# Protocol details

The procedures described in this protocol were first published on the 16/11/2022 in the ISRCTN registry including changes to the protocol implemented on the 19/12/2022. However, this document was created on the 24/02/2025 in line with journal guidelines to describe the research process in more detail.

# Research protocol

## Project summary

It is important to understand how chemistry influences human social interactions because of its many implications for science and society. A large body of literature exists on the role of the olfactory system in emotion processing as well as emotion regulation. However, more detailed research is needed on olfactory processing in this patient group to examine the possible treatment value of human chemosignals. To expand this knowledge, the aim of this study is to assess whether odors extracted from individuals experiencing happy, fearful or neutral emotions can be used to enhance the benefits of mindfulness meditation practice in subjects with social anxiety symptoms. For this aim a single-center single-blind between-subjects randomized trial is will be conducted, investigating the effect that body odors (neutral, fear and happiness) in combination with mindfulness treatment have on the reduction of anxiety symptoms, measured via the State-Trait Anxiety Inventory (STAI).

The target population are women between aged 18 and 35 years, who are normosmic and who score 30 or above in the Liebowitz Social Anxiety Scale (LSAS). Power calculation resulted in a total sample of 96 subjects needed. Subjects will be recruited at the Department of General Psychology at the University of Padua. Each participant is randomized in one of the four groups (fear odor, happiness odor, neutral odor and clean air -control group) at the beginning of the trial and will remain in the same group throughout the study. Subjects undergo two sessions of mindfulness meditation (each practice lasting approx. 24 mins) over two consecutive days, in combination with the odor they have been assigned. Additionally, they complete an online assessment one day after the last session. The current study will bring new knowledge on whether human chemosignals can influence behavior and emotions, in the context of mindfulness practice. This can open new venues for utilizing chemosignals to enhance existing treatments and promote social interaction by increasing feelings of social connectedness.

# Summary of changes to protocol

Version 2: Due to unforeseen recruitment challenges, mainly brought on by the COVID-19 pandemic, screening criteria was changed where the LSAS screening threshold was reduced from > 50 to > 30. This change was implemented on the 19/12/2022.

## General information

Protocol title: Does exposure to body odors increase the effect of mindfulness treatment in patients with social anxiety symptoms?

Registration: https://doi.org/10.1186/ISRCTN98675422

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Sponsor: Karolinska Institutet, National Centre for Suicide Research and Ill-Mental Health Prevention (NASP)

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## Rationale and background information

Chemical signals in human body odors (BOs) hold the potential to convey an array of biological and social information to our surroundings (Dal Bò et al., 2020; Zhou et al., 2014). Chemosignals of individuals, or 'senders', in a particular emotional state have been shown to produce congruent emotional responses in 'receivers', a phenomenon that has been described as an emotion contagion (Calvi et al., 2020; de Groot et al., 2012). Despite this body of evidence, little research has explored whether BOs could aid in regulating emotional states or enhancing well-being — insights that could be valuable for psychiatric treatments.

In light of this, our research group recently conducted an exploratory study (https://doi.org/10.1186/ISRCTN64408867), investigating the prospective advantage of adding fear and happiness BOs (collected from individuals in fearful or joyful emotional states) to two brief mindfulness sessions, practiced by individuals meeting criteria for Social Anxiety Disorder (SAD, n = 48) or Depression n = 30. Findings indicated that both fear and happiness BOs enhanced the anxiety-reducing effects of the mindfulness practice for individuals meeting criteria for SAD, whereas an effect of BOs could not be demonstrated for individuals with depression. Therefore, to build on these findings and expand on this knowledge, we report here a follow-up, hypothesis-driven pilot study to test whether BOs extracted from individuals experiencing happy, fearful, or neutral emotional states could be used to enhance the benefits of two mindfulness sessions in subjects meeting criteria for SAD.

