STUDY PROTOCOL

Mindfulness-Based Intervention for Stress Reduction in Adult Singaporeans (MISRAS)

PROTOCOL VERSION: v1.0
PROTOCOL DATE: 26/08/2024
ClinicalTrials.GOV: NCT06765889

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PROTOCOL SIGNATURE PAGE

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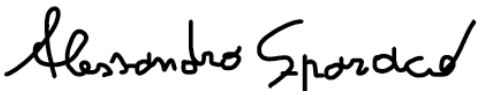
Declaration of Investigator

I confirm that I have read the above-mentioned protocol and its attachments. I agree to conduct the described study in compliance with all stipulations of the protocol and regulations.

Principal Investigator Name: Dr. Alessandro Sparacio

Principal Investigator Signature: 

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1. BACKGROUND AND RATIONALE

Stress dramatically impacts people's lives, influencing how individuals think, behave and experience emotions (Aldwin, 2007; Lazarus & Folkman, 1984). Furthermore, stress is one of the major culprits in the development of pathologies such as depression and chronic anxiety (Yang et al., 2015). According to 'The Ipsos survey for World Mental Health Day' mental health has now become the primary concern for both Singaporeans and individuals worldwide when asked about the most significant healthcare issues in their respective countries. The top health concern among Singaporeans is mental health, with nearly half (46%) identifying it as the most pressing health issue in the country today, followed by cancer (38%) and stress (35%). The far-reaching consequences of this stress epidemic extend beyond personal well-being, impacting both the economic and healthcare sectors. Indeed, the symptoms of depression and anxiety are accountable for a substantial loss of 2.9% of Singapore's GDP (Chodavadia et al., 2023). To proactively address the potential health repercussions linked to chronic stress, the development of stress reduction interventions becomes an imperative undertaking.

Among these, mindfulness stands out. Defined by Jon Kabat-Zinn (2003, 2006) as 'paying attention in a particular way: On purpose, in the present moment, and nonjudgmentally,' mindfulness offers a way to alleviate stress with interventions that can be self-administered. These approaches encompass different aspects such as fostering a non-judgmental attitude and embracing inner experiences, consistent with other mindfulness methods. However, compared to traditional protocols (such as Mindfulness Based Stress Reduction; MBSR; Kabat-Zinn, 2003), these interventions 1) do not require the presence of an instructor, 2) are always available, and 3) are relatively cheap (Spijkerman et al., 2016). As these interventions show promise in effectively managing stress for a significant portion of the population, given their scalability, it is imperative to gain a thorough understanding of their efficacy.

Recent meta-analyses have yielded inconsistent results concerning the presence of these effects for these interventions. One failed to uncover compelling evidence in their favour (Sparacio et al., 2024a), while others identified some evidence, albeit with a small effect size (Cavanagh et al., 2018; Taylor et al., 2021). These discrepancies may stem from various factors such as a medium to high risk of bias, limited sample size and inadequate statistical power of the included studies (Folk & Dunn, 2023). Furthermore, certain studies incorporated only passive control conditions potentially influencing the observed outcomes (Cavanagh et al., 2013) or lacked pre-registration, which raises concerns about the transparency and reliability of their findings (Lee et al., 2018). However, a recent pre-registered high-powered multi-site study (N_{sites} = 37; $N_{participants}$ = 39) has filled most of these literature gaps, finding evidence that brief self-administered mindfulness exercises are more effective in reducing stress than an active control condition (Sparacio et al., 2024b). However, the study's choice of control condition (i.e., participants listening to a story) poses challenges in fully distinguishing the genuine impact of the intervention from potential demand characteristic effects (Nichols et al., 2008).

Our objective is to initiate a preliminary investigation that will lay a solid foundation for a subsequent randomized controlled multi-site study. This upcoming research aims to determine whether the stress-reducing effect of mindfulness is genuine or influenced by participants' expectations when engaging in meditation. To achieve this, we will integrate self-reported data with objective assessments of the autonomic nervous system, specifically focusing on heart rate variability (HRV) as a measure of the stress response. Additionally, we will introduce a sham meditation control condition to further unravel the distinctions between genuine and demand effects.

This pilot study is necessary in laying the groundwork for a comprehensive investigation into mindfulness, aiming to uncover a cost-effective strategy for stress management that could benefit individuals in Singapore, especially those who might find conventional treatments financially prohibitive. Moreover, the success of this methodology has the potential to transcend local confines, offering an impactful solution to stress management that could resonate with a global audience.

2. AIMS AND HYPOTHESES

This pilot study seeks to assess the impact of a three-day mindfulness intervention on the stress response of Singaporean adults aged 21 or more. Specifically, we will anticipate a modest yet discernible reduction in stress levels compared to a control condition, represented by a sham meditation group.

In addition to evaluating the effectiveness of the decentralized mindfulness intervention and its comparison with the sham meditation control group, this study will also incorporate an assessment of the credibility and usability of the survey-based methodology employed.

Participants' feedback regarding the clarity of survey instructions, ease of navigation, and overall user experience will be solicited through post-intervention questionnaires. Insights gleaned from participants' perspectives will inform refinements to the methodology, ultimately optimizing its utility for a fully decentralized study on mindfulness on a larger scale.

3. EXPECTED RISKS AND BENEFITS

The study's primary advantage lies in providing participants from the intervention group with access to a 3-day mindfulness intervention, potentially contributing to stress reduction. Although the study poses no significant risks for participants, a meta-analysis reviewed 36 randomized controlled trials (RCTs) from 7931 search records, including 25 that used Mindfulness-Based Stress Reduction (MBSR) and 11 that used Mindfulness-Based Cognitive Therapy (MBCT), and found that both the mindfulness intervention group and the control group reported 19 adverse events each, accounting for 1% and 0.9% of participants, respectively. In the MBSR group, which had 1231 participants, only three studies (12.0%) reported six adverse events (0.49%) related to the intervention, with no significant adverse events recorded (Wong et al., 2018). In conclusion very few adverse events were reported in RCTs involving MBSR/MBCT, making mindfulness-based interventions generally considered to be safe.

