did not differ from placebo [21]. The current study's comprehensive screening and exclusion criteria aims to reduce the likelihood of adverse events.

2.3.1b Dose

A dose of 150 mg of CBD (i.e., two soft-gel capsules) will be administered orally.

2.3.1c Control

The control is a matched placebo. The placebo is identical to the intervention but contains no CBD and will also be administered via oral ingestion.

2.3.2 Randomisation, Allocation Concealment and Blinding

Participants will be randomised to one of two possible treatments in a 1:1 ratio at the beginning of their test day. Specifically, they will be assigned a unique randomisation number that is linked to a treatment via a pre-populated randomisation schedule. The schedule will be generated in seven balanced blocks of 10 and one balanced block of four by an independent statistician using an online random number generator and stored in a password-protected system inaccessible to blinded study personnel (centralised computerised randomisation). The schedule will only be available to the statistician, an independent researcher and the company that will package and label the treatments (Central Pharmacy Logistics, Coburg North, Victoria).

Treatment allocation will be concealed using numbered containers (or 'sachets'). Each 'dose' will be packaged in a separate, opaque, aluminium sachet labelled with a unique randomisation number.

As this is a double-blind study, participants and the remainder of the research team will not be aware of the assigned treatment. In the event of an emergency, the principal investigator or trial physician may request the unblinding of a participant for medical care.

2.4 Eligibility Screening

A Study Flowchart is presented in **Figure 1**. Willing volunteers will complete a comprehensive online Screening Questionnaire using the 'Research Electronic Data Capture' (REDCap™) web-based system (~20 minutes). Volunteers are required to complete a compulsory online declaration tick-box at the start of the questionnaire consenting to the use of the information they provide to evaluate their eligibility. The questionnaire will assess their eligibility to participate. Each volunteer who attempts the Screening Questionnaire will be assigned a unique screening number to anonymise their identifying information. The trial physician will review volunteers' responses and decide on their eligibility for the trial. They will document their decision by completing an Eligibility

Declaration form and a prescription for the trial drug (valid only when the volunteer has been randomised).

[INSERT FIGURE 1 HERE]

Figure 1. Study flowchart summarising the screening, enrolment and randomisation of participants in the CAPSTAN trial.

2.5 Enrolment

Eligible participants will be invited to complete a telehealth interview with the trial coordinator. Here, they will receive detailed information about the trial procedures and risks and be informed that their enrolment requires a negative urine drug and pregnancy screen at the start of the experimental procedures (i.e., the "Test Day"). The trial coordinator will invite the volunteer to ask questions and to discuss participation in the trial with the trial doctor, or to take additional time to consider their decision to participate. Once the trial coordinator is confident that the volunteer understands the requirements of the trial, they will request that the participant sign the informed consent form (please see Supplementary file). The trial coordinator will then counter-sign the consent form, collect basic demographic information and book the participant for a Test Day.

2.6 Experimental Procedure

Each participant will complete one Test Day (~3 hours) at the Brain and Mind Centre at the University of Sydney (**Table 1**).

Table 1. The CAPSTAN trial experimental procedure

Time (minutes) from start	Approximate Duration	Activity
0	10 min	Introduction and pre-trial compliance checks
10	5 min	Randomisation
15	15 min	Pre-treatment measures
30	10 min	CBD or placebo administration and caloric beverage
40	50 min	Rest period 1
90	15 min	Virtual reality orientation
105	15 min	Baseline measures
120	15 min total (2 min) (3 min) (5 min) (5 min)	**Public Speaking task:** Instructions Speech preparation Speech delivery Arithmetic challenge
135	10 min	Rest period 2
145	5 min total (2 min) (3 min)	**Walk the Plank task:** Instructions Walk the Plank task
150	10 min	Rest period 3
160	5 min total (2 min) (3 min)	**Rollercoaster Ride task:** Instructions Rollercoaster ride task
165	10 min	Rest period 4
175	15 min	Study Close
190	-	**End Of Test Day**

2.6.1 Standardisation Procedures

Prior to each onsite Test Day, participants will be instructed to: (a) abstain from alcohol (≥24 h); (b) avoid greater than one standard serving of caffeine at least 2-hours before the Test Day; (c) ensure they are well hydrated; and (d) ensure that they are adequately fed by consuming a meal at least 2 hours before arrival to the Test Day. These factors aim to reduce the likelihood of malaise, gastrointestinal disturbances or heightened anxiety on the Test Day.

2.6.2 Compliance Checks

Participants will complete a urinary drug screen (to identify any recent use of cannabis and other psychoactive substances such as cocaine, ecstasy (MDMA), amphetamines, benzodiazepines and opioids) and, if they are female, a urine pregnancy screen, on arrival at the study site. The trial coordinator will also confirm compliance with the standardisation procedures and if there have been any changes to the participant's health status or medication use since the last contact. Participants who meet these requirements will be randomised; those who do not may be invited to return at another time if suitable.

2.6.3 Experimental Procedures

Following randomisation, participants will be fitted with the Equivital EQ02+ LifeMonitor belt and the VR headset (detailed below). There will then be a 10-minute collection of pre-treatment measures (see *Study Outcomes* below). After this, the VR headset and Equivital EQ02+ LifeMonitor belt will be temporarily removed, the treatment administered, and the participant given a compulsory standardised caloric beverage to consume; specifically, 500 mL of "Up & Go Liquid Breakfast" (Sanitarium, Berkeley Vale NSW, Australia) containing approximately 1,640 kilojoules, 16.8g of protein, 8.6g of fat and 57g of carbohydrates. For consistency, all participants are required to consume this beverage in its entirety, which aims to potentiate the absorption of CBD in the gastrointestinal tract [6, 48-50] and provide participants with sustenance during the test session. Participants will be provided with a rest period of approximately 50 minutes, during which they will be left alone in a quiet reception area and encouraged to undertake some low-stress reading; this delay is aimed at allowing CBD plasma concentrations to approach a near-maximal level [48]. Following this, participants will be provided with instructions on the three VR tasks and the functionality of the VR hardware during a 15-minute "VR Orientation" session. The VR headset and Equivital EQ02+ LifeMonitor belt will then be re-fitted for a 10-minutes collection of the pre-task measures. The devices will remain fitted to participants until the end of the experimental procedure.

2.6.4 Virtual Reality Tasks

The three tasks developed for this clinical trial are the "Public Speaking" task (for psychosocial stress), the "Walk the Plank" task (for situational anxiety) and the "Rollercoaster Ride" task (for motion sickness). Although these exact VR tasks have not been previously used in clinical trials or laboratory studies, similar tasks have been reported in the broader scientific literature [29, 32, 40-42, 51].

The 15-minute "Public Speaking" task was modelled on previous studies that found CBD to have anxiolytic effects during public speaking challenges [23, 24, 39] and the Trier Social Stress Test (TSST) [52]. The abovementioned public speaking tasks varied in their methodology and involved either "simulated" public speaking tasks whereby participants delivered a speech in front of a video camera or real-life tasks where participants delivered speeches to a live audience [23, 24, 39]. Conversely, the TSST is a structured stress paradigm involving a 10-minute speech preparation period followed by a 10-minute test period during which participants complete a 5-minute job application speech followed by 5 minutes of mental arithmetic [53]. The TSST reliably induces psychosocial stress (i.e., stress involving the perception of one's worth, competence, or status by others) and results in an acute and reliable cortisol response in most participants [53-55]. In one study, a VR adaption of the TSST elicited similar salivary cortisol and subjective stress responses to a real-life TSST, demonstrating that the two were equivalent [29]. The current study utilises a modified VR version of the TSST involving a panel of virtual judges who withhold all feedback or affirmation (**Figure 2A**).

The task involves a three-minute "speech preparation period" (i.e., "please mentally prepare a speech on what attributes and experience you have that makes you the ideal candidate for your dream role") and a 10-minute "test period" consisting of (a) five-minute speech delivery period (i.e., "please deliver your speech and aim to speak for the full five-minutes") and (b) a five-minute mental arithmetic challenge (i.e., "please calculate 2703 – 13. From the result, please subtract 13 again and state your answer out loud. Repeat this process a total of five times. At the end of this process, please recall all five of your answers aloud").

The five-minute "Walk the Plank" task requires participants to virtually walk across a narrow virtual plank between two skyscrapers. Participants will be advised that they have become stranded on the top of the building and are required to signal for help using the safety

beacon placed at the opposite end of the plank. For this task, participants will walk up and down the length of the clinic room with their movement mapped to the virtual plank and guided by virtual boundaries, signifying a safe space (**Figure 2B**). VR studies utilising a similar challenge provided realistic experiences that induce physiological markers of stress that are consistent with acute anxiety (i.e., "situational anxiety") [40-42].

The five-minute "Rollercoaster Ride" task challenges participants to complete a virtual rollercoaster ride (**Figure 2C**). Participants will remain seated throughout this task and can indicate if they would like to stop the task early. Similar VR tasks have been shown to induce motion sickness in participants [32, 43, 44]. Motion sickness will be explored explicitly during the "Rollercoaster Ride" task, while cybersickness will be explored during all three VR scenarios.

[INSERT FIGURE 2A,2B AND 2C HERE]

Figure 2. (A) The panel of virtual judges from the "Public Speaking" task; (B) The roof of the building and plank from the "Walk the Plank" task; and (C) The view from the seat of the "Rollercoaster Ride" task.

2.6.5 Post Trial Procedures

After completing the final VR task, participants have a 10-minute recovery period. After this, they will be queried on any adverse events (AEs) experienced (i.e., "Have you experienced any unfavourable symptoms?"), What treatment do they think they received (i.e., CBD or placebo), and what are their confidence estimates (i.e., How sure are you of your guess on which treatment you received?"). All participants will be provided with the contact details of the research team to self-report any AEs over the next 24 hours. The trial coordinator or research assistant will record all adverse event reports using a REDCap™ AE report form and communicate to the trial physician if required.

The occurrence of AEs will be discussed weekly with the trial physician. Furthermore, a blinded summary of AE reports will be emailed to the trial physician monthly, and the trial physician will indicate if the rate of AEs is unacceptably high. In the unlikely event of a severe

adverse event (SAE), the trial physician and the principal investigator (PI) will be immediately notified, and all SAEs will be reported to the trial sponsor and the HREC within 72 hours.