## References

Calvi, E., Quassolo, U., Massaia, M., Scandurra, A., D'Aniello, B., & D'Amelio, P. (2020). The scent of emotions: A systematic review of human intra- and interspecific chemical communication of emotions. In *Brain and Behavior* (Vol. 10, Issue 5). https://doi.org/10.1002/brb3.1585

Dal Bò, E., Gentili, C., & Cecchetto, C. (2020). Human chemosignals and brain activity: A preliminary meta-analysis of the processing of human body odors. *Chemical Senses*, *45*(9). https://doi.org/10.1093/chemse/bjaa067

de Groot, J. H. B., Smeets, M. A. M., Kaldewaij, A., Duijndam, M. J. A., & Semin, G. R. (2012). Chemosignals Communicate Human Emotions. *Psychological Science*, *23*(11). https://doi.org/10.1177/0956797612445317

Geyer, E. C., Fua, K. C., Daniel, K. E., Chow, P. I., Bonelli, W., Huang, Y., ... Teachman, B. A. (2018). I Did OK, but Did I Like It? Using Ecological Momentary Assessment to Examine Perceptions of Social Interactions Associated With Severity of Social Anxiety and Depression. Behavior Therapy, 49(6), 866–880. doi: 10.1016/j.beth.2018.07.009

Zhou, W., Yang, X., Chen, K., Cai, P., He, S., & Jiang, Y. (2014). Chemosensory communication of gender through two human steroids in a sexually dimorphic manner. *Current Biology*, *24*(10). https://doi.org/10.1016/j.cub.2014.03.035

## Study goals and objectives

The aim of this study is to find out whether odors collected from people who were experiencing happy, fearful or neutral emotions can be used to increase the positive results of mindfulness meditation therapy in subjects with social anxiety symptoms, meeting criteria for SAD.

Study hypotheses:

1. The groups exposed to body odor (neutral, fear and happiness) in combination with mindfulness treatment exhibit lower mean State-Trait Anxiety Inventory (STAI) scores post-treatment compared to the control group (clean air).

2. The group exposed to happiness body odors will show stronger effects in increasing vagally-mediated heart rate variability (HRV) indices compared to neutral and fear body odors.

## Study Design

This study is a single-center, single-blind between-subjects randomized trial, conducted at The University of Padua (Italy) by the Department of General Psychology.

Ethical approval: Approved 20/07/2019, the Ethical Committee for Psychological Research at the University of Padua (Via Venezia 8, 35131, Padova, Italy; +39 (0)498276587; comitato.etico.area17@unipd.it), ref: 3113

Inclusion criteria:

1. Aged between 18 and 35 years
2. Female gender
3. A score over 30 on the Liebowitz Social Anxiety Scale in its self-report formulation (LSAS-SR)
4. A confirmation of the disorder during a face-to-face interview (SCID-5-CV)
5. Normosmic as screened with Sniffin' Stick test
6. No pregnancy
7. Non-smokers

Exclusion criteria

1. Chronic rhinitis or other conditions that may affect the ability to perceive odors
2. Breastfeeding
3. Presence of other mental disorders (including substance abuse disorders) apart from major depression, chronic depression, minor depression, or dysphoria and social anxiety disorder
4. Presence of any severe somatic or neurological conditions
5. Use of psychotropic drugs at the moment of the recruitment (including antidepressants, antipsychotics, anxiolytics and mood stabilizers)
6. Presently undergoing psychological therapy, presence of severe psychotic symptoms (i.e. hallucinations and/or delusions)
7. Presence of suicidal thoughts
8. Incapability to understand and to give an informed consent for the experiment

Trial site: Department of General Psychology, University of Padua (Italy).

## Methodology

Sample size was estimated via a priori power analysis based on effect size extrapolated from the previous exploratory study conducted within the POTION project (https://doi.org/10.1186/ISRCTN64408867). The power calculation was performed using G*Power software (version 3.1.9.7) with the function ANOVA, repeated measurements, between factors. The parameters entered were $\alpha = 0.05$, $1-\beta = 0.95$, and effect size of between subjects' analysis of the first trial day of the above-mentioned study (Cohen's $f = 0.3745$), for a study design of 4 (odor condition; clean air, neutral BO, fear BO, joy BO) x 2 (time; pre, post).