<u>Data privacy</u>

The collection of health-related information via smartphone applications may pose a minor privacy risk in the event of device compromise or data transmission breaches. To mitigate these risks, all participant data will be encrypted to ensure the secure flow and storage of information.

Only essential contact information, including phone numbers and email addresses, will be collected. Phone numbers will primarily be used to reimburse participants and to prevent duplicate survey submissions. At a designated point in the study, participants will be required to send a CSV file to the study coordinator. Upon receipt, the CSV file will be downloaded, and the associated email will be promptly deleted. This information will not be retained. Additionally, all phone numbers will be permanently deleted once data collection is complete.

4. STUDY POPULATION

4.1. Number and nature of participants to be enrolled.

Being this a pilot study, the target recruitment is set at 60 participants, aged 21 or older.

4.2. Criteria for Recruitment and Recruitment Process.

Participants will be recruited from the general Singaporean community. To streamline the recruitment process, we will utilize the broad reach of social media platforms, including Facebook, Instagram, and X (formerly Twitter), where we will post a detailed description of the study.

Interested individuals will be directed to complete an online form (e.g. FormSG, Microsoft Forms etc), where they will answer questions related to the inclusion and exclusion criteria and provide a phone number for potential follow-up by the study coordinator. Those who meet the inclusion criteria will be contacted via phone (e.g. WhatsApp) by the study coordinator, who will guide them through the subsequent steps outlined in the procedure section.

The study coordinator will review the form responses daily and will close the link once 60 participants have been confirmed as meeting the inclusion criteria.

The trial involves a brief, fully anonymized online survey conducted over three consecutive days, with Day 1 and Day 3, requiring a commitment of approximately 35 minutes and Day 2 a commitment of 25 minutes. Given the study's minimal risk, non-invasive nature, and the absence of medication administration, we will seek an exemption from the requirement for traditional written consent.

To further enhance recruitment, we will collaborate with A*STAR's* administrative team to notify potential participants in their database who have consented to receive research-related communications. Information about the study will also be shared through ASTAR's internal channels, in full compliance with digital platform regulations.

4.3. Inclusion criteria

Participants meeting all the following criteria will be included in the study:

- Aged at least 21.
- Possess a smartphone that can run the study applications with an internet access.
- Fluent in the English language to understand the audio tracks, the study instructions and study assessments.
- Sufficient vision and hearing to complete study procedures
- Willing to commit to the study procedures

All inclusion criteria will be assessed based on self-reported information during screening.

4.4. Exclusion Criteria

Participants meeting one or more of the following criteria below will not be included in the study:

- Past (< 6 months prior to the study) or current major neurological and psychiatric disease.
- Having meditated in the 6 months preceding the experiment.

- Participants who are currently taking psychoactive medications, including antidepressants, anxiolytics, hypnotics, and stimulants, or who have used these medications in the past week.
- Members of the research team or their immediate family members. An immediate family member is defined as spouse, parent, child, or sibling, whether biological or legally adopted.

All exclusion criteria will be assessed based on self-reported information during screening.

4.5. Participant discontinuation criteria

The participant has the right to withdraw from the trial at any time without facing any negative consequences.

5. STUDY DESIGN, PROCEDURES AND METHODOLOGY

5.1 Study design

The research will adhere to a randomized, double-blind, 2-arm parallel-groups design. Participants will engage remotely from their home. The entire study duration is set at 3 days.

5.2 Intervention

Participants will be randomly allocated into one of two arms. Participants of the experimental group will listen for three consecutive days to a 10-minute mindfulness track once a day (experimental condition). Participants of the control group will listen for three consecutive days to a ‘sham meditation’ track once a day (control condition). The ‘sham meditation condition’ is an audio track that mimics the structure or elements of a meditation session but lack the authentic content or efficacy associated with genuine meditation practices.

5.2.1. Features of the mindfulness intervention (experimental condition)

The mindfulness intervention is comprised of three tracks of mindfulness practice adapted from a traditional Mindfulness-Based Stress Reduction (MBSR) protocol. The three mindfulness tracks incorporate essential elements of the mindfulness practice: a) Cultivating attentional stability by directing focus to present-moment bodily experiences (Moore et al., 2012), and b) fostering mindful meta-awareness by nonreactivity and nonjudgmentally observing and accepting experiences (Dahl et al., 2015). These components are closely associated with successful mindfulness training and effective stress regulation according to a systematic review of Stein et al., 2020 that investigated the efficacy of different mindfulness components. See Appendix A to find the script of tracks that will be used in the current experiment.
To enhance the protocol's validity, we conducted a focus group comprising 5 certified international mindfulness instructors. Their insights informed decisions on the duration, frequency, and content of the mindfulness tracks. Notably, one instructor that participated in the focus group recorded the audio tracks that will be used in the current study.

5.2.2. Features of the sham meditation intervention (control condition)

We adopted a sham meditation practice aimed at being indistinguishable from meditation practice for newcomers (Zeidan et al., 2021). Ensuring the sham's structural equivalence was a pivotal consideration, with meticulous attention to matching the quantity and timing of instructions found in the mindfulness intervention. We underscored non-specific elements of meditation, like a soothing instructor's voice and terminology designed to create the expectation of authentic mindfulness meditation for those unfamiliar with the practice.
However, the instructions deliberately excluded the training of the two fundamental cognitive/meta-cognitive processes crucial in mindfulness practice. This intentional omission involved 1) removing any attentional stability by providing participants with no specific point to anchor their attention and 2) offering no guidance on cultivating mindful meta-cognitive qualities of attention. Participants in the mindfulness group were taught to observe and accept their present-moment experience without judgment or reaction. In contrast, sham instructions offered no such guidance.

5.3 Study measurements

All questionnaires used in this study will be administered via a Qualtrics survey.