2.7 Data Collection

A summary of the data collected during the CAPSTAN trial and time of collection is provided in **Figure 3**

[INSERT FIGURE 3 HERE]

Figure 3. Data collected during the CAPSTAN trial "Test Day.
IPS = instructions for the "Public Speaking" task, **SP** = speech preparation, **SD** = speech delivery, **AC** = arithmetic challenge, **IWP** = instructions for the "Walk the Plank" task, **WPT** = "Walk the Plank" task, **IRR** = instructions for the "Rollercoaster Ride" task, **RRT** = "Rollercoaster Ride" task

2.7.1 Study Outcomes

The primary outcome measures include:

- Self-reported stress ratings on a VAS (*nervous 0-100*)
- Self-reported nausea ratings on a VAS (*nauseous 0-100*)

The secondary outcome measures include:

- Salivary Cortisol
- Heart rate
- Skin Conductance
- Vomiting or near-vomiting episodes
- Self-reported anxiety ratings on VASs include:
  - (*tense 0-100*)
  - (*calm 0-100*)
  - (*excited 0-100*)
  - (*bored 0-100*)

The exploratory (tertiary) outcome measures are:

- Salivary testosterone and progesterone

- Eye-tracking data including:
  - The frequency and duration of eye-closing
  - The frequency and duration of gaze at areas of interest

2.7.2 Visual Analogue Scales (VASs)

VASs will be used to measure the two primary outcomes: self-reported stress (nervousness) and nausea. They will also be used to measure the secondary outcome of self-reported anxiety (as detailed below) **(Figure 4).** These measures will be recorded before drug administration (pre-treatment measurement), at baseline, prior to the commencement of each VR task, at the completion of each VR task and at the study close. VAS measures will also be recorded prior to the performance of the speech *during* the "Public Speaking" task (Timepoint "SD" Figure 3) and prior to the performance of the walk *during* the "Walk the Plank" (Timepoint "WPT" Figure 3) task. These measures aim to capture "anticipatory anxiety" reported prior to anxiety-inducing tasks in previous studies [23, 24]. As nausea is the main interest during the Rollercoaster Ride task, no VAS measures for anticipatory anxiety will be taken for this task. All VASs will appear on the screen of the VR headset, and participants will use the VR hand controllers to click on the numerical value (0-100) that best describes their current state (**Figure 4**). Using such virtual scales allows seamless execution of self-report without disturbing the participant's sense of immersion within the virtual environment.

For a well-rounded exploration of CBD effects on anxiety, we have utilised VASs that query participant's affect (i.e., the outward expression of an emotion) [56]. Here, we consider 1) the "valence" of an emotion (i.e., its positivity or negativity) and 2) the intensity of the emotion (i.e., whether it is arousing or deactivating). This provides four self-reported anxiety rating VASs, including:

- positively arousing (*excited 0-100*)
- positively deactivating (*calm 0-100*)
- negatively arousing (*tense 0-100*)
- negatively deactivating (*bored 0-100*)

[INSERT FIGURE 4 HERE]

Figure 4. Self-reported Visual Analogue Scales (VASs) as they appear on the virtual reality headset. Values are provided by the participants by clicking on the scales using the hand controllers.

2.7.3 Salivary Cortisol, Testosterone and Progesterone

During the experimental procedure, oral fluid samples will be collected from participants five times using Salivette® collection tubes (Sarstedt AG and Co., Nümbrecht Germany). These samples will be collected immediately before drug administration, before the first VR task, and after each VR task. Participants will be advised to open the Salivette® tube, place the pad in their mouth for two minutes or until soaked with saliva, then return the pad to the tube and firmly seal it. The samples will be centrifuged for 2 minutes at 1,000 x g and analysed simultaneously for cortisol (a secondary outcome) and testosterone and progesterone (exploratory outcomes) using an in-house developed mass spectrometry (MS) method [57, 58].

2.7.4 Heart Rate

Heart rate will be measured for 10 minutes immediately before drug administration (baseline measurement), for 10 minutes at baseline and continuously throughout all three VR tasks using the EQ02+ LifeMonitor and belt, as described in previous studies [59, 60] (Equivital Ltd, Cambridge, United Kingdom; https://www.adinstruments.com/partners/equivital). This is a wireless, medical-grade monitoring system that records a range of physiological measures (such as electrocardiogram, breathing rate, tri-axial acceleration, galvanic skin response, skin temperature) using a wearable vest and permits live data streaming and download using the LabChart software.

2.7.5 Skin Conductance

Skin conductance will be measured for 10 minutes immediately before drug administration (baseline measurement), for 10 minutes at baseline and continuously throughout all three VR tasks.

Skin conductance of the forehead will be measured using the Equivital GSR Sensor connected to the EQ02+ LifeMonitor and belt (as described above). Skin conductance of the fingers will be measured using MLT117F/10 GSR finger electrodes connected to a FE116 GSR Amp with a PLCF1 front-end interface used with PowerLab C software (ADInstruments, Oxford United Kingdom), a system used in previous studies [61, 62]. Research suggests that forehead skin conductance may correlate with motion sickness, while finger skin conductance correlates with acute stress [32, 63]. Therefore, both will be measured in the current study.

2.7.6 Vomiting Episodes

The Test Day source document will record all vomiting episodes throughout the experimental procedure.

2.7.6 Eye-tracking

Eye tracking data will be collected continuously throughout the VR tasks using the VR headset. These measures will provide highly precise, objective data by which to examine participant's compliance (frequency and duration of eye closing) and engagement and response (frequency and duration of gaze at areas of interest, that is, the panel of judges in the “Public Speaking” task or the plank in the “Walk the Plank” task) during the VR challenges [64]. Gazing at pre-defined points of interest (e.g., the judges in the "Public Speaking" task or the plank in the "Walk the Plank" task) is based on the "Eye-Mind Hypothesis" that describes the tendency for people to direct their gaze towards what they are thinking about [64-66]. Both eye closing and gaze away from points of interest may correlate to fear or avoidance behaviours [67].

2.9 Data Management

Participant information collected for this trial will be securely stored and treated as confidential. Participants' identifying information will be anonymised using unique codes: initially, a screening number and, later, a randomisation number. The key to these codes will be securely stored in password-protected files inaccessible from the internet. Clinical trial data will be collected and managed using REDCap™, a secure, online program supported by the University of Sydney; access is password-protected and will only be available to approved

research staff. Hard copies of patient data will be securely stored in locked filing cabinets at the study site. Only the study investigators have access to the participant data. All trial data will be stored securely for at least 15 years. The findings of this clinical trial will be disseminated via conferences, publications, and media, as applicable. Participants will be informed of the results of the study at the conclusion of the trial. No participants will be identified in any report or publication of this study or its results.

2.10 Data and Safety Monitoring

Data monitoring will occur monthly for all new participant entries logged into the REDCap system; a designated team member will adhere to a standardised process of data review, raising queries and locking forms once the review has been completed. Safety monitoring of AEs will be managed according to sponsor and HREC requirements. As this is a small, single-centre, low risk, clinical trial utilising an acute, low-dose CBD, an independent data safety monitoring committee will not be formed. However, an independent 'Expert Group' has been formed and will be consulted by the research team and HREC in the unlikely instance of a severe adverse event. The Expert Group comprises three research physicians with extensive experience with CBD clinical trials. The decision to terminate the trial lies with the principal investigator based on safety data and recruitment targets.

2.11 Roles and Responsibilities

The study investigators have led the design of this study and are responsible for the management and conduct of this clinical trial. The study investigators will conduct the analysis and will make all publication-related decisions.

2.12 Statistical Methods

2.5.1 Sample Size Estimation

The target sample size was determined *a priori* using power calculation software (G*Power Version 3.1.9.6, University of Kiel, Germany). In an earlier investigation, Linares *et al.* (2019) found that 150 mg CBD decreased subjective ratings of anxiety on the Visual

Analogue Mood Scale (VAMS) during a simulated public speaking test, albeit non-significantly (Placebo: 18.6±15.9 mm, n=15; CBD: 7.6±15.5 mm; n=15; Cohen's *d*=0.70) [23].

Using an equivalent effect size (Cohen's *d*=0.70), a power (1-ß) of 0.8 and a two-sided α=0.05, we estimate that n=68 participants will be required to detect a significant effect of CBD on anxiety. Clinical trials conducted by the Lambert Initiative for Cannabinoid Therapeutics have indicated participant retention of ≥90% [48, 68]. Therefore, the n=74 will be recruited to account for attrition.

2.5.2 Statistical Analysis Plan

The primary and secondary outcomes and eye-openness will be analysed using generalized linear mixed models (GLMM), while eye gaze will be analysed using a mixed-effects multinomial logistic regression. Treatment, Time and the Treatment × Time interaction will be included as fixed effects, and the participant will be included as a random effect, with other covartiates included as appropriate to improve goodness of fit (e.g., Sex, Age, Time of day). To refine the models, we will use corrected Akaike Information Criterion [69]. We will calculate Δm between models and exclude models with Δm > 2 as having substantially less support [70]. No covariance structure will be specified (unstructured). To identify the best distribution and link for the GLMM models, the data type, residual plots, Shapiro-Wilk normality test, Levene's test for Homogeneity of Variance, and Pearson's dispersion test will be used. Type III Wald chi-square tests will be used to generate main effects p-values. A priori planned uncorrected pairwise comparisons will be performed to compare:

- subjective ratings of stress on a stress VAS (*nervous 0-100*) across treatments at (Timepoint t=125 (SD), Figure 3)
- subjective ratings of stress on a stress VAS (*nervous 0-100*) across treatments at (Timepoint t=147 (WPT), Figure 3)
- subjective ratings of nausea on a nausea VAS (*nauseous 0-100*) across treatments at (Timepoint t=165, Figure 3)
- subjective ratings of nausea on a nausea VAS (*nauseous 0-100*) across treatments at (Timepoint t=175, Figure 3)

as these are the primary outcome measures.

Dunn-Šidák corrected pairwise comparisons will be performed where additional significant main and interaction effects are present. Statistical significance will be accepted as $p<0.05$. The statistical analysis plan will be finalised before the last participant Test Day and will be available on request.

3. STATEMENTS AND DECLARATIONS

Ethics and dissemination: The study has been approved by the University of Sydney Human Research Ethics Committee (HREC; 2023/307, Version 1.6, 16th February 2024). The findings of this clinical trial will be disseminated via conferences, publications, and media, as applicable. Participants will be informed of the study's results at the trial's conclusion. No participants will be identified in any report or publication of this study or its results.

Author Contributions

I.S.M, D.M, H.M, M.B.P, N.S.L, R.F and Z.B were involved in the conception and design of the research project. Z.B drafted the manuscript, and all authors were involved in critically revising it. I.S.M is the principal investigator who has overall responsibility for the design, conduct and decision to submit for publication. M.B.P is the study statistician, N.S.L will provide medical oversight, H.M and R.F created the virtual reality challenges in collaboration with I.S.M and Z.B. Z.B is the trial coordinator responsible for collecting trial data. All authors have read and approved the final manuscript.

Funding Statement

This work was supported by The Lambert Initiative for Cannabinoid Therapeutics, a philanthropically funded centre for medicinal cannabis research at The University of Sydney.

Competing Interests

Author I.S.M. acts as a consultant to Kinoxis Therapeutics and has received honoraria from Janssen. He has also served as an expert witness in various medicolegal cases involving cannabis and cannabinoids. I.S.M. hold patents on cannabinoid therapies (PCT/AU2018/05089 and PCT/AU2019/050554) and has received consulting fees from the

Medicinal Cannabis Industry Australia (MCIA). D.M. has also received consulting fees from MCIA. Z.B, H.M N.S.L, R.F and M.B.P have no disclosures to report.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Patient consent for publication

Not applicable.

Trial Sponsor

The University of Sydney, Sydney NSW Australia.

Data Availability Statements

The deidentified participant data are available from Professor Iain McGregor (iain.mcgregor@sydney.edu.au) upon reasonable request.

