Aalto yliopisto
Neurotieteen ja lääketieteellisen teknologian laitos
Tutkimuskoodi: AY-NBE-HT-101

Clinical trial protocol, Version 2.0 27/05/2022

A pilot study to assess the effects of mechanic activation of C-tactile afferent nerves on relapse predicting biomarkers and alcohol craving in AUD patients during early abstinence, a 4-week study

Juliana Harkki, MSc (Med), coordinating investigator
Aalto University
Department of Neuroscience and Biomedical Engineering

1 Protocol synopsis

1.1 Investigators and the study site

Principal Investigator:
Hannu Alho, MD, Emeritus professor of addiction medicine, University of Helsinki

Coordinating investigator:
Juliana Harkki, BSC (Pharm), MSc (Med), Aalto University

Investigators:
Pauli Tuovinen, MSc (Tech), Aalto University
2-3 assistant investigators (will be recruited later)

Aalto University
Department of Neuroscience and Biomedical Engineering

1.2 Trial period

1.8.2022-31.12.2025

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

1.3 Study design

A single-center, randomized, single-blind, placebo (non-CT-activating touch) controlled parallel group clinical trial to explore the feasibility, tolerability, and efficacy of mechanic CT-optimal touch to reduce alcohol craving and relapse susceptibility in adult subjects with diagnosed AUD.

1.4 Objectives

Primary objective is to test the hypothesis that CT activation may have therapeutic potential in treatment of AUD. Secondary objective is to investigate the safety and tolerability of mechanic optimal CT activation and its feasibility on modulation of certain biomarkers in AUD population.

1.5 Endpoints

The Surrogate endpoints of the study are HRV, salivary cortisol, pupillary reactivity, and ERP's.

1.6 Inclusion and exclusion

The subjects must be individuals of 18 to 60 years of age who meet the DSM-5 criteria for mild to moderate AUD and who commonly engage in heavy alcohol consumption but have abstained from alcohol for ≥2 weeks before the trial and have BAC 0.00% at visit 1. Normal or corrected-to-normal eyesight. Capability to answer the necessary questioners. Signed informed consent.

Exclusion criteria include current use of other addictive substances (excluding nicotine) or medications that may have affect the results. Diagnosis of other psychiatric diseases than AUD or other clinically significant conditions such as chronic atrial fibrillation. Score ≥26 in AQ questioner. Pregnancy.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

1.7 Treatments

The study treatment is CT-optimal slow (3 cm/s) stroking tactile stimulation produced with a mechanically moving cosmetic brush. The control treatment is mechanically produced non-CT-optimal tactile stimulation, i.e., either vibrating or fast stroking movement.

1.8 Procedure

Experiment 1. - *Stress-related biomarkers in cue-, stress- and neutral stimuli exposure*

HPA-axis and ANS reactivity is assessed before, during and after exposure to alcohol-, stress-, and neutral stimuli. The primary measurements are blood volume pulse (BVP), from which heart rate variability (HRV) will be derived from, and salivary cortisol level. Additional measures that are automatically recorded by the wristband device in use, are electrodermal activity (EDA) and skin temperature (Temp).

Experiment 2 - *Cue-reactivity task test*

In the cue-reactivity task pupillary reactivity, EVP's of EEG and self-reported craving will be measured during alcohol-related and neutral visual stimuli.

The experiments will be followed by a two-week follow-up period during which the participant will keep a drinking and craving diary.

1.9 Safety assessments

Safety will be assessed during each laboratory session by asking the subjects to report any adverse events during and after the tactile stimulation.

1.10 Statistical methods

The results will be analyzed with two-tailed T-tests and, when appropriate, with analysis of variance (ANOVA) and a Chi-square test.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

2 Background

2.1 Alcohol use disorder and craving

Alcohol use disorder (AUD) is a chronic relapsing brain disorder which typically leads to compulsive seeking of the substance despite the known negative consequences. As with all addictive drugs, chronic use of alcohol leads to long-term neuroadaptations in the brain circuits that drive reward, motivation and stress (Gilpin and Koob, 2008; Korpi *et al.*, 2015). The initial trigger that eventually leads to these adaptations is the drug-induced abnormally high level of dopamine in nucleus accumbens (NAc) which is associated with the hedonic state of drug intake (Koob and Volkow, 2016). Long-term alterations in neural functioning result in a high risk of relapse, even after periods of abstinence making AUD an extremely challenging disease to treat. In fact, up to 85% of the patients that attempt to quit drinking with current pharmacological and psychosocial treatments, eventually relapse (Heinz *et al.*, 2010).

Substance craving is one of the characteristic symptoms of an addiction and an important contributor to relapses in both alcohol and other substance use disorders (Sinha, 2013b; Koob and Volkow, 2016). Craving has been described as an intense, urgent abnormal desire that may include both mental and physical need for the drug intake (Sinha, 2013b). Alcohol craving may be triggered by alcohol-related cues, incentive saliences that precipitate the dopaminergic activity in the reward circuits, but also by stress, anxiety and arousal that involve hypothalamic pituitary adrenal (HPA) axis, amygdala and peripheral stress responses (Koob, 2013; Sinha, 2013b; Koob and Volkow, 2016). Especially in the more advanced stages of the disease, the negative reinforcement of the unpleasant mood states that involve the physiological stress systems might dominate the positive reinforcement of the pleasurable effects of alcohol as the driving force of addiction related behavior (Sinha, 2013b; Koob and Volkow, 2016).

2.2 C-tactile touch system and its relation to AUD

It is now known that we have two touch systems in the skin – a fast one and a slow one (Löken *et al.*, 2009; Olausson *et al.*, 2016). The former depends on fast-conducting myelinated nerves that relay tactile information to the brain in tens of milliseconds, performing a discriminative function. The latter depends on slowly conducting unmyelinated nerves where tactile information takes hundreds of milliseconds to arrive in

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

the brain. These nerves, called C-tactile afferents (CT) respond optimally to low velocity and force stimuli that are delivered at skin temperature. CTs have only been found in the hairy skin of the body and are hypothesized to code for the pleasant (rewarding) properties of social and affiliative touch (Pawling *et al.*, 2017a). The fast-touch system projects to the somatosensory cortex subserving its discriminative functions, whereas the slow touch system projects to the insular cortex (IC), a region of high importance in relation to affective mechanisms, considered as a gateway from sensory systems to the emotional systems of the frontal lobe. (Olausson *et al.*, 2002, 2016; Björnsdotter *et al.*, 2009; Löken *et al.*, 2009; Pawling *et al.*, 2017). (Olausson *et al.*, 2002, 2016; Björnsdotter *et al.*, 2009; Löken *et al.*, 2009; Pawling *et al.*, 2017b; Davidovic, Starck and Olausson, 2019). The IC is also known to play an important role in cue-induced drug craving and its stimulation has been suggested as a possible treatment method for AUD (Naqvi *et al.*, 2014; Haaranen *et al.*, 2020; Haaranen *et al.*, 2020). Functionally IC connects to the brain areas involved in reward and motivation and affects the dopaminergic activity in the ventral tegmental area and NAc (Girven *et al.*, 2020). Tactile stimulation of the skin has indeed been shown to increase dopamine level in NAc (Maruyama *et al.*, 2012). Affective touch has also been shown to modulate the endogenous μ-opioid system that has a key role in alcohol-induced dopamine increase and the pleasurable effects of alcohol (Gilpin and Koob, 2008; Korpi *et al.*, 2015; Nummenmaa *et al.*, 2016).