5.3.1 One-time measurement (start of the trial)

The following psychological and self-reported assessments will be taken at baseline. Participants will be starting the survey at home in a quiet place.

Neuroticism subscale of the IPIP (IPIP-20; Goldberg et al., 2006) - 5 NEO domains. The scale consists of 20 items to assess the self-reported levels of neuroticism. Examples of items are “I often feel blue” or “I am filled with doubts about things” (answered on a five-point scale ranging from 1 = Very inaccurate to 5 = Very accurate).

Resilience Scale 14 (RS-14; Miroševič et al., 2019)

The RS-14 is a brief version of the Resilience Scale, comprising 14 items. Each item is rated on a 7-point Likert scale, ranging from 1 to 7. Example of items are “I usually manage one way or another” or “I am determined”. Higher resilience scores are associated with improved mental health, enhanced quality of life, and reduced levels of anxiety and depression.

Demographics Participants will provide demographic information, including age, gender, ethnicity, type of smartphone used for the experiment, as well as its operating system (iOS or Android) and version.

5.3.2 Pre and post measurements

The Five Facet Mindfulness Questionnaire – Short Form (FFMQ-SF; Bohlmeijer et al. 2011). The scale is recommended for comparing pre and post interventions that employ mindfulness training. The scale measures 5 different components; *Observing*; the ability to notice and pay attention to the thoughts, feelings, and surroundings. *Describing*; the attitude to express the experiences in words, to describe thoughts and emotions. *Acting with Awareness*; How well they can act with intention and full awareness in their daily activities, rather than on autopilot. *Non-judging of Inner Experience*; tendency to avoid judging their thoughts and emotions as good or bad. *Non-reactivity to Inner Experience*; tendency to experience thoughts and emotions without reacting impulsively. However, recent hierarchical confirmatory factor analysis indicates a preference for a four-factor FFMQ (excluding the 'observe' subscale) in non-meditative samples, as opposed to the traditional five-factor score (Gu et al., 2016). Consequently, the analysis will focus on four facets: Describing,

acting with awareness, non-judging, and non-reactivity. Examples of items are “I can describe my feelings well” or “I can easily talk about my thoughts and opinions” (answered on a five-point scale ranging from 1 = never or very rarely to 5 = Very often or always true). We will take this assessment at baseline and at the end of the experiment.

Repeated measurements (day 1, day 2, day 3)

State Trait Anxiety Inventory (STAI-6; Marteau TM, Bekker) The STAI-6 is a validated short-form version of the 40-item State-Trait Anxiety Inventory (STAI), consisting of 6 items that measure current anxiety symptoms using a 4-point Likert scale. Example items include statements such as "I feel calm" and "I am tense." The STAI-6 will be administered twice daily: once before and once after listening to each audio track.

Ecological Momentary Assessment – (EMA) Participants will indicate their current state on a 7-point Likert scale, with the options "Very Bad," "Bad," "Fairly Bad," "Same as Before," "Fairly Good," "Good," and "Very Good," encoded as [1, 2, 3, 4, 5, 6, 7], respectively. The following questions will be posed to assess various psychological and physical states:

- "Right now, I feel mentally sharp" – to assess perceived cognitive ability.
- "Right now, I feel fatigued" – to assess fatigue and exhaustion.
- "Right now, I feel stressed" – to assess stress levels.
- "Right now, I feel nervous" – to assess nervousness and tension.
- "Right now, I feel depressed" – to assess depressive symptoms.
- "Right now, I am in a good mood" – to assess positive affect and joy.
- "I slept well last night" – to assess subjective sleep quality.

Ecological Momentary Assessment (EMA) will be conducted twice daily, immediately following each administration of the STAI-6 questionnaires.

Heart Rate Variability. To assess parasympathetic activation, participants' heart rate variability (HRV) will be analyzed. HRV is widely recognized as a sensitive marker of stress, regulated by the autonomic nervous system in response to stressors (Goldberger et al., 2001). Participants will monitor their HRV using the 'Camera Heart Rate Variability' smartphone app, which employs Photoplethysmogram (PPG) technology. Notably, smartphone apps utilizing PPG for heart rate measurement have shown consistency with validated methods such as electrocardiography (ECG) in adult populations during resting states (Guede-Fernández et al., 2020).

Participants will assess their HRV values seven times over the course of the trial (from Day 1 to Day 3). Participants will be instructed to place their index finger over the smartphone's camera and flash, ensuring both are fully covered. They will hold their finger in place for one minute to allow the device to record their heart rate variability (HRV). Upon completion, the HRV measurement will be saved in the app's local folder. If the finger placement is inadequate, resulting in poor assessment quality, the app will indicate that the CSV file has not been created. In such cases, participants will be asked to repeat the assessment up to two more times. If they are still unable to obtain a valid measurement after the third attempt, they will be instructed to proceed to the next section of the survey.

Participants will then send the corresponding CSV file via email to the study coordinator using a feature included in the app, following the instructions provided at the beginning of the experiment. To ensure that HRV data is not lost, participants will also self-report the corresponding values in the Qualtrics survey.
The first HRV measurement will serve as testing purposes and will be taken immediately after the survey that will prompt participants to go to a quiet place just after the start of the experiment. The additional HRV measurements will be taken twice daily, immediately following the completion of each Ecological Momentary Assessment (EMA).