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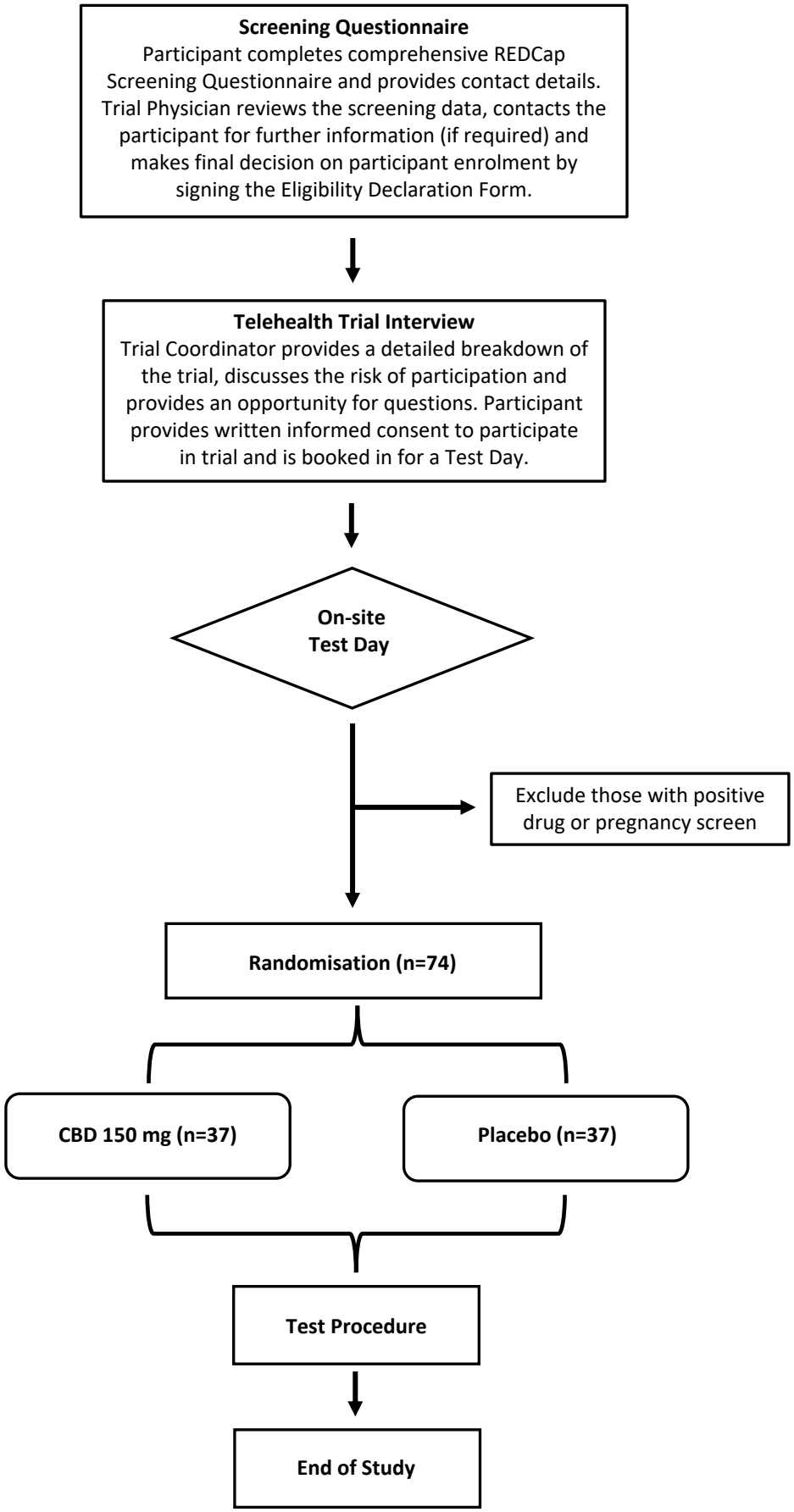

Screening Questionnaire
Participant completes comprehensive REDCap Screening Questionnaire and provides contact details. Trial Physician reviews the screening data, contacts the participant for further information (if required) and makes final decision on participant enrolment by signing the Eligibility Declaration Form.
Telehealth Trial Interview
Trial Coordinator provides a detailed breakdown of the trial, discusses the risk of participation and provides an opportunity for questions. Participant provides written informed consent to participate in trial and is booked in for a Test Day.
On-site Test Day
Exclude those with positive drug or pregnancy screen
Randomisation (n=74)
CBD 150 mg (n=37)
Placebo (n=37)
Test Procedure
End of Study

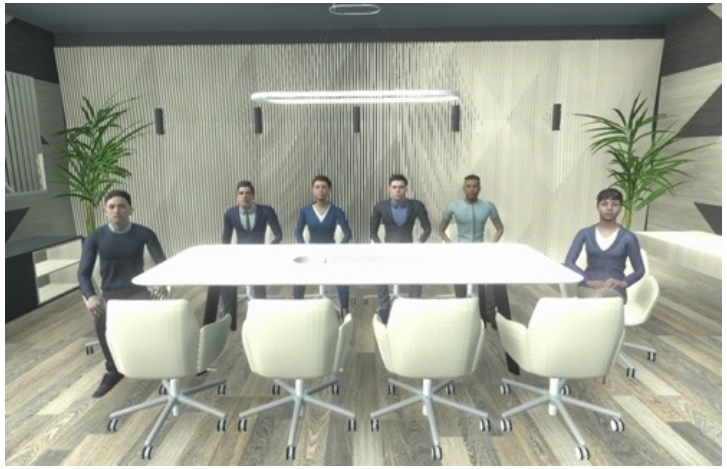


Figure 2, Panel A

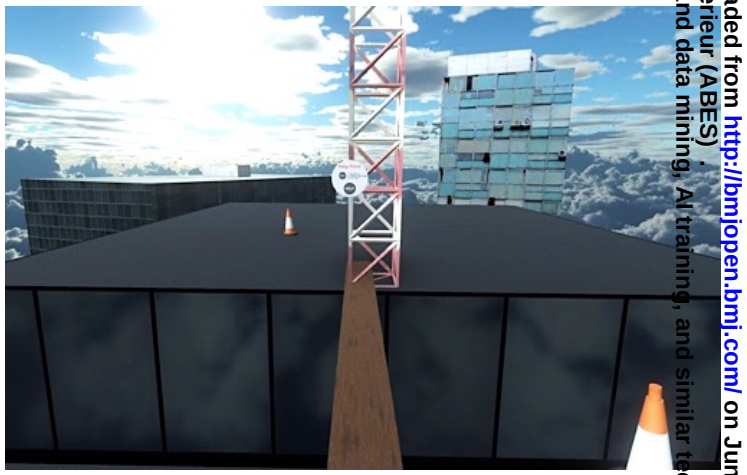
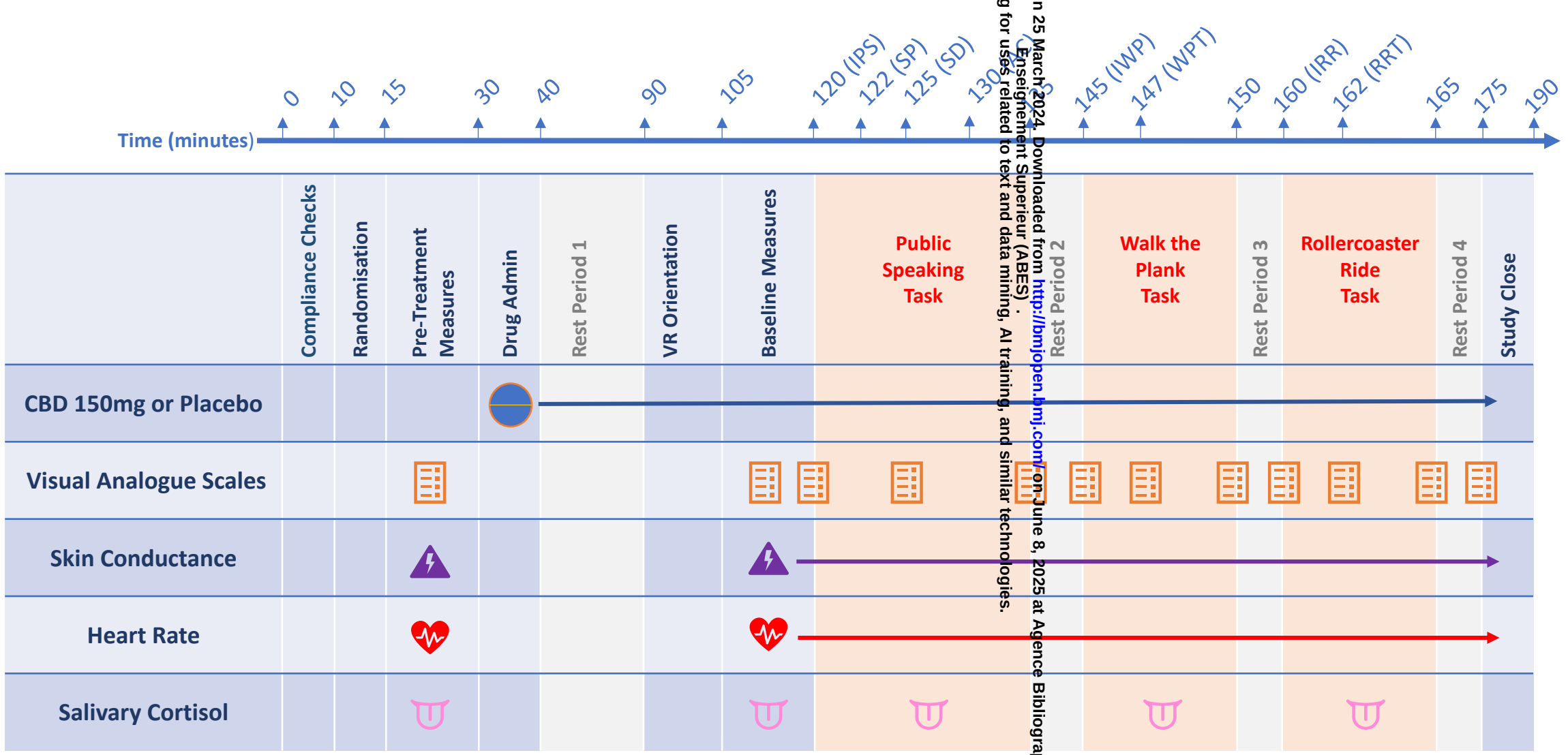