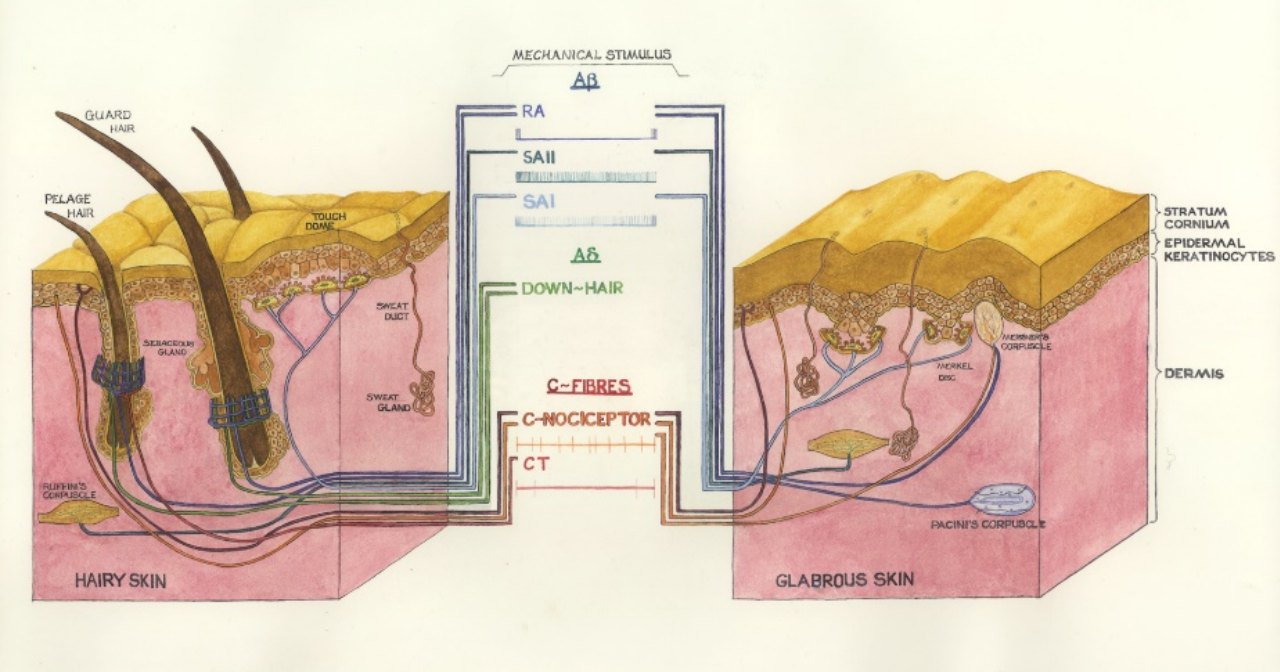


Figure 1. CT fibers are only found on the hairy skin of the body.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

In addition to the rewarding properties of dopamine and opioid peptide related mechanisms, pleasant tactile stimulation also modulates stress response systems that, as mentioned earlier, provide a major contribution to addiction, alcohol craving and relapses (Koob, 2013; Sinha, 2013b; Blaine, Seo and Sinha, 2017; Pawling *et al.*, 2017b). CT stimulating touch is known to increase oxytocin and reduce stress system activity and cortisol levels, all of which have been demonstrated to have a beneficial effect on prevention of alcohol craving (Ditzen *et al.*, 2007; Uvnäs-Moberg, Handlin and Petersson, 2014; Walker *et al.*, 2017; King and Becker, 2019; Eckstein *et al.*, 2020). Most importantly, a rat study by Walker *et al* (2020) showed that just 10 minutes of slow stroking touch per day had a striking effect on stress-resilience and it nearly abolished the anxiety-related behavior in a rodent model of chronic mild stress.

Against this scientific background, it is of interest to examine the therapeutic potential of CT-optimal tactile stimulation delivered by a neuro-designed haptic device. In this pilot study, we aim to gather preliminary data for the feasibility and safety of such stimulation in the prevention of alcohol relapses by testing its effect on relapse-predicting biomarkers in a laboratory environment. However, the final aim is to develop a haptic therapeutic device that could be used in managing alcohol and other addiction related compulsive behaviour, and to prevent relapses.

2.3 Relapse-predicting biomarkers

Psychological stress is an important contributor of alcohol craving and relapses. Early abstinence from alcohol is associated with aversive stress-related symptoms, such as anxiety, negative mood and aggressive behavior (Sinha, 2013a; Koob and Volkow, 2016). Chronic use of alcohol is known to alter both central and peripheral stress systems which manifests e.g. as dysfunction of HPA-axis and autonomic nervous system (ANS) (Sinha, 2009; Sinha *et al.*, 2009; Koob, 2013; Blaine, Seo and Sinha, 2017). Certain biomarkers of central and peripheral nervous system functioning have been shown to be valuable in predicting a susceptibility to an alcohol relapse (Junghanns *et al.*, 2005; Fox *et al.*, 2007; Garland, Ingmar and Howard, 2012; Sinha, 2013a; Petit *et al.*, 2015; Kvamme *et al.*, 2019). The following measures have been previously used for this purpose in a controlled laboratory environment and therefore have been selected to be used in this pilot study.

Salivary cortisol is considered a reliable, yet indirect, biomarker of HPA-axis activity (Hellhammer, Wüst and Kudielka, 2009; Bozovic, Racic and Ivkovic, 2013). With healthy individuals, HPA-axis activity and cortisol secretion increases during a stress reaction (Chida

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

and Steptoe, 2009). The stressor stimulates the release of corticotrophin releasing factor (CRF) in hypothalamus, leading to secretion of adrenocorticotropic hormone (ACTH) in the frontal lobe of pituitary gland, which then results in synthesis and release of cortisol in adrenal cells. However, studies have shown that with alcohol dependent individuals the HPA-axis reactivity is blunted when exposed to stressors but hyperactive in a neutral relaxing state (Sinha, 2009). Blunted cortisol responses in stress or cue exposure have been associated a susceptibility to relapse (Junghanns *et al.*, 2005; Sinha, 2013a). Affective touch is known to lower cortisol levels in humans and low threshold C-fiber mechanoreceptor targeted touch lowers corticosterone levels in stressed rats suggesting that CT-optimal touch may have a potent ability to modulate HPA-axis activity (Eckstein *et al.*, 2020; Walker *et al.*, 2020).

The autonomic nervous system (ANS) plays an important role in regulating physiological responses to emotional stress. The ANS consists of sympathetic and parasympathetic nervous systems (SNS, PNS). SNS is activated in states of arousal and stress adapting body to a “fight or flight” mode, whereas PNS contributes to the opposite “rest and digest” state.