One-time measurement (end of the trial)
Usability of the intervention to assess the usability of the current pilot trial, a set of questions will be asked to assess the participants' experiences with the mindfulness and sham meditation tracks in terms of ease of use, clarity of instructions, relevance to daily life, engagement levels, comfort, perceived efficacy, willingness to continue usage, any technical issues encountered, and suggestions for improvement.
Perceived Awareness of the Research Hypothesis (PARH; Rubin 2016) is a four-item quantitative self-report tool designed to assess the possible impact of demand characteristics in research settings. Its purpose is to assist in challenging the notion that observed effects can be attributed to demand characteristics. Participants provide their responses to these statements using a 7-point Likert-type scale, where the options range from strongly disagree (1) to strongly agree (7). Examples of statements include: “I was aware of the researchers' objectives in this study” and “I was uncertain about the researchers' intentions in conducting this research”.
Expectancy and credibility of the intervention
To assess expectancy, we will use a single item adapted from the Credibility/Expectancy Questionnaire (Devilly & Borkovec, 2000) during the final phase of post-intervention testing: “On a scale from 0 to 10, How effective do you believe the meditation training has been in alleviating stress? (0 = Not at all successful, 10 = Extremely successful).”
To evaluate credibility, participants will respond to a two-part question: “If you were informed that you might have received either meditation training or control training, which type do you believe you received? (Meditation, Control)”. “How confident are you in your answer? (0 = Not at all confident, 10 = Extremely confident)”.

5.4 Study procedures

Participants expressing interest will first complete an initial online screening form (e.g., via FormSG or Microsoft Forms), containing non-sensitive questions to assess eligibility based on study criteria such as English proficiency, age, meditation experience, vision and hearing capabilities, willingness to commit to the study procedures, and any relation to A*STAR research team members. Participants will also provide their phone number, which will be used to notify them of their eligibility status. The phone number will be retained until the end of data collection to prevent repeat participation, facilitate the delivery of study materials, and ensure compensation via e-vouchers. The study coordinator will check whether a mobile number has been previously used and stop participants if a duplicate number is detected.
Once the study coordinator reviews the responses daily, eligible participants (I.e., those meeting inclusion and exclusion criteria and those who provided a phone number not previously entered in the survey), will receive a standardized text

message (e.g., via WhatsApp) confirming their preliminary eligibility. For ineligible participants, their collected data will be discarded. If eligible, participants will be informed that the study will begin within three weeks. Upon the recruitment of the 20th participant or at the end of the third week, the coordinator will notify the cohort, instructing them to start the study within two days. The study will consist of three cohorts of 20 participants each. If fewer than 60 participants are recruited after the third cohort, an additional cohort will be organized to reach the target number.

At this point, participants will receive a message containing the following: a link to access the Qualtrics survey, an access code (which serves as an anonymous identifier), and a link to instructions for downloading the 'Camera Heart Rate Variability' app, used for heart rate variability (HRV) measurement. The instructions will detail how to take measurements and submit the generated HRV.csv data files to the study team. Participants will also receive a code to download the app for free. They will be advised to complete the survey each day at the same time, between 9 am and 9 pm.

The Qualtrics link contains the written informed consent form and the actual experiment. Participants will be able to review all relevant information about the study and either accept or decline to participate. If they do not provide consent, they will be opted out of the study. If consent is provided, participants will proceed to the second screening, which involves answering additional questions about their use of concomitant medication. If they meet the criteria for this stage, they will proceed to answer questions about any neurological or psychiatric conditions. If any of the screening criteria are not satisfied at any point, the study will automatically terminate. Once consent and second screening are complete, participants will then be instructed to move to a quiet environment where they will not be disturbed for the next 35 minutes. They will be asked to download the ‘Camera Heart Rate Variability’ app and take an initial HRV measurement for testing purposes. Participants will then submit the generated CSV file to the study coordinator via Whatsapp and self-report their rMSSD values in the Qualtrics survey. Participants are instructed to label their CSV files using their anonymous identifier provided by the study coordinator via Whatsapp at the start. Afterward, they will complete a series of questionnaires, including the 20-item IPIP scale, the RS-14, the FFMQ, and various demographic questions such as age, gender, ethnicity, smartphone type used during the experiment, and its operating system (iOS or Android) and version. Pre-intervention assessments of STAI-6, EMA will also be conducted. They will then use the ‘Camera Heart Rate Variability’ app to measure HRV, submit the generated CSV file to the study coordinator, and self-report their rMSSD values in the Qualtrics survey, as outlined in the instructions.

Participants will then be randomly assigned by the Qualtrics engine to either the experimental group, which listens to a mindfulness audio track, or the control group, which listens to a ‘sham meditation’ track. Both tracks are embedded within the Qualtrics survey. Participants cannot skip this portion of the survey for 10 minutes, ensuring they listen to the entire 10-minute audio track. Afterward, participants will be asked to sit quietly for three minutes without engaging in any activities. This pause is necessary because, some of the audio track encompasses the application of mindfulness principles while performing a routine activity (e.g., brushing teeth) during the listening of a mindfulness track. Although the movement involved in this activity is minimal, we want participants to sit still for three minutes to avoid introducing any confounding variables in the subsequent HRV assessments. The survey will be programmed to remain stuck during this period to prevent participants from skipping this step.

Following the quiet period, participants will complete post-intervention assessments for STAI-6, EMA and HRV. Participants will be thanked and instructed to reopen the survey link the following day, ideally at the same time between 9am and 9pm. On Day 2, they will complete the same pre-intervention assessments (STAI-6, EMA and HRV), listen to a different audio track, either mindfulness or sham meditation, depending on their group allocation, sit for three minutes, and then complete the post-intervention assessments mirroring Day 1.On Day 3, participants will undergo the same procedure as Day 2 but will listen to another audio track (mindfulness or sham). Additionally, they will complete the FFMQ, answer questions about their expectations of the intervention, its credibility, the PARH scale, and the usability questionnaire. Finally, participants will be thanked for their participation in the study and debriefed.

6. Ethics information

This study will be conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and in compliance with applicable regulatory requirements. The final study protocol, along with the latest version of the Participant Information and Consent Form, will receive written approval from the A*STAR Institutional Review Board (A*STAR IRB) before any participants are enrolled.

Participants who express interest in the study will first complete an initial screening, where non-sensitive questions will be asked to assess their English proficiency, age, meditation experience, vision and hearing capabilities, willingness to commit to study procedures, and any relation to A*STAR research team members. If participants do not meet the inclusion/exclusion criteria at this stage, their responses will be promptly disposed of to ensure anonymity. If they qualify, they will be sent a link to the Qualtrics platform, which will contain the written informed consent form and the actual experiment.