Figure 2, Panel B

Figure 2, Panel C



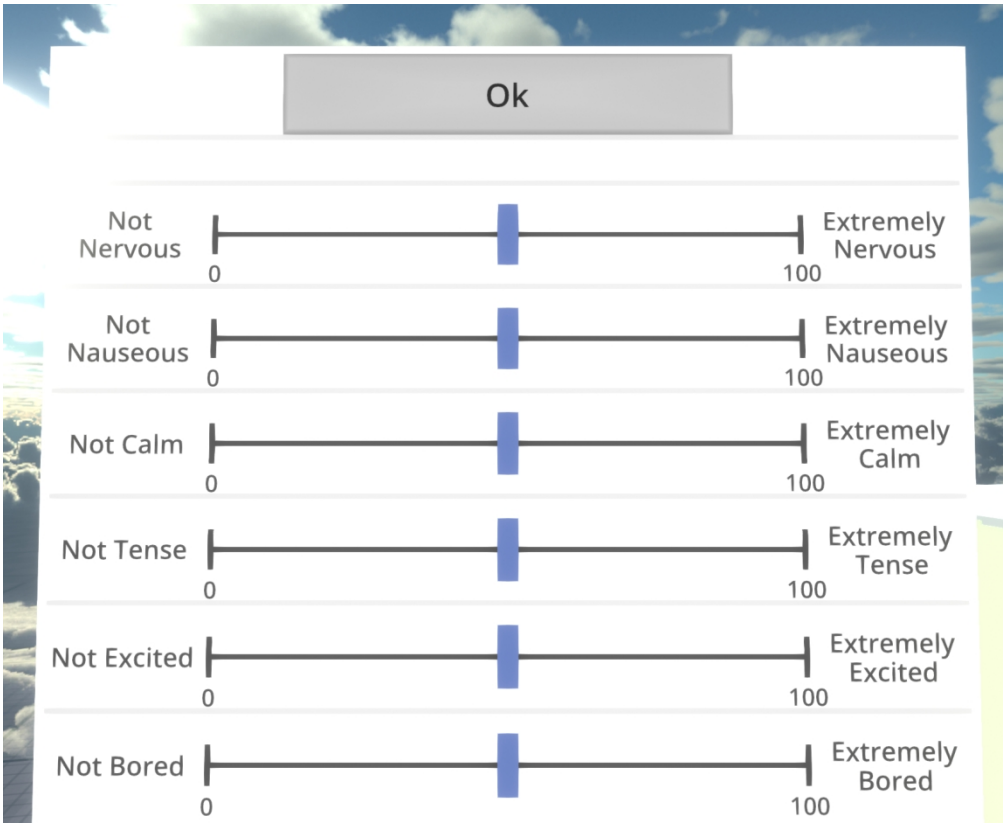


Figure 4. Self-reported Visual Analogue Scales (VASs) as they appear on the virtual reality headset. Values are provided by the participants by clicking on the scales using the hand controllers.

451x369mm (96 x 96 DPI)

ABN 15 211 513 464

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The Cannabidiol for Acute Psychosocial Stress and Nausea (CAPSTAN) Trial
ENROLMENT CONSENT FORM (HREC Approval No.: 2023/307)

1. I have read and understood the Participant Information Sheet and agree to take part in the CAPSTAN trial.

2. I have had the project, so far as it affects me, and the potential risks and burdens fully explained to my satisfaction by the research team. I have had the opportunity to ask any questions I may have about the project and my participation. My consent is given freely.

3. I have no history of allergy or other adverse reactions to the drug, cannabidiol.

4. Although I understand the purpose of the research project is to improve the quality of health/medical care, it has also been explained to me that my involvement may not be of any benefit to me.

5. I realise my participation in this study is voluntary and that I have the right to withdraw from the study at any stage without prejudice. I also understand that the research team has the right to terminate the study at any stage before completion, without jeopardising my medical care, if they believe this is in my best interests, for non-compliance with study procedures or for other legitimate reasons. If I decide to withdraw from the study, I agree that information collected about me up to the point when I withdraw may continue to be processed.

6. I understand that all information will remain confidential and while the information gained during the study may be published in journal articles, conferences, presentations etc, I will not be identified in any way. I agree to my non-identifiable information being used for future research purposes limited to the work of the University of Sydney.

7. My information will only be used for the purposes of this research, and it will only be disclosed according to the consent provided, except where disclosure is required by law.

8. I understand that access may be required to my medical records for the purpose of this study as well as for quality assurance, auditing and in the event of a serious adverse event and I consent to this access.

9. I am aware that I should keep a copy of this Consent Form, when completed, and a copy of the Patient Information Sheet.

10. I agree to have my General Practitioner informed of my participation in this study if required for my medical care and that he/she may divulge details of my past medical history, as he/she sees relevant, to the Investigator.

11. I am aware that I should keep a copy of the completed Consent Form and the attached Participant Information Sheet.

12. I am 18 years of age or over.

This consent form relates to Participant Information Statement Version 1.6_1 December 2023

Participant to complete:

I consent to participate in the CASTAN trial screening procedure and if eligible, I consent to participate in the CASTAN trial.

Name: _____Signature: _____Date: _____

Trial Coordinator to complete:

I have described the nature of the research to _____

print name of participant

and in my opinion, she/he understood the explanation.

Name: _____Signature: _____Date: _____

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Manuscript Page and Notes
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	Yes, ANZCTR public trials registry, ACTRN12623000872639. Registered 15 August 2023.
Protocol version	3	Date and version identifier	2, 6 and 25
Funding	4	Sources and types of financial, material, and other support	6, 25
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,25
	5b	Name and contact information for the trial sponsor	6
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	25
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	23
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5-6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA, intervention is a single acute dose of active or placebo with no ongoing involvement of participants.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA, intervention is a single acute dose of active or placebo with no ongoing involvement of participants.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12-13

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	19
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10 (Figure1)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	23
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	22
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7,17
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	22-23
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	23-24
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	23-24
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	23-24
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	23
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22-23
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	2, 6, 25
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	6
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	25
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	17
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	23
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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The effects of cannabidiol on psychosocial stress, situational anxiety and nausea in a virtual reality environment: A protocol for a single-centre randomised clinical trial

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The effects of cannabidiol on psychosocial stress, situational anxiety and nausea in a virtual reality environment: A protocol for a single-centre randomised clinical trial

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ABSTRACT

Introduction: The non-intoxicating plant-derived cannabinoid, *cannabidiol* (CBD), has demonstrated therapeutic potential in a number of clinical conditions. Most successful clinical trials have utilised relatively high (≥ 300 mg) oral doses of CBD. Relatively few studies have investigated the efficacy of lower (<300 mg) oral doses, typical of those available in over-the-counter CBD products.

Methods: We present a protocol for a randomised, double-blind, placebo-controlled, parallel-group clinical trial investigating the effects of a low oral dose (150 mg) of CBD on acute psychosocial stress, situational anxiety, motion sickness and cybersickness in healthy individuals. Participants (n=74) will receive 150 mg of CBD or a matched placebo 90 minutes before completing three virtual reality (VR) challenges (tasks) designed to induce transient stress and motion sickness: (1) a 15-minute "Public Speaking" task; (2) a 5-minute "Walk the Plank" task (above a sheer drop); and (3) a 5-minute "Rollercoaster Ride" task. The primary outcomes will be self-reported stress and nausea measured on 100-mm visual analogue scales. Secondary outcomes will include salivary cortisol concentrations, skin conductance, heart rate, and vomiting episodes (if any). Statistical analyses will test the hypothesis that CBD reduces nausea and attenuates subjective, endocrine, and physiological responses to stress compared to placebo. This study will indicate whether low-dose oral CBD has positive effects in reducing acute psychosocial stress, situational anxiety, motion sickness and cybersickness.

Ethics and dissemination: The University of Sydney Human Research Ethics Committee has granted approval (2023/307, Version 1.6, 16th February 2024). Study findings will be disseminated in a peer-reviewed journal and at academic conferences.

Trial Registration Number: ACTRN12623000872639 (ANZCTR public trials registry, registered 15th August 2023).

Keywords: cannabidiol (CBD), nausea, anxiety, virtual reality.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study will use a rigorous randomised, double-blind, placebo-controlled design to investigate the effects of a low oral dose (150 mg) of cannabidiol.
- The dose of cannabidiol provided (i.e., 150 mg) will be comparable to those available over-the-counter in many countries.
- Virtual reality (VR) will be used to administer safe, realistic, and precisely reproducible test paradigms that induce transient stress and nausea in volunteers.
- Blood samples will not be obtained to verify plasma cannabidiol concentrations as venepuncture has the potential to induce extraneous stress.

1. INTRODUCTION

Cannabidiol (CBD) is a non-intoxicating constituent of the *Cannabis sativa* plant [1, 2]. It has a good safety and tolerability profile [3-7] and a diverse range of pharmacological targets, including the serotonin receptors (e.g., 5-HT1A), G protein-coupled receptors (e.g., GPR55 and GPR18) and transient receptor potential ion channels (e.g. TRPA1) [8-10]. CBD has demonstrated therapeutic potential in several clinical conditions [4, 5], including anticonvulsant effects in paediatric epilepsy [11-13], anxiolytic effects in anxiety disorders [14, 15], antipsychotic effects in schizophrenia [16, 17] and "anti-addiction" effects in substance use disorders [18, 19]. These clinical benefits are typically observed at relatively high oral doses of CBD (e.g., ~300-1500mg) [4, 5].

In regions such as North America and Europe, CBD is available as both a prescription drug and a 'nutraceutical product' [20]. These nutraceutical or "wellness" products are typically oral formulations (e.g., oils, capsules, gummies) that contain low doses of CBD (i.e., ≤150 mg/day) and are widely available in health food stores and pharmacies [20, 21]. In Australia, regulation of "low-dose CBD products" (containing ≤150 mg/day) was recently eased to allow patients without a prescription access to registered products in pharmacies. However, registration of such products requires approval by Australia's medicines regulator based on demonstrated efficacy and safety [22]. With no products having been registered in the ~3 years since these regulatory changes were enacted (December 2020) [22], questions about whether CBD can demonstrate efficacy at these lower doses have been raised.

Indeed, a recent review found little high-quality evidence to support the efficacy of CBD (in any conditions) at doses ≤200 mg. This review acknowledged, however, that such doses were under-studied [21]. Some promising results have been obtained with public speaking tasks designed to induce "psychosocial stress" in healthy volunteers when 300 mg CBD was administered [23, 24]. Therefore, further research investigating the anxiolytic effects of low to moderate oral doses of CBD is warranted. It should be noted that the oral bioavailability of CBD is limited (~13-19%) but may be enhanced by certain lipid-rich formulations or by administration with fatty foods [6, 25]. The current study will utilise a proprietary blend of tocopherol phosphates (so called 'Tocopheryl Phosphate Mixture' (TPM®)), which has been shown to increase the bioavailability of lipid-soluble substances [26, 27].

Virtual reality (VR) technologies are increasingly being used to investigate psychosocial stress and anxiety in laboratory studies [28, 29]. This approach allows for minimal resourcing relative to "real-world" studies, customisable and reproducible test paradigms and the accurate monitoring and recording of key outcomes [29, 30]. Furthermore, VR provides the advantage of simulating physiologically-provoking activities without real danger e.g., walking a plank over a sheer drop. However, the use of VR is often accompanied by motion sickness (a pattern of symptoms that arise from exposure to stimuli involving significant visual or physical motion) and cybersickness (a sub-type of motion sickness that arises specifically due to exposure to VR) [31-34]. CBD has shown anti-nausea and anti-emetic effects in preclinical studies involving laboratory animals [35-37]. Interestingly, two of these studies demonstrated that CBD administered intraperitoneally at low doses (2.5 - 10 mg/kg) but not higher doses (25 and 40 mg/kg) reduced toxin-induced vomiting in house musk shrews [35, 36]. CBD also reduced vomiting in human studies when used in combination with Δ^9-tetrahydrocannabinol to treat chemotherapy-induced nausea and vomiting [38]. Accordingly, the current study will investigate CBD's possible anti-nausea effects in participants by exposure to VR scenarios [29].