Total sample size estimation led to n= 96 subjects.

The intervention consists of two sessions of mindfulness meditation in combination with odors or clean air, conducted over two consecutive days (approximately 60 min each, including the mindfulness practice (≈ 25 minutes) and the completion of baseline and post-treatment questionnaires). Additionally, one follow-up session online the day after the end of the last session in the lab, is conducted.

Procedure: On the first day of the study, all participants will answer several questionnaires that measure how anxious, stressed, and mindfully aware they are. Next, they will be placed at random in one of the four odor groups (happy, fearful, neutral or clear air). Then, all participants will follow the mindfulness intervention while smelling the odor through an odor-delivery system designed for this study. Their skin conductance and heart rate are measured during the intervention, for 3 minutes before and for 3 minutes after the intervention. When they are done with the intervention, the participants will have to answer the questionnaires about how anxious they are.

The next day, all participants will answer the same questionnaire as the first day, assessing their level of anxiety. Then, participants will follow the mindfulness intervention while smelling the same type of odor as they did the previous day, with their skin conductance and heart rate being measured again as the previous day. When they are done, they will have to answer the questionnaires about how anxious they are one more time. Then participants will be told that they will have to give a short presentation in front of a small audience (stress induction), and they will be administered questionnaires about anxiety one last time. At the end, participants are told that they do not have to make a presentation.

The day after, participants will be invited for an online follow-up session. They will fill out the same questionnaires as they did at the beginning of the trial, as well as an additional questionnaire about how helpful they think mindfulness meditation will be for them in the future. This session will also show if fewer participants of one odor group show up for the experiment than another.

Questionnaires list and time points at which they were administered:

Primary outcome

1. Anxiety symptoms measured by the State-Trait Anxiety Inventory (STAI) at baseline, at the end of day 1, at the beginning of day 2, after mindfulness intervention at day 2, after stress induction and during day 3

Secondary outcomes

1. Awareness of social odors measured using the Social Odor Scale (SOS) at baseline during day 1
2. Heart rate variability measured using ECG at baseline, during treatment and post-treatment of day 1; at baseline, during treatment, post-treatment and post-stress induction of day 2.
3. Skin conductance measured using electrodermal activity (EDA) at baseline, during treatment and post-treatment of day 1; at baseline, during treatment, post-treatment and post-stress induction of day 2
4. Previous experience with mindfulness meditation (answering the question "Do you practice or have you ever practiced mindfulness meditation?", if yes there is a follow-up question "how often do you practice?" with possible answers "not currently", "practice less than one time a week", "less than three times a week", "practice three times a week or more", "practice every day") measured at baseline during day 1
5. State mindfulness assessed with the State Mindfulness Scale (SMS) at post-treatment during day 2
6. Manipulation check of anxiety induction (after stress induction): Social anxiety; Task-related anxiety; Task importance. Done after the stress induction during day 2
7. Perceived helpfulness of the intervention (0 to 10 rating scale from "not useful at all" to "very useful") measured after the stress induction on day 2
8. Intensity, pleasantness and familiarity of the odor (each one on a scale from 1 to 10 from "not at all" to "very much") measured after the stress induction on day 2
9. Number of dropouts in each odor group measured at the follow-up online session during day 3

Treatment intervention:

The mindfulness intervention will be done using the APP "Con tatto" (developer LifeSTech research team). Participants will be asked to install the app on their mobile phones one week before the first meeting and to practice with the different proposed mindfulness practices. During the two interventions participants will complete two practices for a total time of about 25 minutes each. During these practices, happiness body odor, fear body odor, neutral body odor or clean air will be presented to the participants.

Odor delivery system:

The odor delivery system consists of an olfactometer, which will deliver the odor in 72-second-long pulses separated by 216 seconds of clean air at constant airflow between 50 and 70 ml/min. The odors are obtained from previously collected sweat samples. The participants are exposed to the odor for the entire duration of the intervention.