Given that the study poses minimal risk to participants, does not involve the use of medication, and is not expected to result in any incidental findings, we will implement a written informed consent process. Participants will be able to review all relevant information about the study and provide their consent through the online survey used for the experiment. A study coordinator and witness will not be present during the consent process. If participants choose not to consent, they will be opted out of the study. Those who do provide consent will be asked additional questions regarding their use of concomitant medication. If the participant satisfies this inclusion criteria he will pass at the next window of the survey where he will answer a question related to neurological or psychiatric conditions. If any screening criteria are not satisfied, the study will automatically terminate at that point.

As part of standard ethical procedures, participants will provide their consent by completing a written form administered through the online survey platform. We will collect participants' phone numbers to facilitate compensation and to prevent multiple survey submissions. All phone numbers will be deleted at the end of the trial. Although participants will email the study coordinator with the generated CSV file, their email addresses will not be collected, and once the CSV file is downloaded, the email will be permanently deleted. All other data collected will be de-identified to protect participants' anonymity.

6.2 Data protection

Data will be processed in accordance with the Data Protection Act 2018 and the General Data Protection Regulation 2016 (GDPR). All information collected will be kept strictly confidential. The data we will collect for this study will be made anonymous from the start. All electronic data will be stored on password-protected computers belonging to the research team. The researchers will abide by local data protection laws when collecting personal data. All research data will be captured as de-identified (coded) electronic data. A designated folder will be created for the study team to store the research data files on an SOD (Storage on demand) space. The A*STAR SOD is created on secured A*STAR servers and is designed with strict access controls. Once the data is no longer necessary (maximum of 10 years), it will be appropriately discarded (e.g., deleted off from A*STAR's SOD).

7. SAFETY MEASUREMENTS

7.1 Definitions

Serious adverse event (SAE) in relation to human biomedical research means any untoward medical occurrence because of any human biomedical research which:

- Results in or contributes to death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in or contributes to persistent or significant disability/incapacity
- Results in or contributes to a congenital anomaly /birth defect
- Results in the transmission of a communicable disease
- Results in any misidentification or mix-up of any type of tissue, gametes or embryo
- Any other events that may be prescribed

7.2 Safety Monitoring Plan

The Principal Investigator (PI) will collect feedback from participants at the end of the trial. Given that this study involves minimal risk to participants and does not include the use of medications, we do not plan to systematically record adverse events. Therefore, we do not anticipate significant risks of adverse events.

7.4. Complaint Handling

Any feedback from participants of each site will be taken seriously and concerns will be brought up to the PI to follow up.

8. SAMPLE SIZE AND STATISTICAL METHODS

8.1. Determination of Sample Size.

Since this is a pilot trial, we aim to recruit 60 participants to account for a ~20% dropout rate, ensuring we meet our target of at least 50 participants.

8.2. Statistical analyses.

Our confirmatory statistical analyses will include:

- Bayesian mixed-effects models to investigate the longitudinal changes in self-reported stress measured via STAI-6. The model will have STAI-6 as the

dependent variable, with 'Group' (mindfulness intervention or control), 'Day' (1 to 3), and their interaction term as independent variables. Participant ID will be used as a random intercept to control for individual differences. A BF10 greater than 1 will indicate slightly positive evidence supporting the effectiveness of the mindfulness condition over the control condition in stress reduction.

We will also conduct the following exploratory statistical analyses:

- A Bayesian mixed-effects model to assess longitudinal changes in HRV, using HRV as the dependent variable and 'Group', 'Day' (1 to 3), and their interaction as independent variables. Participant ID will again serve as a random intercept. A BF10 greater than 1 will suggest positive evidence for the mindfulness intervention's effectiveness compared to the control.

- An additional Bayesian mixed-effects model will analyze longitudinal changes in HRV, with the dependent variable being HRV (calculated by the difference in pre- and post-meditation HRV scores) and independent variables including 'Group', 'Day' (1 to 3), and their interaction. Participant ID will account for individual variability in baseline HRV levels.

- Correlational analyses to explore the relationship between changes in mindfulness scores and stress levels, as well as between HRV changes and stress levels.

- Descriptive statistics to summarize baseline participant characteristics, such as neuroticism, RS-14 scores, FFMQ scores related to mindfulness training, and demographic details.

- Analysis of responses regarding the intervention's credibility and participant awareness of the research hypothesis to assess equal credibility of the intervention for the mindfulness condition and the sham.

9. PUBLICATIONS

This pilot study will adhere to A*STAR publication policy and will be registered in a public registry (www.clinicaltrials.gov) and the Open Science Framework (www.osf.io).

10. RETENTION OF STUDY DOCUMENTS

Documents pertaining to the study, such as protocols, regulatory papers, participant logs, safety reports, financial and contractual records, data collection sheets, and case report forms, will be organized into distinct folders known as Investigator Site Files. These files will be securely stored in designated locked cabinets and/or rooms, ensuring access is restricted to authorized study personnel exclusively. Authorized authorities will have the privilege to inspect and copy these records. Upon completion of the study or when the data is no longer required (up to a maximum of 10 years), appropriate disposal procedures will be implemented, such as deleting the data from A*STAR's secure storage system (SOD).

11. References

Aldwin, C. M. (2007). Stress, Coping and Development; An Intergrative Perspective. *Library of Congress Cataloging-in-Publication Data*, 449.

Bohlmeijer, E., ten Klooster, P. M., Fledderus, M., Veehof, M., & Baer, R. (2011). Psychometric properties of the five facet mindfulness questionnaire in depressed adults and development of a short form. *Assessment*, *18*(3), 308–320.