We have developed a series of three unique VR challenges for the current trial. The "Public Speaking" task was adapted from studies that explored the anxiolytic effects of CBD during public speaking challenges [23, 24, 39] and will allow us to determine the effects of CBD during social threat (i.e., psychosocial stress). The 5-minutes "Walk the Plank" task has been modified from previous VR studies requiring participants to walk along a narrow virtual plank above a precipitous drop, inducing physiological markers of acute anxiety [40-42] (i.e., "situational anxiety"). Finally, the "Rollercoaster Ride" task challenges participants to complete two rounds of a virtual rollercoaster ride and has been modelled from VR rollercoaster ride paradigms that have been reliably used to induce motion sickness and cybersickness in participants [32, 43, 44].

In summary, the current protocol describes a study that aims to investigate the effects of low-dose (150 mg) CBD *versus* placebo on virtual reality (VR)-induced acute psychosocial stress, situational anxiety, acute motion sickness and cybersickness in healthy individuals.

2. METHODS

2.1 Study design

This study is a randomised, double-blind, placebo-controlled, parallel-group, clinical trial comparing the efficacy of low-dose CBD (150 mg) *versus* placebo. The study is known as the CAPSTAN (Cannabidiol for Acute Psychosocial Stress and Nausea) clinical trial. A crossover design was deemed unsuitable due to the high likelihood of trial-order effects (e.g., habituation to the VR scenarios) [32].

The trial sponsor is the University of Sydney, and the trial site is the Brain and Mind Centre in Sydney, Australia. The study has been approved by the University of Sydney Human Research Ethics Committee (HREC; 2023/307, Version 1.6, 16th February 2024) and registered on the Australian New Zealand Clinical Trials Registry (ACTRN12623000872639, 15th August 2023). The study is financially supported by the Lambert Initiative for Cannabinoid Therapeutics, a philanthropically funded centre for cannabinoid research at the University of Sydney. Recruitment commenced on 9th October 2023 and is anticipated to conclude in mid-2024.

2.2 Participant Population

2.2.1 Inclusion Criteria

We aim to recruit 74 participants who will be: (a) healthy adults aged between 18 and 50 years; (b) proficient in English and able to provide informed consent; (c) residing in the Greater Sydney region of New South Wales, Australia; and (d) willing to follow the protocol requirements.

2.2.2 Exclusion Criteria

The following exclusion criteria will apply:

a. Self-reported regular use (i.e., more than twice weekly) in the past two weeks of:
    i. Cannabinoid-containing products (e.g., cannabis or CBD)

ii. Psychotropic drugs (prescriptive or illicit) (e.g., cannabis, amphetamines, cocaine, ecstasy (MDMA), LSD (acid), antidepressants, antiepileptics, opioids, benzodiazepines)

iii. Medication that may affect the stress response (e.g., corticosteroids, beta-blockers)

b. Self-reported history of allergic reaction (e.g., urticaria or anaphylaxis) to cannabis or cannabinoid-containing products.

c. Self-reported history of liver disease, renal disease, epilepsy or heart disease (medically controlled high blood pressure <140/90mmHg is acceptable).

d. Current (i.e., within the past 2 weeks) otologic (vestibular) disease.

e. A history of repeated episodes of syncope.

f. Pregnant, lactating, or trying to conceive.

g. Self-reported history of drug and/or alcohol dependence (or suspected drug and/or alcohol dependence as determined by the trial physician).

h. A medically diagnosed anxiety disorder (e.g., social anxiety disorder) within the past 12 months.

i. Current suicidal ideation (i.e., a score >0 on Question 9 of the Patient Health Questionnaire) or suspected suicidal ideation as determined by the trial physician.

j. Current depression, anxiety, and stress scores outside the 'healthy range' on the Depression Anxiety Stress Scale-21 [45] (i.e., > moderate scores for depression (>20), anxiety (>14) and stress (>25)).

k. A chronic medical condition (mental or physical) that is uncontrolled i.e., has been either newly diagnosed, or previously diagnosed and remains symptomatic.

l. Self-reported high vulnerability to cybersickness or motion sickness.

m. Frequent (i.e., more than weekly) use of VR technologies, which tends to produce desensitisation towards cybersickness [32].

n. Self-reported intense fear of heights.

2.2.3 Recruitment and Retention

Participants will be recruited via social media, word of mouth, printed or online study advertisements and direct emails to individuals who have previously registered their

interest in participating in clinical trials with the Lambert Initiative for Cannabinoid Therapeutics. Participation is voluntary, and participants can withdraw at any time. Participants will be reimbursed with a $200 gift voucher as compensation for time and expenses incurred as a result of study participation.

2.3 Treatments

The treatments will be purchased from Avecho Biotechnology Limited (Clayton, Victoria), manufactured (Catalent Pharma Solutions, St. Petersburg, FL), as well as packaged and labelled (Central Pharmacy Logistics, Coburg North, VIC) at GMP-licenced facilities, stored at the Brain and Mind Centre (in a secure, temperature-controlled room), and dispensed by the trial coordinator (who is also a registered pharmacist) and another blinded investigator.

As this trial utilises a non-clinical (healthy) population, a placebo comparator is the most suitable and ethical choice. Accordingly, an acute dose of CBD (as opposed to chronic administration) will be utilised. Indeed, the treatment of many ailments utilises an *ad hoc* treatment regime.

2.3.1 Intervention

The investigational product (Avecho Biotechnology Limited, Victoria, Australia) is an oil-based, soft-gel capsule. Each gel capsule contains 75 mg of pure, synthetic (-)-CBD enantiomer and 75 mg of TPM® in medium chain triglyceride (MCT) oil. The capsules do not contain any other cannabinoids or cannabis constituents.

2.3.1a Safety

CBD is generally considered to have a good safety profile [3, 4, 21, 46, 47]. In previous studies, 150 mg CBD caused a very low frequency of mild adverse events that did not differ from placebo [21]. The current study's comprehensive screening and exclusion criteria aims to reduce the likelihood of adverse events.

2.3.1b Dose

A dose of 150 mg of CBD (i.e., two soft-gel capsules) will be administered orally.

2.3.1c Control

The control is a matched placebo. The placebo is identical to the intervention but contains no CBD and will also be administered via oral ingestion.

2.3.2 Randomisation, Allocation Concealment and Blinding

Participants will be randomised to one of two possible treatments in a 1:1 ratio at the beginning of their test day. Specifically, they will be assigned a unique randomisation number that is linked to a treatment via a pre-populated randomisation schedule. The schedule will be generated in seven balanced blocks of 10 and one balanced block of four by an independent statistician using an online random number generator and stored in a password-protected system inaccessible to blinded study personnel (centralised computerised randomisation). The schedule will only be available to the statistician, an independent researcher and the company that will package and label the treatments (Central Pharmacy Logistics, Coburg North, Victoria).

Treatment allocation will be concealed using numbered containers (or 'sachets'). Each 'dose' will be packaged in a separate, opaque, aluminium sachet labelled with a unique randomisation number.

As this is a double-blind study, participants and the remainder of the research team will not be aware of the assigned treatment. In the event of an emergency, the principal investigator or trial physician may request the unblinding of a participant for medical care.

2.4 Eligibility Screening

A Study Flowchart is presented in **Figure 1**. Willing volunteers will complete a comprehensive online Screening Questionnaire using the 'Research Electronic Data Capture' (REDCap™) web-based system (~20 minutes). Volunteers are required to complete a compulsory online declaration tick-box at the start of the questionnaire consenting to the use of the information they provide to evaluate their eligibility. The questionnaire will assess their eligibility to participate. Each volunteer who attempts the Screening Questionnaire will be assigned a unique screening number to anonymise their identifying information. The trial physician will review volunteers' responses and decide on their eligibility for the trial. They will document their decision by completing an Eligibility

Declaration form and a prescription for the trial drug (valid only when the volunteer has been randomised).

[INSERT FIGURE 1 HERE]

Figure 1. Study flowchart summarising the screening, enrolment and randomisation of participants in the CAPSTAN trial.

2.5 Enrolment

Eligible participants will be invited to complete a telehealth interview with the trial coordinator. Here, they will receive detailed information about the trial procedures and risks and be informed that their enrolment requires a negative urine drug and pregnancy screen at the start of the experimental procedures (i.e., the "Test Day"). The trial coordinator will invite the volunteer to ask questions and to discuss participation in the trial with the trial doctor, or to take additional time to consider their decision to participate. Once the trial coordinator is confident that the volunteer understands the requirements of the trial, they will request that the participant sign the informed consent form (please see Supplementary file). The trial coordinator will then counter-sign the consent form, collect basic demographic information and book the participant for a Test Day.

2.6 Experimental Procedure

Each participant will complete one Test Day (~3 hours) at the Brain and Mind Centre at the University of Sydney (**Table 1**).

Table 1. The CAPSTAN trial experimental procedure

Time (minutes) from start	Approximate Duration	Activity
0	10 min	Introduction and pre-trial compliance checks
10	5 min	Randomisation
15	15 min	Pre-treatment measures
30	10 min	CBD or placebo administration and caloric beverage
40	50 min	Rest period 1
90	15 min	Virtual reality orientation
105	15 min	Baseline measures
120	15 min total (2 min) (3 min) (5 min) (5 min)	**Public Speaking task:** Instructions Speech preparation Speech delivery Arithmetic challenge
135	10 min	Rest period 2
145	5 min total (2 min) (3 min)	**Walk the Plank task:** Instructions Walk the Plank task
150	10 min	Rest period 3
160	5 min total (2 min) (3 min)	**Rollercoaster Ride task:** Instructions Rollercoaster ride task
165	10 min	Rest period 4
175	15 min	Study Close
190	-	**End Of Test Day**

2.6.1 Standardisation Procedures

Prior to each onsite Test Day, participants will be instructed to: (a) abstain from alcohol (≥24 h); (b) avoid greater than one standard serving of caffeine at least 2-hours before the Test Day; (c) ensure they are well hydrated; and (d) ensure that they are adequately fed by consuming a meal at least 2 hours before arrival to the Test Day. These factors aim to reduce the likelihood of malaise, gastrointestinal disturbances or heightened anxiety on the Test Day.

2.6.2 Compliance Checks

Participants will complete a urinary drug screen (to identify any recent use of cannabis and other psychoactive substances such as cocaine, ecstasy (MDMA), amphetamines, benzodiazepines and opioids) and, if they are female, a urine pregnancy screen, on arrival at the study site. The trial coordinator will also confirm compliance with the standardisation procedures and if there have been any changes to the participant's health status or medication use since the last contact. Participants who meet these requirements will be randomised; those who do not may be invited to return at another time if suitable.