Heart rate variability (HRV) is a standard measure for ANS functioning (Shaffer and Ginsberg, 2017; Ghiasi *et al.*, 2020; Schuurmans *et al.*, 2020). HRV describes the variability of the interval between the consecutive heartbeats. Both PNS and SNS contribute to cardiovascular functioning including heartbeat frequency and inter-beat interval. HRV is considered to most reliably reflect PNS functioning, increase in HRV indicating increase in PNS activity. Furthermore, HRV provides an estimation of the ratio between SNS and PNS activity. With alcohol dependent population, a greater high frequency HRV reactivity to alcohol cues has been previously shown to predict a relapse (Garland, Ingmar and Howard, 2012). A study by Triscoli *et al.* (2017) has shown that HRV is increased by CT activating stroking touch.

Electrodermal activity (EDA), also called skin conductance, is widely considered a reliable biomarker of SNS activity (Shu *et al.*, 2018; Ghiasi *et al.*, 2020; Posada-Quintero and Chon, 2020). SNS modulates sudomotor nerves which control the sweat glands and therefore affect the electrical conductance of the skin (Posada-Quintero and Chon, 2020). As sweat consists of mostly water, increased sweating increases the conductivity. EDA reactivity due to stress or cue exposure in AUD population has been reported (Kaplan *et al.*, 1985; Alinia *et al.*, 2021). However, to our knowledge, there are no studies demonstrating that changes in EDA due to cue or stress exposure would directly predict a relapse.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

Pupillary reactivity may be used as a biomarker of the Locus Coeruleus (LC) – Noradrenalin (NA) system activity (Laeng, Sirois and Gredebäck, 2012). Alongside, and in collaboration with, HPA-axis, LC–NA system plays an important role in physiological stress reactions (Laeng, Sirois and Gredebäck, 2012). In addition, this system is activated e.g. by attention, arousal and cognitive tasks. A pilot study by Kvamme *et al.* (2019) showed that greater pupillary dialation to alcohol-related images in a cue-reactivity task test predicts a susceptibility to a relapse with AUD patients.

Event related potentials (ERP's) of **electroencephalography (EEG)** have also been demonstrated to be valuable in predicting a vulnerability to relapses in laboratory environment (Petit *et al.*, 2015; Matheus-Roth *et al.*, 2016). Patients with AUD, as well as other substance use disorders, have larger P3 amplitudes elicited by substance-related than neutral stimuli which indicates that P3 amplitudes reflect the neural correlates of substance craving (Namkoong *et al.*, 2004; Littel *et al.*, 2012). In addition, the AUD patients with reduced P3 amplitudes to alcohol cues are less likely to relapse than those with larger P3 amplitudes (Petit *et al.*, 2015). P3 is a component that refers to a large positive deflection of the ERP which is typically maximal at medial central and parietal electrode sites and arises about 300–800 ms after stimulus presentation. P3 is generally associated with task-related and motivated attention which is necessary for the survival of the individual (Littel *et al.*, 2012). Larger P3 amplitudes are hence thought to reflect attentional bias to substance-related stimuli which has been associated with a poor treatment outcome in multiple studies (Littel *et al.*, 2012).

3 Objectives and endpoints

The primary aim of this pilot study is to test the hypothesis that mechanically produced CT-optimal touch will reduce stress- and cue-induced alcohol craving and affect physiological predictors of relapse with alcohol dependent individuals. To test the hypothesis in laboratory environment, the subjects will be exposed to alcohol-related, stress-inducing, and neutral stimuli while biomarkers that have been previously shown to have relapse-predicting value will be measured. These biomarkers include HRV, salivary cortisol, pupillary reactivity, and ERP's. In addition, we will assess the self-reported subjective alcohol craving. The mentioned biomarkers are the surrogate endpoints of this study.

The secondary aim of the study is to assess the safety, tolerability and feasibility of the CT-optimal mechanically produced tactile stimulation.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

4 Study design

This is a single-center, randomized, single-blind, placebo (non-CT-activating touch) controlled parallel group clinical trial to explore the safety, tolerability, and feasibility of mechanic CT-optimal tactile stimulation to reduce alcohol craving and relapse susceptibility in adult subjects with diagnosed AUD.

5 Power calculation

The power calculation was conducted using clinicalc.com sample size calculator (https://clincalc.com/stats/samplesize.aspx. Accessed March 3, 2022) with the means of relapsers' and abstainers' post-stimulation week 3 salivary cortisol levels (μg/dl) from Junghanns *et al.* (2005). The calculation can be seen in Figure 2. The following values were used in the calculation: α=0.05, power=0.80, ratio=1.0. Based on the calculation the minimum number of subjects in each group is 19. As the study consists of several measures in addition to salivary cortisol, it is possible that a larger number of subjects might be required to reach the statistical power in those tests. However, as this is the first pilot study if its kind, 20 subjects per group throughout the study has been considered a sufficient number also for financial and time-related reasons.

$$k = \frac{n_2}{n_1} = 1$$

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

$$n_1 = \frac{(1.42^2 + 1.42^2/1)(1.96 + 0.84)^2}{1.29^2}$$

$$n_1 = 19$$

$$n_2 = K * n_1 = 19$$

Figure 2. The power calculation.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

6 Test device

The test device that is used for producing the haptic stimulation is designed in a similar way as in the study by Eriksson Hagberg *et al* (2019) where the effect of brushing was studied to activate skin neural fibers and related brain activity. The primary actuation is performed with a hypoallergenic cosmetic brush (Flaer, 208 Highlighter fan brush) operated by three pneumatic artificial muscles. The three pneumatic muscles are used to ensure appropriate distance and force to the skin and movement along the skin. The pneumatic muscles are operated with compressed air that is controlled with a pneumatic control system located at a safe distance from the participant. The muscles are reinforced and supported with glass-fiber composite tubes and plastic polypropylene connectors. The stimulator is equipped with three optoswitches, and a load cell to measure the skin contact, velocity of the brush, and load on the skin. In the secondary actuation, the brush is replaced with a vibrating monofilament (frequency range 100-500 Hz). The device by Eriksson Hagberg *et al* does not have CE marking but has received ethical approval for investigational purposes at Karolinska Institutet in Sweden. The device is electromagnetically compatible with neurolobiological studies and causes no electromagnetic irradiation to the participant.

7 Trial population

40 early abstinent (≥ 2 weeks) female and male individuals of 18 to 60 years of age who meet the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) criteria for mild to moderate AUD and who commonly engage in heavy alcohol consumption. Heavy alcohol consumption is defined as ≥6 heavy drinking days (HDDs) in the 4 weeks prior to the online screening. HDD is defined as a day with alcohol consumption of 60 g or more for males and 40 g or more for females. However, the participants should precede two weeks of abstinence. The participants will be randomly assigned to active treatment (CT-optimal touch, n=20) and control treatment (non-CT-optimal touch, n=20) groups.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

8 Recruitment, screening and diagnosis

Subjects will be recruited by a newspaper and/or social media advertisement. The recruitment advertisement will guide the potential subjects to a website where they will be able to anonymously answer a screening questioner (Appendix 1). If the inclusion criteria are fulfilled, the potential subject will be directed to leave their contact information. The research personnel will contact them to provide more information about and to send them the participant information sheet.