Cavanagh, K., Strauss, C., Cicconi, F., Griffiths, N., Wyper, A., & Jones, F. (2013). A randomised controlled trial of a brief online mindfulness-based intervention. *Behaviour Research and Therapy*, *51*(9), 573–578. https://doi.org/10.1016/J.BRAT.2013.06.003

Cavanagh, K., Churchard, A., O'Hanlon, P., Mundy, T., Votolato, P., Jones, F., Gu, J., & Strauss, C. (2018). A Randomised Controlled Trial of a Brief Online Mindfulness-Based Intervention in a Non-clinical Population: Replication and Extension. *Mindfulness*, *9*(4), 1191. https://doi.org/10.1007/S12671-017-0856-1

Chodavadia, P., Teo, I., Poremski, D., Fung, D. S. S., & Finkelstein, E. A. (2023). Prevalence and economic burden of depression and anxiety symptoms among Singaporean adults: results from a 2022 web panel. *BMC Psychiatry*, *23*(1). https://doi.org/10.1186/s12888-023-04581-7

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, *24*(4), 385–396. https://doi.org/10.2307/2136404

Council of Europe (2001). Common European framework of reference for languages: Learning, teaching, assessment. Retrieved from https://www.coe.int/en/web/common-european-framework-reference-languages

Dahl, C. J., Lutz, A., & Davidson, R. J. (2015). Reconstructing and deconstructing the self: Cognitive mechanisms in meditation practice. *Trends in Cognitive Sciences*, *19*(9), 515. https://doi.org/10.1016/J.TICS.2015.07.001

Devilly, G. J., & Borkovec, T. D. (2000). Psychometric properties of the credibility/expectancy questionnaire. *Journal of behavior therapy and experimental psychiatry*, *31*(2), 73–86. https://doi.org/10.1016/s0005-7916(00)00012-4

Farias, M., Maraldi, E., Wallenkampf, K. C., & Lucchetti, G. (2020). Adverse events in meditation practices and meditation-based therapies: a systematic review. *Acta Psychiatrica Scandinavica*, *142*(5), 374–393. https://doi.org/10.1111/ACPS.13225

Folk, D., & Dunn, E. (2023). A systematic review of the strength of evidence for the most commonly recommended happiness strategies in mainstream media. *Nature Human Behaviour 2023 7:10*, *7*(10), 1697–1707. https://doi.org/10.1038/s41562-023-01651-4

Goldberger JJ, Challapalli S, Tung R, Parker MA, Kadish AH. Relationship of heart rate variability to parasympathetic effect. *Circulation.* 2001;103:1977–1983.

Goldberg, L. R., Johnson, J. A., Eber, H. W., Hogan, R., Ashton, M. C., Cloninger, C. R., & Gough, H. C. (2006). The International Personality Item Pool and the future of public-domain personality measures. *Journal of Research in Personality*, 40, 84-96.

Gu, J., Strauss, C., Crane, C., Barnhofer, T., Karl, A., Cavanagh, K., & Kuyken, W. (2016). Examining the factor structure of the 39-item and 15-item versions of the Five Facet Mindfulness Questionnaire before and after mindfulness-based cognitive therapy for people with recurrent depression. *Psychological assessment*, *28*(7), 791–802.

Guede-Fernández, F., Ferrer-Mileo, V., Mateu-Mateus, M., Ramos-Castro, J., García-González, M. Á., & Fernández-Chimeno, M. (2020). A photoplethysmography smartphone-based method for heart rate variability assessment: device model and breathing influences. *Biomedical Signal Processing and Control*, 57, 101717.

Kabat-Zinn, J. (2003). Mindfulness-based interventions in context: Past, present, and future. *Clinical Psychology: Science and Practice*, *10*(2), 144–156. https://doi.org/10.1093/CLIPSY.BPG016

Kabat-Zinn, J., Massion, A. O., Kristeller, J., Peterson, L. G., Fletcher, K. E., Pbert, L., Lenderking, W. R., & Santorelli, S. F. (2006). Effectiveness of a meditation-based stress reduction program in the treatment of anxiety disorders. *Https://Doi.Org/10.1176/Ajp.149.7.936*, *149*(7), 936–943.

Lazarus, R., & Folkman, S. (1984). *Stress, Appraisal, and Coping. New York Springer. - References - Scientific Research Publishing*.

Lee, R. A., & Jung, M. E. (2018). Evaluation of an mHealth App (DeStressify) on University Students' Mental Health: Pilot Trial. *JMIR Mental Health*, *5*(1), e2. https://doi.org/10.2196/mental.8324

Lindsay, E. K., Chin, B., Greco, C. M., Young, S., Brown, K. W., Wright, A. G. C., Smyth, J. M., Burkett, D., & Creswell, J. D. (2018). How mindfulness training promotes positive emotions: Dismantling acceptance skills training in two randomized controlled trials. *Journal of Personality and Social Psychology*, *115*(6), 944. https://doi.org/10.1037/PSPA0000134

Marteau TM, Bekker H. (1992).The development of a six-item short-form of the state scale of the Spielberger State—Trait Anxiety Inventory (STAI). *British Journal of Clinical Psychology*. *31*(3):301-306. doi:10.1111/j.2044-8260.1992.tb00997.

Miroševič, Š., Klemenc-Ketiš, Z.& Selič, P. (2019). The 14-item resilience scale as a potential screening tool for depression/anxiety and quality of life assessment: A systematic review of current Research. *Family Practice*, *36* (3), 262–268. https://doi.org/10.1093/fampra/cmy081.

Moore, A., Gruber, T., Derose, J., & Malinowski, P. (2012). Regular, brief mindfulness meditation practice improves electrophysiological markers of attentional control. *Frontiers in Human Neuroscience*, *6,* 18.