Collection of sweat samples:

Sweat samples were previously obtained from 26 healthy volunteers by the Instituto Superior de Psicologia Aplicada (Lisbon, Portugal), during two sessions, separated by one week. Before each session, absorbent pads (10 cm x 10 cm) were attached to each armpit of donors, after which donors watched one of two types of state-inducing film clips: fear-inducing, happiness-inducing or neutral (nature documentaries). The donors in fear condition sat alone while watching the film clips, while those in the happiness condition sat in groups of three participants. Afterwards, they were asked to rate how angry, fearful, sad, happy, disgusted, neutral, surprised, calm and amused they felt on 7-point Likert scales, which were used to indicate low/high arousal and positive/negative emotions. Pads were then stored at -80 degrees and transported to the University of Padua.

## Ethical Approval

Approved 20/07/2019, the Ethical Committee for Psychological Research at the University of Padua (Via Venezia 8, 35131, Padova, Italy; +39 (0)498276587; comitato.etico.area17@unipd.it), ref: 3113

## Safety considerations

The questionnaires and the physiological measures are non-invasive measures. The study involves a stress induction procedure which may increase symptoms of anxiety. However, the participants will not have to undergo a presentation (stress induction), which is likely to reduce their stress response substantially by the end of the session.

If a moderate mental disorder is detected the participants will be referred to the local clinical services. In addition, if some participants in the study show severe psychological symptoms, such as severe depressive symptoms, suicidal ideation, or psychotic symptoms, colleagues with specific psychotherapeutic skills will be contacted for further examination and, if necessary, appropriate treatment or support. Finally, all participants will be reminded of the presence of clinical services at the SCUP (Servizi Clinici Universitari Psicologici) of the University of Padova.

Overall, the potential risks for the participants (diagnosis of a psychological disorder, being subjected to smells, and possible temporary discomfort due to the stress induction) are outweighed by the benefits and scientific value of the studies.

## Follow-up

The study involves a follow-up after completion of the intervention session. This will assess the level of anxiety of the participants, perceived usefulness of the mindfulness training together with general acceptability of the intervention.

## Data management

The data will be collected by clinical psychologists at the University of Padova, Italy. The ethical application needed to collect the data was submitted by the responsible researchers to the ethics committee at the University of Padova, including the informed consent, which will be used prior to data collection. This written informed consent is obtained from each subject after explaining all aspects of the study that are relevant for the participant to make an informed decision.

The data will be stored according to the GDPR and will be stored securely according to the regulations at KI. KI will receive each subject's pseudonymized demographic and clinical data from the research team handling data collection at University of Padua. These data a will stored, according to KI rules, on OneDrive, a secured data storage system protected with 2 factor-authentication. The key of the study will not be shared not be accessible to researchers at KI.

In addition, all partners involved in the project have signed the Grant Agreement to comply with the data protection legislation: General Data Protection Regulation (GDPR) and Data Protection Act (2018:2018): Lag (2018) med kompletterande bestämmelser till EU:s Dataskyddsförordning. Guidelines of data handling have been developed within the project, which all partners obliged to conform to. These guidelines include confidentiality of all personal information. All personal information is subject to the GDPR, which came into force in May 2018, irrespective of where the information is stored (computer or hard copy) or its format (audio or video recordings or emails). Karolinska Institutet complies with the principles related to processing of personal data.

Participants are informed that the information obtained from the questionnaires, as well as the information about identity and personal data (such as telephone and email) are shared exclusively by the principal investigator in Italy, who is bound by professional secrecy and bound by privacy law (D. Lgs. 196/2003, 'Personal Data Protection Code'). The protection of personal data is designated by Decree of Director General 4451 of 19 December 2017, in which the Chief Executive of the University of Padova was appointed Data protection (privacy@unipd.it).

Participants are also informed that they are free to change their mind at any time and interrupt the completion of the questionnaire, without any penalty. Participants can also at any moment request access to their data, request for modifications and for their data to be deleted.