The informed consent will be signed, **Autism Spectrum Quotient (AQ)** (Appendix 2) questioner taken and **DSM-5 diagnosis** (Appendix 3) confirmed, before the beginning of the experiment in the Visit 1. Possible withdrawal symptoms will be monitored with **Withdrawal Assessment for Alcohol (CIWA-ar)** (Appendix 4) interview and **breath alcohol concentration (BAC)** of 0.00% will be confirmed with a breathalyser. In case the subject has current withdrawal symptoms or BAC above 0.00% the experiment will not be carried out and a new visit will be scheduled.

The alcohol consumption and craving within the previous 4 weeks will be backtracked with the **Timeline Followback method (TLFB)** and the **Obsessive Compulsory Drinking Scale (OBCD)** (Appendix 5) respectively (Anton, Moak and Latham, 1996; Sobell *et al.*, 1996).

9 Criteria for inclusion and exclusion

9.1 Inclusion

The inclusion criteria are:
1. The subject has BAC of 0.00% at the Visit 1.
2. The subject has mild or moderate diagnosis of AUD as measured by
DSM-5 criteria.
3. Males and females aged 18 to 60.
4. Able to provide TLFB alcohol consumption information for the 28-day period prior to the Visit 1.
5. A subject has had ≥6 HDDs in the 4 weeks prior to the online screening.
6. The subject has had ≤14 consecutive abstinent days preceding the Visit 1.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

7. Willingness to provide a saliva sample for cortisol analysis
8. Able to read and understand, and complete the rating scales and questionnaires accurately, follow instructions, and make use of the relapse counselling.
9. The subjects must have normal or corrected-to-normal eyesight.

9.2 Exclusion

The exclusion criteria are:
1. Patients with withdrawal symptoms at the Visit 1, monitored with Ciwa-ar.
2. Subjects with diagnosis of any of the following concomitant psychiatric disorders: non-treated, unstable schizophrenia, bipolar disorder, other psychotic disorder during the lifetime of the subject.
5. Subjects with current use of any other addictive substances (excluding nicotine).
6. The subject has been treated with any investigational medicinal product within 30 days.
7. The subject is currently participating or has recently (4 weeks prior to the Visit 1) participated in a treatment program for alcohol use disorders.
8. Dermatological conditions, especially on the targeted anatomical area. E.g., sun burns, cuts, eczema and fungal or other contagious skin diseases.
9. The subject has a clinically significant untreated and unstable illness, for example, hepatic or renal insufficiency, or a cardiovascular, pulmonary, gastrointestinal, endocrine, neurological, infectious, neoplastic, ~~or~~ metabolic disturbance or chronic atrial fibrillation.
10. Current use of systemic steroids
11. Autism spectrum disorder (ASD). Score 26 or more in AQ test.
13. The subject reports treatment, either current or within 28 days prior to the online screening, with any medications having a potential effect on alcohol consumption and related behaviours or mood. These include opiate antagonists (e.g., naltrexone, Vivitrol, Selincro), glutamate antagonists (e.g., acamprosate), anticonvulsants (e.g., topiramate), serotonin reuptake inhibitors (e.g., fluoxetine), serotonin antagonists (e.g., buspirone), other antidepressants (e.g., tricyclic antidepressants or monoamine oxidase inhibitors), dopamine antagonists (e.g., haloperidol), and disulfiram (Antabuse).
14. Current abuse of benzodiazepines. Benzodiazepines are allowed if used chronically.
15. Pregnancy by information given by the patient (non-invasive study).
16. Any other condition or therapy that in the investigator's opinion may pose a risk to the subject, prevent the subject from completing the required study procedures or interfere with the study objectives.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

10 Study visits

For each participant, four visits to the study site are required (Figure 3). All together the participation will take approximately 5 to 6 hours of the participant's time. The content of the individual visits is shown in detail in Figures 4 and 5. Three visits are required for the Experiment 1 and one visit for the Experiment 2. The aim is to arrange the first three visits (Experiment 1) on consecutive days and the last visit (Experiment 2) within 7 days from the third visit. However, exceptions may be made in case this does not suit the participant's personal schedule. After the last visit the participant will be asked to fill in a daily online (or manual, if preferred) diary in which they will report the amount of alcohol consumed and to rate the craving they have experienced with a visual analog scale (VAS) for the following two weeks.

For the visits 1 to 3 (Experiment 1) the subjects will be instructed not to consume any caffeinated drinks or energy drinks or take beta blocker medication for 18 hours before arriving to the study site. The subjects will also be asked to restrain from physical exercise for 3 hours and to not to eat or drink for 1 hour before the visits. Brushing teeth, flossing, or doing anything that may cause bleeding of gums should also be avoided before the visits.

For the visit 4 the subjects will be asked to wash their hair in the morning before the experiment and to not to consume any caffeinated drinks or energy drinks for 18 hours before arriving to the study site.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

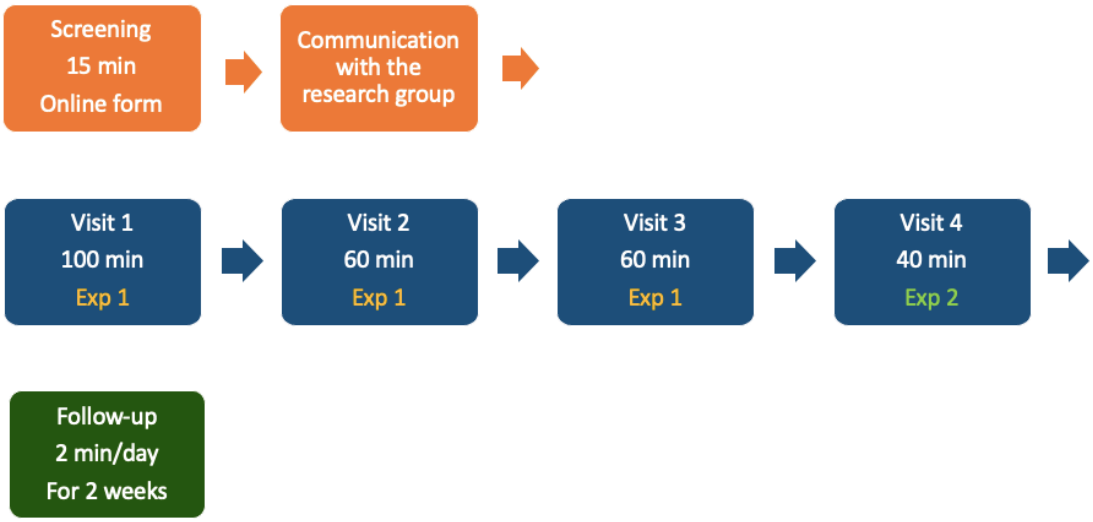


Figure 3. After the online screening, four visits to the study site are required for each participant. After the last study visit, the participants are asked to keep a daily drinking and craving diary for the following two weeks. All together the participation will take approximately 5 to 6 hours of the participant's time (plus travelling).