Murray, A. L., Xiao, Z., Zhu, X., Speyer, L. G., Yang, Y., Brown, R. H., Katus, L., Eisner, M., & Ribeaud, D. (2023). Psychometric evaluation of an adapted version of the

perceived stress scale for ecological momentary assessment research. *Stress and Health*, *39*(4), 841–853. https://doi.org/10.1002/SMI.3229

Nichols, A. L., & Maner, J. K. (2008). The Good-Subject Effect: Investigating Participant Demand Characteristics. *The Journal of General Psychology*, *135*(2), 151–166. https://doi.org/10.3200/GENP.135.2.151-166

Rubin, M. (2017). *The Perceived Awareness of the Research Hypothesis Scale: Assessing the influence of demand characteristics.* https://doi.org/10.31234/OSF.IO/M2JGN

Sparacio, A., Ropovik, I., Jiga-Boy, G., Forscher, P. S., Paris, B., & IJzerman, H. (2024a). *Stress regulation via self-administered mindfulness and biofeedback interventions in adults: A pre-registered meta-analysis*. https://doi.org/10.31234/OSF.IO/ZPW28

Sparacio, A., IJzerman, H., Ropovik, I., Giorgini, F., Spiessens, C., Uchino, B. N., Landvatter, J., Tacana, T., Diller, S. J., Derrick, J. L., Segundo, J., Pierce, J. D., Ross, R. M., Francis, Z., LaBoucane, A., Ma-Kellams, C., Ford, M. B., Schmidt, K., Wong, C. C., Higgins, W. C., … Jiga-Boy, G. M. (2024b). Self-administered mindfulness interventions reduce stress in a large, randomized controlled multi-site study. *Nature Human Behaviour*, 10.1038/s41562-024-01907-7. Advance online publication.

Spijkerman, M. P. J., Pots, W. T. M., & Bohlmeijer, E. T. (2016). Effectiveness of online mindfulness-based interventions in improving mental health: A review and meta-analysis of randomised controlled trials. *Clinical Psychology Review*, *45*, 102–114. https://doi.org/10.1016/J.CPR.2016.03.009

Stein, E., & Witkiewitz, K. (2020). Dismantling Mindfulness-Based Programs: A Systematic Review to Identify Active Components of Treatment. *Mindfulness*, *11*(11), 2470–2485. https://doi.org/10.1007/s12671-020-01444-0

Taylor, H., Strauss, C., & Cavanagh, K. (2021). Can a little bit of mindfulness do you good? A systematic review and meta-analyses of unguided mindfulness-based self-help interventions. *Clinical Psychology Review*, *89*, 102078. https://doi.org/10.1016/J.CPR.2021.102078

Van der Meulen, E., van der Velden, P. G., van Aert, R. C. M., & van Veldhoven, M. J. P. M. (2020). Longitudinal associations of psychological resilience with mental health and functioning among military personnel: A meta-analysis of prospective studies. *Social Science & Medicine (1982)*, *255*, 112814.

Warttig, S.L., Forshaw, M.J., South, J., White, A.K., 2013. New, normative, English sample
data for the short form perceived stress scale (PSS-4). J. Health Psychol. 18 (12), 1617–1628.

Wong, S. Y. S., Chan, J. Y. C., Zhang, D., Lee, E. K. P., & Tsoi, K. K. F. (2018). The safety of mindfulness-based interventions: A systematic review of randomized controlled trials. *Mindfulness, 9*(5), 1344–1357

Yang, L., Zhao, Y., Wang, Y., Liu, L., Zhang, X., Li, B., & Cui, R. (2015). Send Orders for Reprints to reprints@benthamscience.ae The Effects of Psychological Stress on Depression. *Current Neuropharmacology*, *13*, 494–504.

Zeidan, F., Emerson, N. M., Farris, S. R., Ray, J. N., Jung, Y., McHaffie, J. G., & Coghill, R. C. (2015). Mindfulness Meditation-Based Pain Relief Employs Different Neural Mechanisms Than Placebo and Sham Mindfulness Meditation-Induced Analgesia. *Journal of Neuroscience*, *35*(46), 15307–15325. https://doi.org/10.1523/JNEUROSCI.2542-15.2015

11. Appendix A

Day 1

Intro (2 min)

We live our life in such a hurry, that we often allow moments to just pass us by.
Most of the time, the mind is preoccupied, and we are only paying partial attention to everything.
We tend not to be very present with our environment, with others, and especially with ourselves.

Paying attention is important for our wellbeing.
It is only with attentiveness that we know how we are choosing to interact with life itself.
It is only with attentiveness that we realise, most of the time we are unnecessarily dwelling in the past and the future.
When the mind is only concerned with the past and future, we miss out on what is going on in the present.
We miss the joyful, magical moments of every day life.
We miss our connectedness with people and this world we inhabit.
We ignore signs in the mind and the body telling us that we need change, that we need to live better.

So let's practice being present today, by tuning in to our experience from moment to moment.
Let's pay attention to everything that is going on, inside and outside of us.

Practice (8 min)

Let's begin with a sitting posture. If you are seated on a chair, make sure that your legs are uncrossed, and your feet are planted flat on the floor, for stability.
Take a few moments to settle the body into stillness.
Invite a sense of gentleness and lightness to this practice, by softening your gaze.

Take a deep breath in, and as you breathe out...
Allow your awareness to take in the visuals of your surroundings.
Notice colours, shapes, patterns and textures around you.
Get a sense of the space in front of you. The space to your right. And to your left.
The space behind you.

One moment at a time, inviting presence to the sense of sight.

(long pause)

And now allow the eyes to gently close if you like.
When you're ready, open your awareness to the presence of sounds.
Allow the ears to just receive sounds from your environment.

If there are sounds present, just know that sound is present.
If there are no sounds present, just know that no sound is present.

One moment at a time, inviting presence to the sense of hearing.

(long pause)

And now bring your attention to this body in this sitting posture.
Get a sense of the whole body just sitting and resting in this stillness.
Invite curiosity to what the body may be experiencing from moment to moment.

You might bring your attention to the immediate space around the body
How the air comes in contact with the skin - is there temperature in the air?
Notice how the body is coming in contact with the chair or what you are resting on
Maybe also notice the way the body is breathing

One moment at a time, inviting presence to the sense of touch.