2.6.3 Experimental Procedures

Following randomisation, participants will be fitted with the Equivital EQ02+ LifeMonitor belt and the VR headset (detailed below). There will then be a 10-minute collection of pre-treatment measures (see *Study Outcomes* below). After this, the VR headset and Equivital EQ02+ LifeMonitor belt will be temporarily removed, the treatment administered, and the participant given a compulsory standardised caloric beverage to consume; specifically, 500 mL of "Up & Go Liquid Breakfast" (Sanitarium, Berkeley Vale NSW, Australia) containing approximately 1,640 kilojoules, 16.8g of protein, 8.6g of fat and 57g of carbohydrates. For consistency, all participants are required to consume this beverage in its entirety, which aims to potentiate the absorption of CBD in the gastrointestinal tract [6, 48-50] and provide participants with sustenance during the test session. Participants will be provided with a rest period of approximately 50 minutes, during which they will be left alone in a quiet reception area and encouraged to undertake some low-stress reading; this delay is aimed at allowing CBD plasma concentrations to approach a near-maximal level [48]. Following this, participants will be provided with instructions on the three VR tasks and the functionality of the VR hardware during a 15-minute "VR Orientation" session. The VR headset and Equivital EQ02+ LifeMonitor belt will then be re-fitted for a 10-minutes collection of the pre-task measures. The devices will remain fitted to participants until the end of the experimental procedure.

2.6.4 Virtual Reality Tasks

The three tasks developed for this clinical trial are the "Public Speaking" task (for psychosocial stress), the "Walk the Plank" task (for situational anxiety) and the "Rollercoaster Ride" task (for motion sickness). Although these exact VR tasks have not been previously used in clinical trials or laboratory studies, similar tasks have been reported in the broader scientific literature [29, 32, 40-42, 51].

The 15-minute "Public Speaking" task was modelled on previous studies that found CBD to have anxiolytic effects during public speaking challenges [23, 24, 39] and the Trier Social Stress Test (TSST) [52]. The abovementioned public speaking tasks varied in their methodology and involved either "simulated" public speaking tasks whereby participants delivered a speech in front of a video camera or real-life tasks where participants delivered speeches to a live audience [23, 24, 39]. Conversely, the TSST is a structured stress paradigm involving a 10-minute speech preparation period followed by a 10-minute test period during which participants complete a 5-minute job application speech followed by 5 minutes of mental arithmetic [53]. The TSST reliably induces psychosocial stress (i.e., stress involving the perception of one's worth, competence, or status by others) and results in an acute and reliable cortisol response in most participants [53-55]. In one study, a VR adaption of the TSST elicited similar salivary cortisol and subjective stress responses to a real-life TSST, demonstrating that the two were equivalent [29]. The current study utilises a modified VR version of the TSST involving a panel of virtual judges who withhold all feedback or affirmation (**Figure 2A**).

The task involves a three-minute "speech preparation period" (i.e., "please mentally prepare a speech on what attributes and experience you have that makes you the ideal candidate for your dream role") and a 10-minute "test period" consisting of (a) five-minute speech delivery period (i.e., "please deliver your speech and aim to speak for the full five-minutes") and (b) a five-minute mental arithmetic challenge (i.e., "please calculate 2703 – 13. From the result, please subtract 13 again and state your answer out loud. Repeat this process a total of five times. At the end of this process, please recall all five of your answers aloud").

The five-minute "Walk the Plank" task requires participants to virtually walk across a narrow virtual plank between two skyscrapers. Participants will be advised that they have become stranded on the top of the building and are required to signal for help using the safety

beacon placed at the opposite end of the plank. For this task, participants will walk up and down the length of the clinic room with their movement mapped to the virtual plank and guided by virtual boundaries, signifying a safe space (**Figure 2B**). VR studies utilising a similar challenge provided realistic experiences that induce physiological markers of stress that are consistent with acute anxiety (i.e., "situational anxiety") [40-42].

The five-minute "Rollercoaster Ride" task challenges participants to complete a virtual rollercoaster ride (**Figure 2C**). Participants will remain seated throughout this task and can indicate if they would like to stop the task early. Similar VR tasks have been shown to induce motion sickness in participants [32, 43, 44]. Motion sickness will be explored explicitly during the "Rollercoaster Ride" task, while cybersickness will be explored during all three VR scenarios.

[INSERT FIGURE 2A,2B AND 2C HERE]

Figure 2. (A) The panel of virtual judges from the "Public Speaking" task; (B) The roof of the building and plank from the "Walk the Plank" task; and (C) The view from the seat of the "Rollercoaster Ride" task.

2.6.5 Post Trial Procedures

After completing the final VR task, participants have a 10-minute recovery period. After this, they will be queried on any adverse events (AEs) experienced (i.e., "Have you experienced any unfavourable symptoms?"), What treatment do they think they received (i.e., CBD or placebo), and what are their confidence estimates (i.e., How sure are you of your guess on which treatment you received?"). All participants will be provided with the contact details of the research team to self-report any AEs over the next 24 hours. The trial coordinator or research assistant will record all adverse event reports using a REDCap™ AE report form and communicate to the trial physician if required.

The occurrence of AEs will be discussed weekly with the trial physician. Furthermore, a blinded summary of AE reports will be emailed to the trial physician monthly, and the trial physician will indicate if the rate of AEs is unacceptably high. In the unlikely event of a severe

adverse event (SAE), the trial physician and the principal investigator (PI) will be immediately notified, and all SAEs will be reported to the trial sponsor and the HREC within 72 hours.

2.7 Data Collection

A summary of the data collected during the CAPSTAN trial and time of collection is provided in **Figure 3**

[INSERT FIGURE 3 HERE]

Figure 3. Data collected during the CAPSTAN trial "Test Day.
IPS = instructions for the "Public Speaking" task, **SP** = speech preparation, **SD** = speech delivery, **AC** = arithmetic challenge, **IWP** = instructions for the "Walk the Plank" task, **WPT** = "Walk the Plank" task, **IRR** = instructions for the "Rollercoaster Ride" task, **RRT** = "Rollercoaster Ride" task

2.7.1 Study Outcomes

The primary outcome measures include:

- Self-reported stress ratings on a VAS (*nervous 0-100*)
- Self-reported nausea ratings on a VAS (*nauseous 0-100*)

The secondary outcome measures include:

- Salivary Cortisol
- Heart rate
- Skin Conductance
- Vomiting or near-vomiting episodes
- Self-reported anxiety ratings on VASs include:
  - (*tense 0-100*)
  - (*calm 0-100*)
  - (*excited 0-100*)
  - (*bored 0-100*)

The exploratory (tertiary) outcome measures are:

- Salivary testosterone and progesterone

- Eye-tracking data including:
  - The frequency and duration of eye-closing
  - The frequency and duration of gaze at areas of interest

2.7.2 Visual Analogue Scales (VASs)

VASs will be used to measure the two primary outcomes: self-reported stress (nervousness) and nausea. They will also be used to measure the secondary outcome of self-reported anxiety (as detailed below) **(Figure 4).** These measures will be recorded before drug administration (pre-treatment measurement), at baseline, prior to the commencement of each VR task, at the completion of each VR task and at the study close. VAS measures will also be recorded prior to the performance of the speech *during* the "Public Speaking" task (Timepoint "SD" Figure 3) and prior to the performance of the walk *during* the "Walk the Plank" (Timepoint "WPT" Figure 3) task. These measures aim to capture "anticipatory anxiety" reported prior to anxiety-inducing tasks in previous studies [23, 24]. As nausea is the main interest during the Rollercoaster Ride task, no VAS measures for anticipatory anxiety will be taken for this task. All VASs will appear on the screen of the VR headset, and participants will use the VR hand controllers to click on the numerical value (0-100) that best describes their current state (**Figure 4**). Using such virtual scales allows seamless execution of self-report without disturbing the participant's sense of immersion within the virtual environment.

For a well-rounded exploration of CBD effects on anxiety, we have utilised VASs that query participant's affect (i.e., the outward expression of an emotion) [56]. Here, we consider 1) the "valence" of an emotion (i.e., its positivity or negativity) and 2) the intensity of the emotion (i.e., whether it is arousing or deactivating). This provides four self-reported anxiety rating VASs, including:

- positively arousing (*excited 0-100*)
- positively deactivating (*calm 0-100*)
- negatively arousing (*tense 0-100*)
- negatively deactivating (*bored 0-100*)

[INSERT FIGURE 4 HERE]

Figure 4. Self-reported Visual Analogue Scales (VASs) as they appear on the virtual reality headset. Values are provided by the participants by clicking on the scales using the hand controllers.

2.7.3 Salivary Cortisol, Testosterone and Progesterone

During the experimental procedure, oral fluid samples will be collected from participants five times using Salivette® collection tubes (Sarstedt AG and Co., Nümbrecht Germany). These samples will be collected immediately before drug administration, before the first VR task, and after each VR task. Participants will be advised to open the Salivette® tube, place the pad in their mouth for two minutes or until soaked with saliva, then return the pad to the tube and firmly seal it. The samples will be centrifuged for 2 minutes at 1,000 x g and analysed simultaneously for cortisol (a secondary outcome) and testosterone and progesterone (exploratory outcomes) using an in-house developed mass spectrometry (MS) method [57, 58].

2.7.4 Heart Rate

Heart rate will be measured for 10 minutes immediately before drug administration (baseline measurement), for 10 minutes at baseline and continuously throughout all three VR tasks using the EQ02+ LifeMonitor and belt, as described in previous studies [59, 60] (Equivital Ltd, Cambridge, United Kingdom; https://www.adinstruments.com/partners/equivital). This is a wireless, medical-grade monitoring system that records a range of physiological measures (such as electrocardiogram, breathing rate, tri-axial acceleration, galvanic skin response, skin temperature) using a wearable vest and permits live data streaming and download using the LabChart software.

2.7.5 Skin Conductance

Skin conductance will be measured for 10 minutes immediately before drug administration (baseline measurement), for 10 minutes at baseline and continuously throughout all three VR tasks.

Skin conductance of the forehead will be measured using the Equivital GSR Sensor connected to the EQ02+ LifeMonitor and belt (as described above). Skin conductance of the fingers will be measured using MLT117F/10 GSR finger electrodes connected to a FE116 GSR Amp with a PLCF1 front-end interface used with PowerLab C software (ADInstruments, Oxford United Kingdom), a system used in previous studies [61, 62]. Research suggests that forehead skin conductance may correlate with motion sickness, while finger skin conductance correlates with acute stress [32, 63]. Therefore, both will be measured in the current study.

2.7.6 Vomiting Episodes

The Test Day source document will record all vomiting episodes throughout the experimental procedure.

2.7.6 Eye-tracking

Eye tracking data will be collected continuously throughout the VR tasks using the VR headset. These measures will provide highly precise, objective data by which to examine participant's compliance (frequency and duration of eye closing) and engagement and response (frequency and duration of gaze at areas of interest, that is, the panel of judges in the “Public Speaking” task or the plank in the “Walk the Plank” task) during the VR challenges [64]. Gazing at pre-defined points of interest (e.g., the judges in the "Public Speaking" task or the plank in the "Walk the Plank" task) is based on the "Eye-Mind Hypothesis" that describes the tendency for people to direct their gaze towards what they are thinking about [64-66]. Both eye closing and gaze away from points of interest may correlate to fear or avoidance behaviours [67].