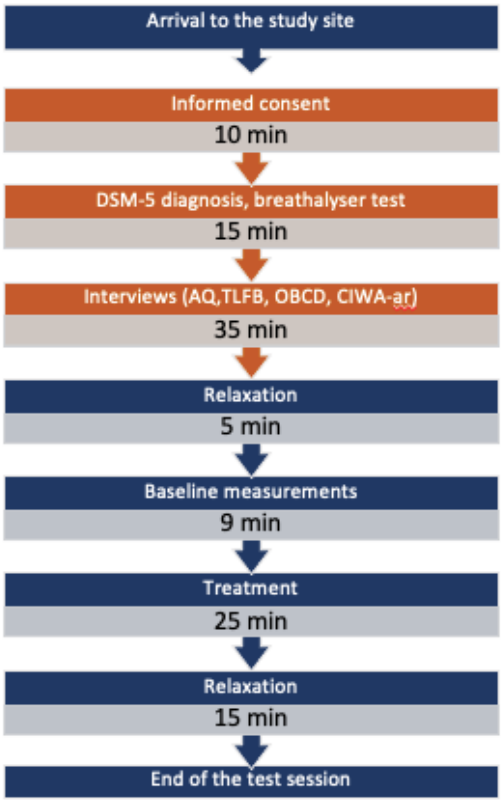


Figure 4. The timeline of the visits 1 to 3. The events on the orange background only take place at visit 1.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

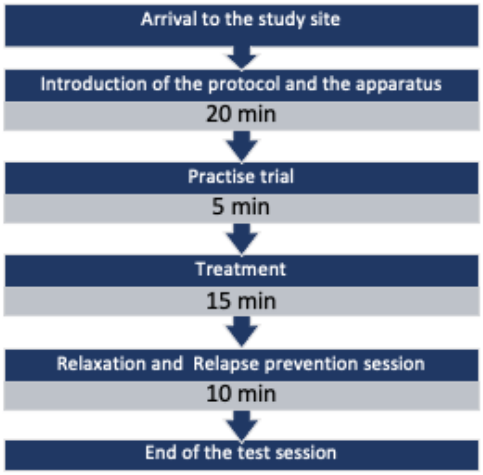


Figure 5. The timeline of the visit 4.

11 Trial treatments

The active treatment is CT-optimal slow (3 cm/s) stroking haptic stimulation on the upper back or forearm of the participant. Non-CT-optimal vibrating haptic stimulation will be used as a control treatment.

12 Trial assessments

12.1 Experiment 1. - *Stress-related biomarkers in cue-, stress- and neutral stimuli exposure*

Experiment 1 aims to assess HPA-axis and ANS reactivity before, during and after exposure to alcohol-, stress-, and neutral stimuli. The primary biomarkers that will be monitored during the experiment are blood volume pulse (BVP), from which heart rate variability (HRV) will be derived from, and salivary cortisol level. Additional measures that are automatically recorded by the wristband device in use, are electrodermal activity (EDA) and skin temperature (Temp).

HPA-axis reactivity: To assess the HPA-axis reactivity, five salivary cortisol samples will be collected at time points -20, -5, 0, +10 and +25. The saliva will be collected by placing a synthetic swab (Salivette Cortisol, Sarstedt Inc. Nümbrecht, Germany) between the tongue

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

and the cheek of the participants for ~60 s until completely saturated with saliva after which it will be placed in a plastic tube (Salivette Cortisol, Sarstedt Inc. Nümbrecht, Germany) and stored in -20 °C temperature until the analysis. The salivary samples will be analysed with a competitive enzyme-linked immunosorbent assay (Cortisol free in Saliva ELISA DES6611, Demeditec Diagnostics GmbH, Kiel, Germany).

ANS reactivity: To assess ANS reactivity BVP, EDA and Temp will be monitored with a biosensor wristband (E4, Empatica Inc., Milan, Italy). HRV will be derived from BVP and analysed with Kubios HRV Premium software (Kubios Oy, Kuopio, Finland) and Matlab computational software (The MathWorks Inc., Natick, Massachusetts, USA).

Subjective craving: The subject will self-report the current craving for alcohol by rating it on a ten-point visual analog scale (VAS) (Figure 5.). The first rating will take place in the beginning of the experiment, and the second one directly after the visual stimuli.

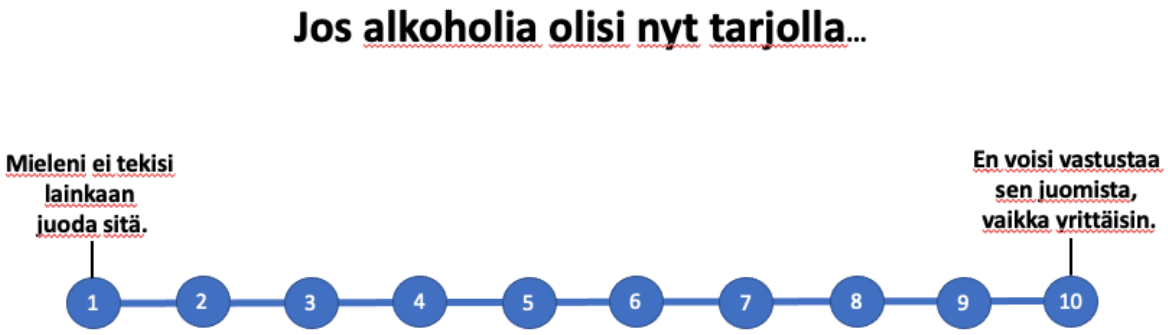


Figure 5. Visual analog scale to assess self-reported subjective craving before and after exposure to the stimuli. *If alcohol was available to me right now… 1 = I would not want to drink it at all, 10 = I would not be able to resist drinking even if I tried.*

Safety assessments: The subject is asked to report any adverse reactions in the end of the laboratory session. She/he will also be instructed to inform the research staff immediately if severe adverse reactions take place at any point of the experiment.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

12.2 Experiment 2 - *Cue-reactivity task test*

In the cue-reactivity task pupillary reactivity, EVP's of EEG and self-reported craving will be measured during alcohol-related and neutral visual stimuli.

Pupillary reactivity will be measured using the EyeLink1000 plus eye tracking system (SR Research Ltd., Ottawa, Canada)

EEG will be recorded using an BrainAmp amplifier and an ActiCap 32-channel electrode set (both by Brain Products GmbH, Gilching, Germany).

Subjective craving will be self-reported by rating it on a 5-point VAS after each alcohol-related image (Figure 6.).

Safety assessments: (see Experiment 1).