See if you can be with the body as it is, without the need to change anything in your experience.

Full awareness of the body, from moment to moment.

(long pause)

From time to time, you may notice your attention getting pulled away by sounds around you.

When that happens, just know that sound is here, and invite your attention back to the body.

Perhaps your attention gets pulled away by thoughts.
And just know that thought is here, and gently guide your attention back to the body.

(long pause)

One moment at a time.

(long pause)

Now let's bring this practice to an end, by taking a deep breath in.
As you breathe out, gently opening or widening the eyes.

Have a lovely day!

(End)

Day 2

Intro (2 min)

Let's learn to be curious today.
When we invite curiosity to any moment of our experience, we begin to connect more deeply with ourselves and the world.
When we are curious, we are more likely to stay present.
With curiosity we approach an activity with interest and alertness, rather than allow the mind to operate on autopilot or habitual tendencies.

We are so used to judging everything that comes into our awareness, and jumping to conclusions about how things should or should not be.
Curiosity helps us to see things as they are.
When we bring in curiosity, we suspend any unnecessary judgement and we become more open to new perspectives and possibilities.

The practice of curiosity should begin in everyday life.
It requires us to turn towards the whole of our experience and acknowledge what is happening, even when we have experienced it many times before.
We learn to take a step back and simply watch and observe ourselves.

Today, we are going to cultivate curiosity as we brush our teeth.
You must have brushed your teeth countless times in your life, and this time you are invited to be curious about the way you engage in this routine activity.
Allow this audio to play in the background, and simply follow along the guidance as best as you can while brushing your teeth.
If you have a different way of cleaning your teeth, feel free to adapt the instructions, with the understanding that when we brush our teeth with curiosity, we should be fully present with the entire experience.

Practice (8 min)

Let's start by paying attention to how you are preparing the toothbrush and toothpaste.

(pause)

As you begin to brush your teeth, engage with the experience by inviting awareness to your five senses.

How does the toothpaste taste? You might notice sensations of mintiness and the taste of sweetness.
And how the toothpaste begins to foam inside your mouth.
Stay with this experience for a few moments, and just observe.

(pause)

Are there also thoughts arising in the mind? Is your attention being pulled away by sounds or events happening around you?
Whenever you find yourself getting distracted, simply bring your attention back to the experience of brushing your teeth.

(pause)

With curiosity, pay attention to the movements of your arm and hand as you brush the teeth.

How is the brush coming in contact with the inside of the mouth? How do the bristles feel against the teeth and the gums?
You might even bring awareness to the sounds of brushing.

(pause)

How do you know the teeth are now clean enough, and you are ready to rinse and gargle?

(pause)

And now when you're ready, tune in to the sound of the tap water running.
Invite curiosity to the temperature of the water with your hand and fingers.
Is there coolness? Is there warmth?

(pause)

As you rinse and gargle, turn your attention inwards to notice the sounds and sensations of gargling.
Notice how many times you rinse the mouth.

(pause)

And when the teeth feels clean, maintain your awareness on the way you are bringing this activity to an end.

(pause)

Have a lovely day!

(End)

Day 3

Intro (2 min)

Have you ever paid much attention to your breathing?
Usually not, at least not until we are feeling short of breath.

But what might you notice when you intentionally bring awareness to your body breathing?
Nothing much, I suppose, and this is what today's practice is all about.
We are not looking for anything special or unusual in the breath.
We simply invite curiosity to how the body is breathing, and learn to observe the breath just the way it is.

We allow each breath that comes into our awareness to remind us that we are present, we are in the here and now.

When we bring awareness to the breath, we might notice that we don't actually like the way we are breathing, and we may want to change the breathing experience or try to control our breath and breathe a certain way.
No need for any of that. Not for our practice today.

Keep in mind that there is nothing we need to do to the breath. There is no need to deepen the breath or lengthen the breath.
We are simply going to cultivate curiosity by gently turning our attention towards the natural breath.
And see if you can bring in a whole lot of curiosity to what you are observing. Watch how each breath enters the body, and then leaves the body. Trust that the body knows how to breathe on its own.

Practice (8 min)

Have a seat somewhere, adopting a wakeful sitting posture. You may choose to close your eyes for this practice, or leave them open, resting your gaze on the floor in front of you.Take a few moments to settle the body into stillness.

(pause)

And now bring your attention to your body in this sitting posture, and with curiosity notice the way the body is breathing in and breathing out.

(long pause)

You may choose to rest your attention on a smaller part of the body, where you best feel the sensations of breathing, like at the nose - noticing the air flowing in and out through the nostrils,

(pause)

Or you might pay attention to the movements of the breath at the chest, at the shoulders, or at the belly - rising, expanding as you breathe in, and releasing as you breath out.

(long pause)

Notice if there’s a tendency to want to deepen or lengthen the breath in any way, and see if it's possible to allow the breath to just be natural.

(pause)

We’re not trying to control the breath here, we’re just learning to rest with the natural flow of the breath.

(pause)

Awareness of one breath at a time.
Simply follow the movements of the breath, the physical sensations of the breath.

(long pause)

Full awareness of the in breath. Full awareness of the out breath.

One breath at a time.

(long pause)

From time to time, you might notice your mind getting distracted by sounds aroud you, or by your own thoughts. Each time your attention wanders off, simply bring it back to your breath in the body.

(pause)

Rest your attention on the body, breathing in, breathing out.

(pause)

If you notice any judgmental thoughts about how restless the mind is, simply know that these thoughts come and go, just like any other thoughts you've had.

So simply invite your attention back to the breath in the body.

(long pause)

Over and over again, bringing your attention back to the breath.

Gently paying attention to this experience, one breath at a time.

(long pause)

Now when you're ready to end this practice, take a deep breath in through the nose, and exhale slowly through the mouth. And gently opening or widening your eyes.

Have a lovely day!

(End)