## Statistical analysis

Data is analyzed using R statistical software (version 4.1). Differences in demographic data, baseline characteristics and odor rating are explored by means of one-way ANOVA or Kruskal-Wallis test when normality was violated. For descriptive purposes, the magnitude of change (mean difference and Cohen's d, with respective 95% CIs) of pre- to post-treatment changes within groups, as well as between-group comparisons, are assessed.

Changes in STAI scores are examined by applying linear mixed modelling. Fixed effect on odor condition, day of trial and time (pre- and post-intervention), random effect on subject ID number.

## Expected outcomes of the study

We hope that this study will be able to bring new knowledge on the mechanism of how human chemosignals influence behavior and emotions. This will open new venues for utilizing chemosignals to enhance existing treatments and promote social interaction by increasing feelings of social connectedness.

In addition, mental health disorders impact on social and emotional well-being and can lead to stigma, loss of productivity and costly resources needed for treatment and management. With our study we aim at improving access to different forms of treatments that can be done independently.

This has multiple benefits as pressure on the health care system is reduced, whilst also improving patient choice regarding available treatment options.

## Dissemination of results

Results are disseminated in the form of journal articles. Additional dissemination activities may include presentations during scientific conferences.

## Duration of the project

Over-all study start date: 01/01/2019

Over-all study end date: 20/06/2023

Recruitment start date: 17/10/2022

Recruitment end date: 30/05/2023.

Analysis of the data will take place after data collection and data cleaning is completed and is expected to end October 2023.

## Project management

**Claudio Gentili**, MD, PhD is a young Associate Professor of clinical psychology from 2015 at the University of Padua. He graduated in Medicine (2002) and Psychology (2009) and obtained his PhD in 2006. From 2010 to 2015 was Assistant Professor in Clinical Psychology at the University of Pisa. From 2012 he is boarded psychotherapist. His main research interests are Clinical Psychophysiology of mood and anxiety disorders; Neurobiology of face perception in mood and anxiety disorders; Psychophysiological remote monitoring of mood disorders; FMRI resting state correlates of psychological and personality traits; Meta-analysis of fMRI data. He recently started a research line aimed at estimate clinical diagnosis and clinical severity of mental disorders through multi feature analysis of heart rate variability. He has published more than 65 peer-review papers. He has many international collaborations including Stanford University (Department of Psychology) and the Vrjie University Amsterdam. He is also member of The Platform for Advanced Imaging – MRI/EEG – in Clinical Cognitive Sciences (the SkyRa Platform) at the University Babes-Bolyai in Cluj-Napoca, Romania.

**Vladimir Carli**, MD, PhD, Senior Lecturer at the National Centre for Suicide Research and Prevention of Mental Ill-Health (NASP), Karolinska Institutet (KI). He is the chair of the WPA Section of Suicidology. VC is Co-Director of the WHO Collaborating Centre for Research, Training and Methods Development in Suicide Prevention. He recently collaborated with the WHO in the development of the mhGAP intervention guide, by scrutinizing and reviewing the existing medical literature on suicide prevention and producing the evidence-based recommendations that are part of the guide. VC is the project leader of the project Suicide Prevention through Internet and Media-based Suicide Prevention (SUPREME), funded by the European Agency for Health and Consumers (CHAFEA). He is also Assistant Project Leader of the 7th Framework Programme EU funded project Saving and Empowering Young Lives in Europe (SEYLE) and Working in Europe to Stop Truancy Among Youth (WE-STAY).

## Ethics

Ethical approval for data collection and analysis was granted on the 20/07/2019, by the Ethical Committee for Psychological Research at the University of Padua (Via Venezia 8, 35131, Padova, Italy; +39 (0)498276587; comitato.etico.area17@unipd.it), ref: 3113.

Additional ethical approval for data analysis carried out by the Karolinska Institutet team was granted on the 16/01/2023 by the Swedish Ethical Review Authority, Etikprövningsmyndigheten (Dnr 2022-05346-01).

## Funding

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