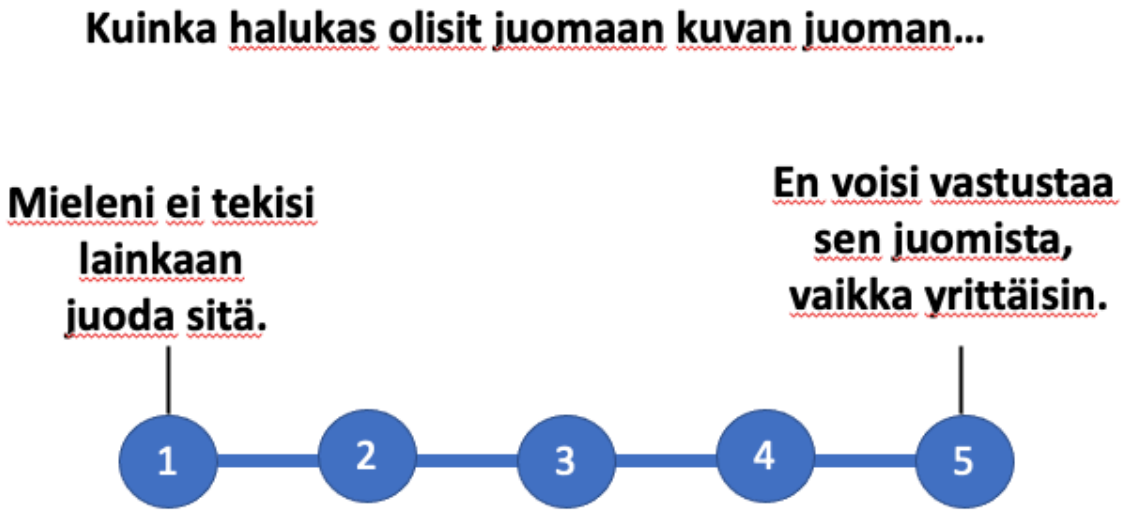


Figure 6. The visual analog scale for rating the alcohol craving after each alcohol-related image in the cue reactivity task. *How willing are you the drink the drink in the previous image… 1 = I would not want to drink it at all, 5 = I would not be able to resist drinking even if I tried.*

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

12.3 Follow-up

The experiments will be followed by a two-week period during which the participant will report **subjective craving**, **alcohol consumption** and possible **adverse events** by filling an online diary (Appendix 6). If the participant does not have access to the internet, the diary may be filled manually.

13 Study procedures

13.1 Experiment 1. - *Stress-related biomarkers in cue-, stress- and neutral stimuli exposure*

The experiment consists of three laboratory sessions. The experimental design and the timeline of the sessions is illustrated in the Figure 7.

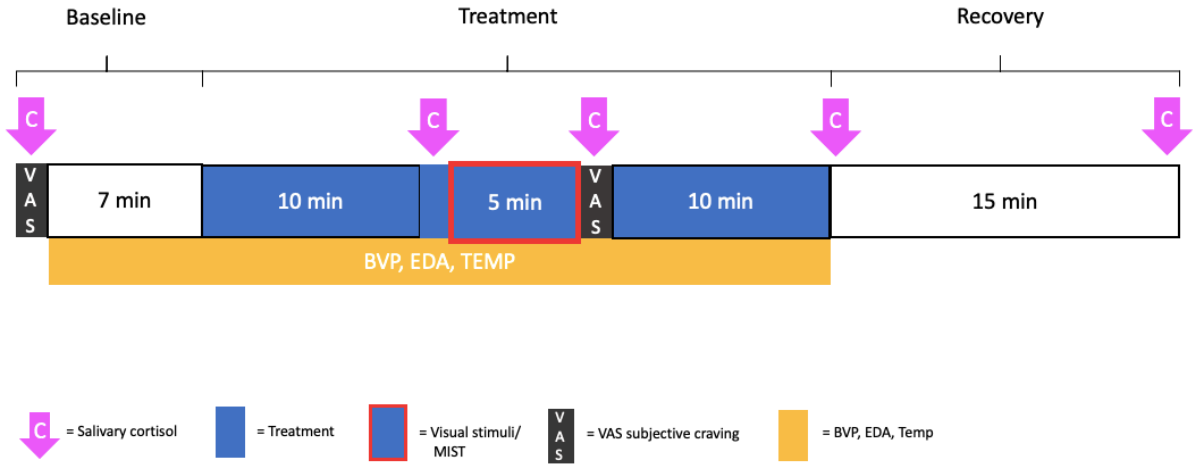


Figure 7. The study procedure of the experiment 1. The procedure will be repeated on three days with different type stimuli (neutral, alcohol-related or stress-inducing) on each day.

Stimuli: The subject will be exposed to 5-minute set of **alcohol-related** or **neutral/relaxing** images or social stress induced by **Montreal Imaging Stress Task (MIST)** (Figure 8.) (Dedovic *et al.*, 2005). MIST is a validated method for inducing social stress in a laboratory environment and is well suited for simultaneous HRV measurement (Brugnera *et al.*, 2018).

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

It is a non-verbal arithmetic task test which has been adapted from the Trier Social Stress Test (Allen *et al.*, 2017). Each set of alcohol-related or neutral images will contain 30 pictures of one type of stimuli, each image being visible for 10 s. All participants will be exposed to all three types of stimuli on separate days in random order. The images for the neutral/relaxing stimuli will be chosen from International Affective Picture System database (IAPS), the alcohol-related images will be chosen from The Geneva Appetitive Alcohol Picture database (GAAP) (Figure 9.) (Billieux *et al.*, 2011). The alcohol-related visual material will be adapted to suit the subject's personal preference by ensuring that the material contains images of their preferred type of alcoholic beverage. When necessary, the material will also be modified to be recognisable for the Finnish AUD patients for example by replacing an image of a Swiss beer bottle label with a Finnish equivalent.

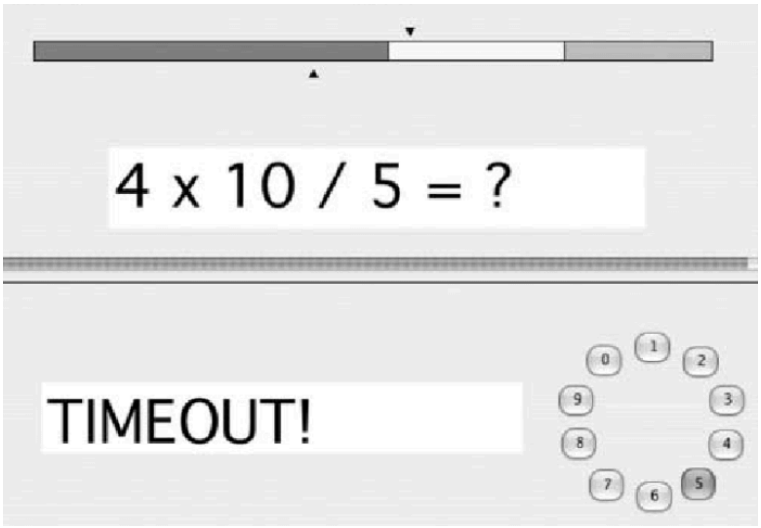


Figure 8. Graphical user interface and an example of an arithmetic task of the Montreal Imaging Stress Task (MIST). (Dedovic *et al.*, 2005)

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.