2.9 Data Management

Participant information collected for this trial will be securely stored and treated as confidential. Participants' identifying information will be anonymised using unique codes: initially, a screening number and, later, a randomisation number. The key to these codes will be securely stored in password-protected files inaccessible from the internet. Clinical trial data will be collected and managed using REDCap™, a secure, online program supported by the University of Sydney; access is password-protected and will only be available to approved

research staff. Hard copies of patient data will be securely stored in locked filing cabinets at the study site. Only the study investigators have access to the participant data. All trial data will be stored securely for at least 15 years. The findings of this clinical trial will be disseminated via conferences, publications, and media, as applicable. Participants will be informed of the results of the study at the conclusion of the trial. No participants will be identified in any report or publication of this study or its results.

2.10 Data and Safety Monitoring

Data monitoring will occur monthly for all new participant entries logged into the REDCap system; a designated team member will adhere to a standardised process of data review, raising queries and locking forms once the review has been completed. Safety monitoring of AEs will be managed according to sponsor and HREC requirements. As this is a small, single-centre, low risk, clinical trial utilising an acute, low-dose CBD, an independent data safety monitoring committee will not be formed. However, an independent 'Expert Group' has been formed and will be consulted by the research team and HREC in the unlikely instance of a severe adverse event. The Expert Group comprises three research physicians with extensive experience with CBD clinical trials. The decision to terminate the trial lies with the principal investigator based on safety data and recruitment targets.

2.11 Roles and Responsibilities

The study investigators have led the design of this study and are responsible for the management and conduct of this clinical trial. The study investigators will conduct the analysis and will make all publication-related decisions.

2.12 Statistical Methods

2.5.1 Sample Size Estimation

The target sample size was determined *a priori* using power calculation software (G*Power Version 3.1.9.6, University of Kiel, Germany). In an earlier investigation, Linares *et al.* (2019) found that 150 mg CBD decreased subjective ratings of anxiety on the Visual

Analogue Mood Scale (VAMS) during a simulated public speaking test, albeit non-significantly (Placebo: 18.6±15.9 mm, n=15; CBD: 7.6±15.5 mm; n=15; Cohen's *d*=0.70) [23].

Using an equivalent effect size (Cohen's *d*=0.70), a power (1-ß) of 0.8 and a two-sided α=0.05, we estimate that n=68 participants will be required to detect a significant effect of CBD on anxiety. Clinical trials conducted by the Lambert Initiative for Cannabinoid Therapeutics have indicated participant retention of ≥90% [48, 68]. Therefore, the n=74 will be recruited to account for attrition.

2.5.2 Statistical Analysis Plan

The primary and secondary outcomes and eye-openness will be analysed using generalized linear mixed models (GLMM), while eye gaze will be analysed using a mixed-effects multinomial logistic regression. Treatment, Time and the Treatment × Time interaction will be included as fixed effects, and the participant will be included as a random effect, with other covartiates included as appropriate to improve goodness of fit (e.g., Sex, Age, Time of day). To refine the models, we will use corrected Akaike Information Criterion [69]. We will calculate Δm between models and exclude models with Δm > 2 as having substantially less support [70]. No covariance structure will be specified (unstructured). To identify the best distribution and link for the GLMM models, the data type, residual plots, Shapiro-Wilk normality test, Levene's test for Homogeneity of Variance, and Pearson's dispersion test will be used. Type III Wald chi-square tests will be used to generate main effects p-values. A priori planned uncorrected pairwise comparisons will be performed to compare:

- subjective ratings of stress on a stress VAS (*nervous 0-100*) across treatments at (Timepoint t=125 (SD), Figure 3)
- subjective ratings of stress on a stress VAS (*nervous 0-100*) across treatments at (Timepoint t=147 (WPT), Figure 3)
- subjective ratings of nausea on a nausea VAS (*nauseous 0-100*) across treatments at (Timepoint t=165, Figure 3)
- subjective ratings of nausea on a nausea VAS (*nauseous 0-100*) across treatments at (Timepoint t=175, Figure 3)

as these are the primary outcome measures.

Dunn-Šidák corrected pairwise comparisons will be performed where additional significant main and interaction effects are present. Statistical significance will be accepted as $p<0.05$. The statistical analysis plan will be finalised before the last participant Test Day and will be available on request.

3. STATEMENTS AND DECLARATIONS

Ethics and dissemination: The study has been approved by the University of Sydney Human Research Ethics Committee (HREC; 2023/307, Version 1.6, 16th February 2024). The findings of this clinical trial will be disseminated via conferences, publications, and media, as applicable. Participants will be informed of the study's results at the trial's conclusion. No participants will be identified in any report or publication of this study or its results.

Author Contributions

I.S.M, D.M, H.M, M.B.P, N.S.L, R.F and Z.B were involved in the conception and design of the research project. Z.B drafted the manuscript, and all authors were involved in critically revising it. I.S.M is the principal investigator who has overall responsibility for the design, conduct and decision to submit for publication. M.B.P is the study statistician, N.S.L will provide medical oversight, H.M and R.F created the virtual reality challenges in collaboration with I.S.M and Z.B. Z.B is the trial coordinator responsible for collecting trial data. All authors have read and approved the final manuscript.

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Competing Interests

Author I.S.M. acts as a consultant to Kinoxis Therapeutics and has received honoraria from Janssen. He has also served as an expert witness in various medicolegal cases involving cannabis and cannabinoids. I.S.M. hold patents on cannabinoid therapies (PCT/AU2018/05089 and PCT/AU2019/050554) and has received consulting fees from the

Medicinal Cannabis Industry Australia (MCIA). D.M. has also received consulting fees from MCIA. Z.B, H.M N.S.L, R.F and M.B.P have no disclosures to report.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Patient consent for publication

Not applicable.

Trial Sponsor

The University of Sydney, Sydney NSW Australia.

Data Availability Statements

The deidentified participant data are available from Professor Iain McGregor (iain.mcgregor@sydney.edu.au) upon reasonable request.

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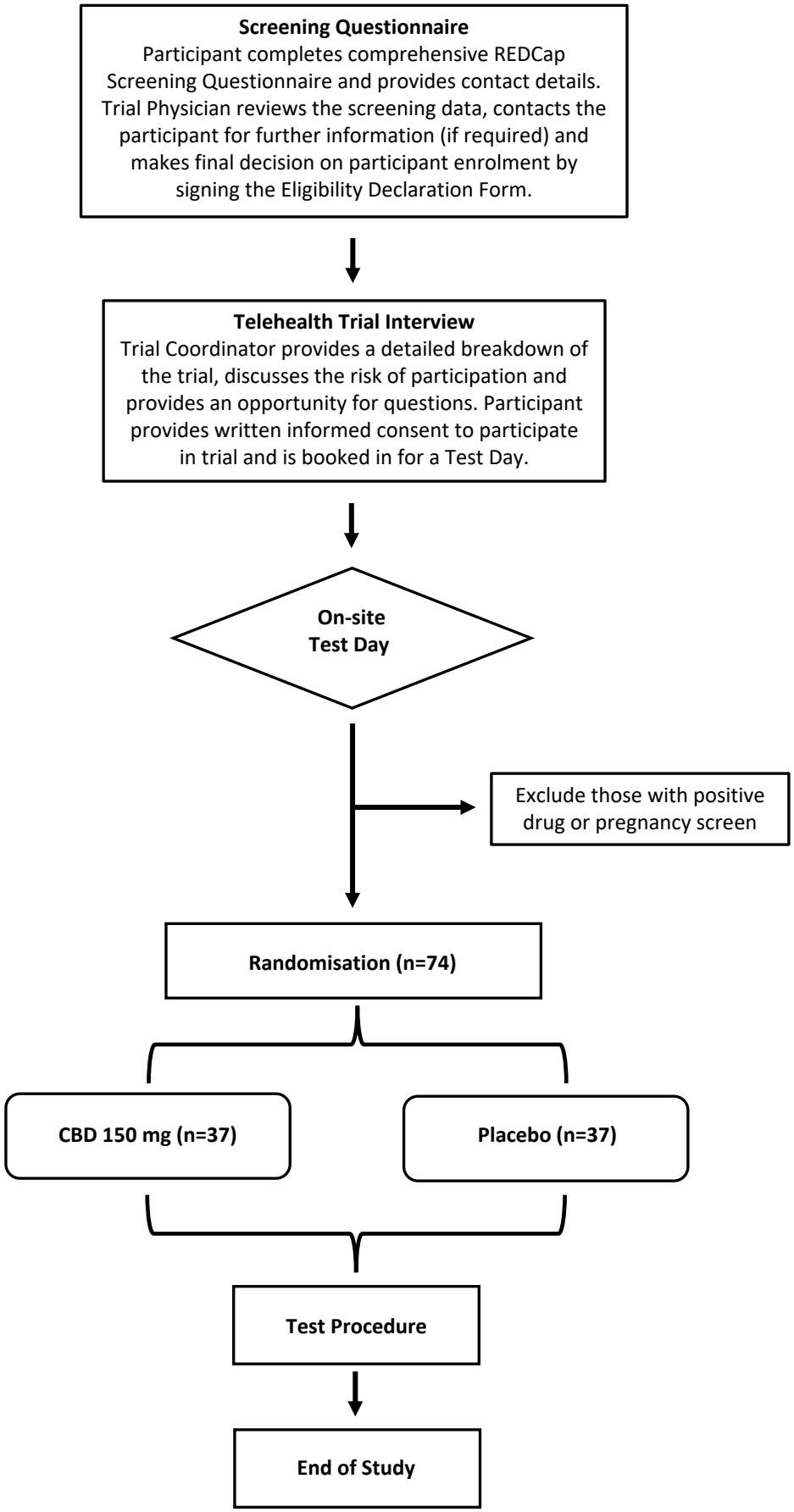

Screening Questionnaire
Participant completes comprehensive REDCap Screening Questionnaire and provides contact details. Trial Physician reviews the screening data, contacts the participant for further information (if required) and makes final decision on participant enrolment by signing the Eligibility Declaration Form.
Telehealth Trial Interview
Trial Coordinator provides a detailed breakdown of the trial, discusses the risk of participation and provides an opportunity for questions. Participant provides written informed consent to participate in trial and is booked in for a Test Day.
On-site Test Day
Exclude those with positive drug or pregnancy screen
Randomisation (n=74)
CBD 150 mg (n=37)
Placebo (n=37)
Test Procedure
End of Study