Figure 9. Examples of the GAAP database alcohol-related images. (Billieux *et al.*, 2011)

Procedure: Before the beginning of the experiment the subject is instructed to sit still without speaking during the session (excluding the TSST) and to pay close attention to the images presented during the visual stimulation. Each test session will start with a collection of a salivary sample, self-report of the current craving for alcohol using VAS, followed by 7-minute baseline recording of BVP, EDA and Temp. BVP, EDA and Temp monitoring will continue throughout the whole test session. Next, the tactile stimulation will be activated. The subject will remain sitting without speaking for 10 min after which the second salivary sample will be collected. Next, either neutral/relaxing or alcohol-related images will be shown on a screen/laptop placed in front of the subject for 5 minutes or 5-minute TSST will be performed for stress induction. The third salivary sample will be collected directly after the exposure to the visual stimuli and at the same time point the subject is again asked to rate the subjective craving with the VAS. The fourth salivary sample will be collected after a 10-minute period during which the test device remains active. BVP, EDA and Temp will be

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

monitored throughout the whole session. The fifth salivary sample will be collected after a 15-minute recovery period approximately 25 minutes after the exposure to the stimuli.

13.2 Experiment 2 - *Cue-reactivity task test*

The pupillary reactivity protocol will be modified from the experimental set-up previously described by Kvamme *et al.* (2019). The experiment will take place in an electrically shielded room provided by Aalto Behavioral Laboratory. The pupillary reactivity tracking and the EEG measuring systems will be connected to the PC providing the stimuli. The experimental design and the timeline of the session is illustrated in the Figure 10.

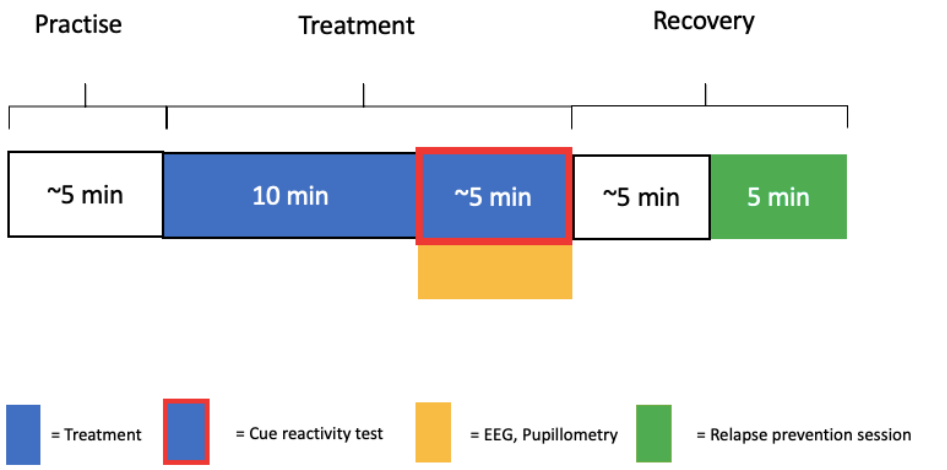


Figure 10. The experimental design of the experiment 2.

Stimuli: Two types of stimuli – 48 alcohol-related and 48 neutral images - will be presented so that each alcohol related image will be followed by a neutral image. Examples of the images are presented in the Figure 11. The images will be centered on a white background and the colour, size and brightness of the alcohol and neutral stimuli will be matched. The alcohol-related images presenting typical alcoholic beverages available in Finland will either be sourced from the internet or taken by a professional photographer in the research team. The neutral images will be sourced from the food.pics database (Blechert *et al.*, 2019). The stimulation sequence and the interface will be programmed using Python based PsychoPy2 application

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.



Figure 11. Examples of the alcohol-related and neutral stimuli that may be used in the cue reactivity task**.** Modified from Kvamme *et al.* (2018)

Procedure: Before the beginning of the experiment, the subject will be provided a detailed description of the procedure and introduction of the apparatus. The subject will be seated in front of a monitor and a short practice trial will be performed to familiarize the subject with the protocol.

Once the subject has understood the protocol the treatment will be started by activating the test device. The subject will sit still for 10 minutes before the beginning of the recordings and the stimulus presentation. The EEG and the pupillary reactivity will be recorded simultaneously. The EEG cap will be positioned according to the extended 10/20 system.

The stimulus presentation protocol will be modified from the protocol presented in the Figure 12. In the beginning of each trial a fixation cross will be presented on the screen until the eye position is stable within 1.5° visual degrees of the fixation cross for a minimum of 200 ms. Next, a neutral or alcohol-related stimulus image will be presented for 2.0 s. After each alcohol-related image, a 5-point VAS will appear on the screen and the subject will rate their will to drink the particular drink in the image by pressing a corresponding button on a

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

response pad (BG-610, Cedrus, San Pedro, USA). After each neutral image, the subject will be presented a question about the predominant colour in the image, which they will answer with the response pad. Once the subject has responded, the next stimulus image will be presented. The trial and the EEG and pupillometry recordings will finnish when the subject has responded to all images. In the end of the laboratory session, the subject will go through a relapse prevention session with an addiction medicine specialist to ensure that the distress caused by the experiments will not trigger a relapse outside of the laboratory.

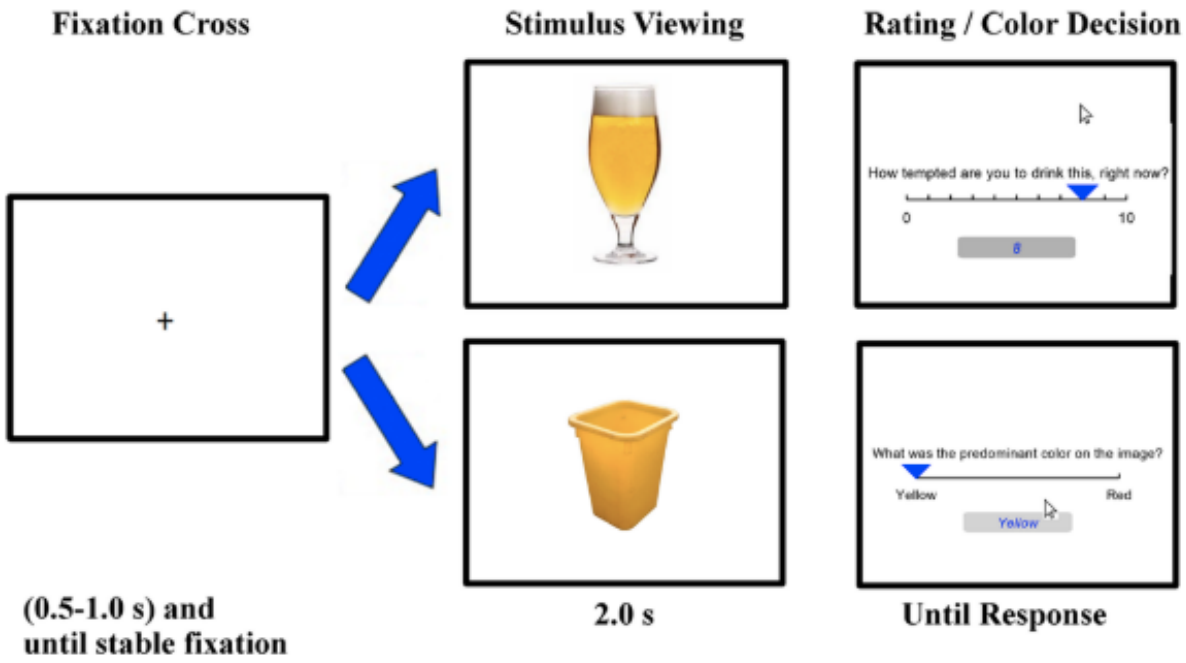


Figure 12. The cue-reactivity task test will be modified from the procedure previously described by Kvamme *et al.* (2019).