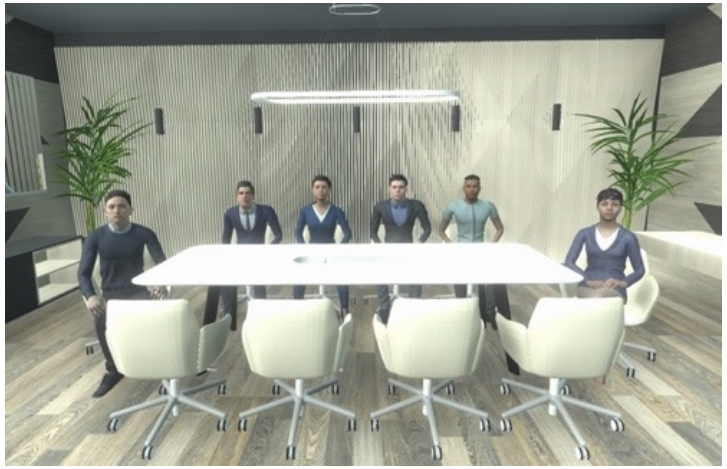


Figure 2, Panel A

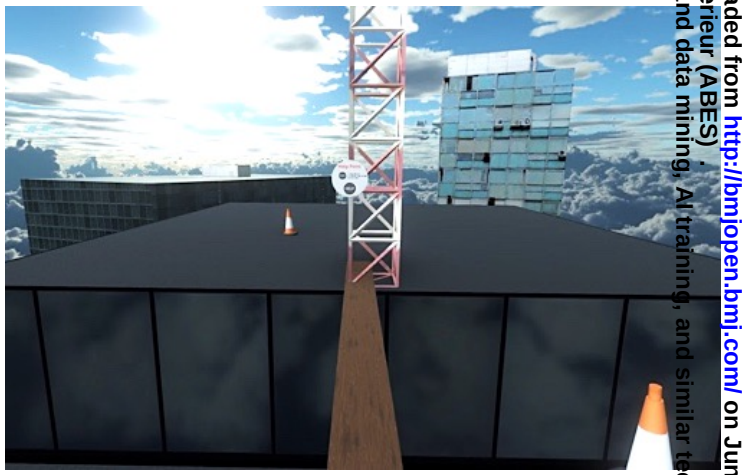
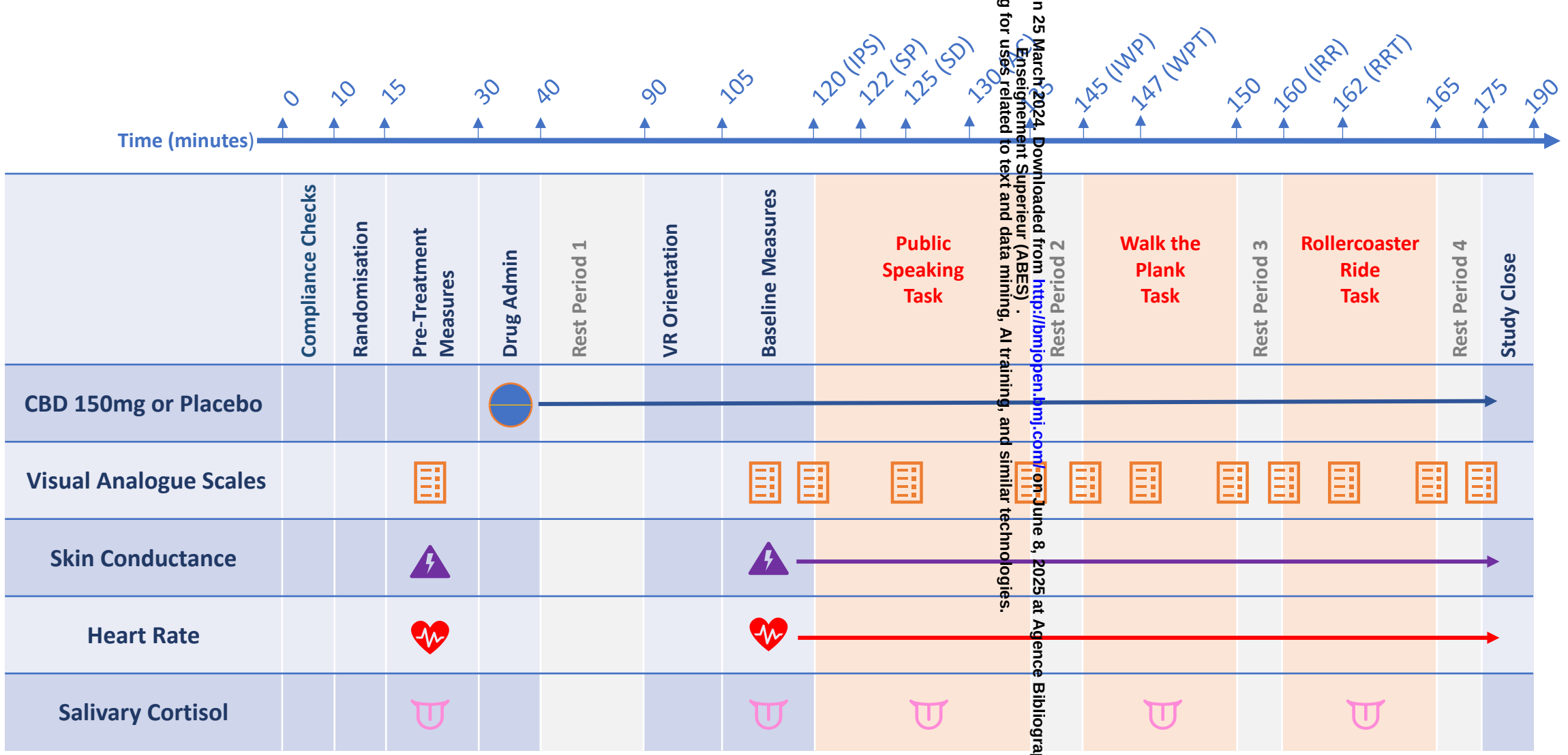


Figure 2, Panel B

Figure 2, Panel C



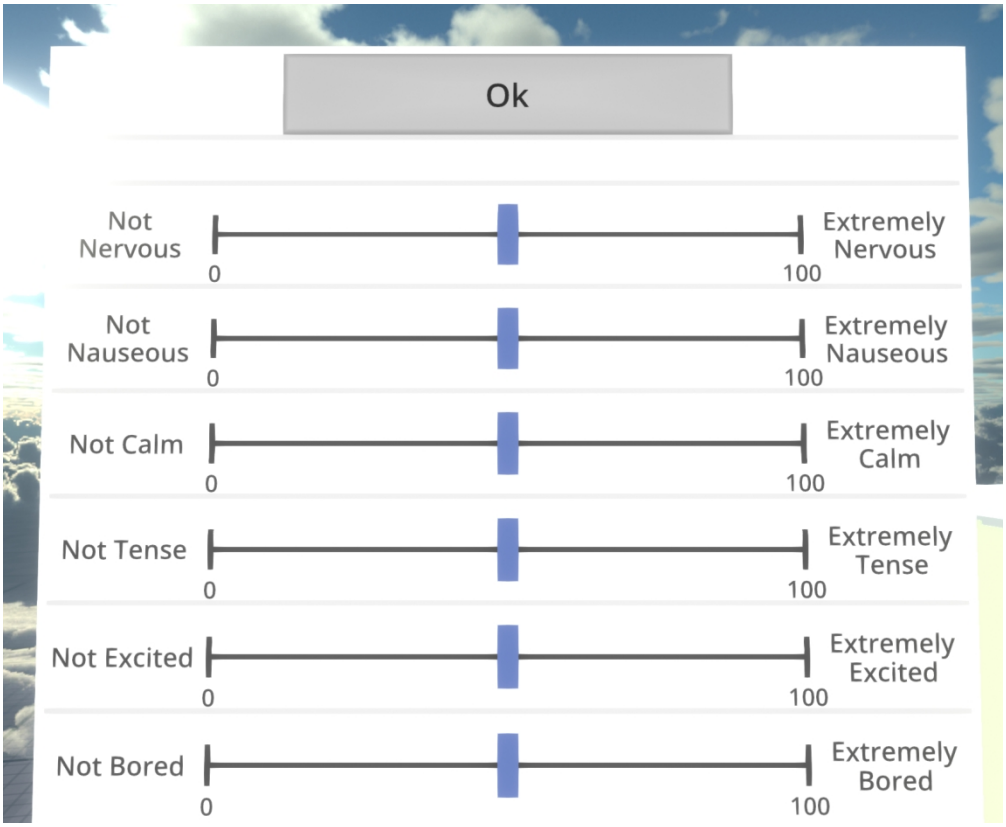


Figure 4. Self-reported Visual Analogue Scales (VASs) as they appear on the virtual reality headset. Values are provided by the participants by clicking on the scales using the hand controllers.

451x369mm (96 x 96 DPI)

ABN 15 211 513 464

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The Cannabidiol for Acute Psychosocial Stress and Nausea (CAPSTAN) Trial
ENROLMENT CONSENT FORM (HREC Approval No.: 2023/307)

1. I have read and understood the Participant Information Sheet and agree to take part in the CAPSTAN trial.

2. I have had the project, so far as it affects me, and the potential risks and burdens fully explained to my satisfaction by the research team. I have had the opportunity to ask any questions I may have about the project and my participation. My consent is given freely.

3. I have no history of allergy or other adverse reactions to the drug, cannabidiol.

4. Although I understand the purpose of the research project is to improve the quality of health/medical care, it has also been explained to me that my involvement may not be of any benefit to me.

5. I realise my participation in this study is voluntary and that I have the right to withdraw from the study at any stage without prejudice. I also understand that the research team has the right to terminate the study at any stage before completion, without jeopardising my medical care, if they believe this is in my best interests, for non-compliance with study procedures or for other legitimate reasons. If I decide to withdraw from the study, I agree that information collected about me up to the point when I withdraw may continue to be processed.

6. I understand that all information will remain confidential and while the information gained during the study may be published in journal articles, conferences, presentations etc, I will not be identified in any way. I agree to my non-identifiable information being used for future research purposes limited to the work of the University of Sydney.

7. My information will only be used for the purposes of this research, and it will only be disclosed according to the consent provided, except where disclosure is required by law.

8. I understand that access may be required to my medical records for the purpose of this study as well as for quality assurance, auditing and in the event of a serious adverse event and I consent to this access.

9. I am aware that I should keep a copy of this Consent Form, when completed, and a copy of the Patient Information Sheet.

10. I agree to have my General Practitioner informed of my participation in this study if required for my medical care and that he/she may divulge details of my past medical history, as he/she sees relevant, to the Investigator.

11. I am aware that I should keep a copy of the completed Consent Form and the attached Participant Information Sheet.

12. I am 18 years of age or over.

This consent form relates to Participant Information Statement Version 1.6_1 December 2023

Participant to complete:

I consent to participate in the CASTAN trial screening procedure and if eligible, I consent to participate in the CASTAN trial.

Name: _____Signature: _____Date: _____

Trial Coordinator to complete:

I have described the nature of the research to _____

print name of participant

and in my opinion, she/he understood the explanation.

Name: _____Signature: _____Date: _____

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Manuscript Page and Notes
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	Yes, ANZCTR public trials registry, ACTRN12623000872639. Registered 15 August 2023.
Protocol version	3	Date and version identifier	2, 6 and 25
Funding	4	Sources and types of financial, material, and other support	6, 25
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,25
	5b	Name and contact information for the trial sponsor	6
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	25
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	23
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5-6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA, intervention is a single acute dose of active or placebo with no ongoing involvement of participants.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA, intervention is a single acute dose of active or placebo with no ongoing involvement of participants.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12-13

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	19
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10 (Figure1)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	23
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	22
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7,17
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	22-23
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	23-24
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	23-24
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	23-24
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	23
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22-23
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	2, 6, 25
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	6
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	25
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	17
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	23
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.