13.3 Relapse prevention sessions

In both experiments the subjects will go through a relapse prevention session with an experienced addiction medicine specialist. The relapse prevention session will take place at the end of each laboratory session, after the exposure to the stimuli. The session consists of short motivational enhancement counselling designed to prevent relapsing.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

14 Statistical analyses

The results will be analyzed by comparing the groups with two-tailed T-tests and, when appropriate, with analysis of variance (ANOVA) and a Chi-square test.

15 Risk assessment

15.1 Physical and psychological risk to the subjects

The subjects are likely to experience some level of psychological stress during the experiment due to the experimental protocol. This will be mitigated by the recovery and relaxation period, as well as the possibility to discuss with the research team health care professionals in the end of each visit. At the last visit all participants will go through the relapse prevention session with an addiction specialist. In case of severe psychological stress, the subjects will be guided to appropriate treatment.

Adverse reaction caused by the study treatment are unlikely, however they will be carefully monitored, and necessary actions will be taken immediately in case of such reactions. A risk of skin irritation caused by test device cannot be ruled out and the experiment will be terminated immediately if irritation occurs.

During the experiment, at least one of the researchers will be present either in the study room or in the control room, where they can be reached immediately e.g. by calling them by name.

15.2 Unexpected risks

In case of a fire or other emergency situation in the study location, we will act according to the fire safety and evacuation protocols of the building which will be familiarized beforehand. In case of a sudden illness or trauma, first aid will be used and, if necessary, emergency services (112) will be called. The participants are insured by patient insurance provided by Aalto University (Appendix 7).

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

15.3 Clinically significant incidental findings

It is possible that e.g abnormal heart beat or fever would be detected during the experiments. In such situation the participant in question would be directed to consult a physician. The health care professionals in the research group will be able to give health advice and instruct the participants to seek medical care if necessary. However, as all data will be stored and analyzed anonymously, we will not be able to inform the participants in case of clinically significant findings that are detected after the study visits.

16 Ethical considerations

16.1 Ethical review

A trial protocol, subject information sheet, informed consent form, online screening form and any other document provided to subjects will be submitted to HUS Ethics Committee for evaluation and approval. A trial will not commence until favorable opinion has been obtained from the appropriate Committee. If any alterations, other than changes of administrative nature only, are made to the trial protocol, a formal protocol amendment will be issued and submitted for relevant approval. The amendment will not be implemented until the approval has been obtained, except in cases where immediate implementation is necessary to eliminate or prevent imminent hazard to the subjects.

16.2 Ethical conduct of the trial

The trial will be conducted in compliance with the protocol, International Conference of Harmonization Good Clinical Practice (ICH GCP) and the Declaration of Helsinki.

16.3 Subject data protection

All subject data will be managed in compliance with the GDPR regulations. Strong identifiers, such as full name or contact information will be collected only in informed consent form which will be stored in a secure place that may be accessed only by the investigators. The salivary samples will be pseudonymized until they will be disposed of. All other data gathered during the study will be immediately anonymized with a random

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

identifier and it will be stored securely at Aalto University Network Drive. We will not create a key for opening the anonymization ~~code~~ identifiers. The data security of the drive is managed through confidential level encryption by Aalto IT Services. Data in the storage may be accessed only by the research group members. The access is controlled by research group member Pauli Tuovinen in collaboration with Aalto IT Services. Once the results of the study are published the unidentifiable data will be available in an open data repository.

16.4 Subject participation

Participation in the experiment is voluntary. All subjects will be asked to carefully read through the Informed Consent, Participant Information Sheet and Research Data Privacy Notice before signing the Informed Consent. Possible adverse reactions will be monitored throughout the experiments and the two-week follow-up period. The subjects have a possibility to seize the experiment at any point without a need for an explanation.

16.5 Subject compensation

The subjects will have no remuneration. The travel expenses are compensated according to the documents. The subjects will be offered snacks at the study site during the visits.

17 Funding

The study is funded by Business Finland Research to Business funding and Aalto University. The estimated expenses of the study are presented in the Appendix 8.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

References

Alinia, P. *et al.* (2021) 'Associations between physiological signals captured using wearable sensors and self-reported outcomes among adults in alcohol use disorder recovery: Development and usability study', *JMIR Formative Research*, 5(7). doi: 10.2196/27891.
Allen, A. P. *et al.* (2017) 'The Trier Social Stress Test: Principles and practice', *Neurobiology of Stress*. Elsevier Inc, 6, pp. 113–126. doi: 10.1016/j.ynstr.2016.11.001.
Anton, R. F., Moak, D. H. and Latham, P. K. (1996) 'The obsessive compulsive drinking scale: A new method of assessing outcome in alcoholism treatment studies', *Archives of General Psychiatry*. American Medical Association, 53(3), pp. 225–231. doi: 10.1001/archpsyc.1996.01830030047008.
Billieux, J. *et al.* (2011) 'The Geneva Appetitive Alcohol Pictures (GAAP): Development and preliminary validation', *European Addiction Research*, 17(5), pp. 225–230. doi: 10.1159/000328046.
Björnsdotter, M. *et al.* (2009) 'Somatotopic organization of gentle touch processing in the posterior insular cortex', *Journal of Neuroscience*, 29(29), pp. 9314–9320. doi: 10.1523/JNEUROSCI.0400-09.2009.
Blaine, S. K., Seo, D. and Sinha, R. (2017) 'Peripheral and prefrontal stress system markers and risk of relapse in alcoholism', *Addiction Biology*, 22(2), pp. 468–478. doi: 10.1111/adb.12320.
Blechert, J. *et al.* (2019) 'Food-pics_extended-an image database for experimental research on eating and appetite: Additional images, normative ratings and an updated review', *Frontiers in Psychology*, 10(MAR), p. 2019. doi: 10.3389/fpsyg.2019.00307.
Bozovic, D., Racic, M. and Ivkovic, N. (2013) 'Salivary cortisol levels as a biological marker of stress reaction.', *Medicinski arhiv*, 67(5), pp. 374–377. doi: 10.5455/medarh.2013.67.374-377.
Brugnera, A. *et al.* (2018) 'Heart rate variability during acute psychosocial stress: A randomized cross-over trial of verbal and non-verbal laboratory stressors', *International Journal of Psychophysiology*. Elsevier, 127(March), pp. 17–25. doi: 10.1016/j.ijpsycho.2018.02.016.
Chida, Y. and Steptoe, A. (2009) 'Cortisol awakening response and psychosocial factors: A systematic review and meta-analysis', *Biological Psychology*, 80(3), pp. 265–278. doi: 10.1016/j.biopsycho.2008.10.004.
Davidovic, M., Starck, G. and Olausson, H. (2019) 'Processing of affective and emotionally neutral tactile stimuli in the insular cortex', *Developmental Cognitive Neuroscience*. Elsevier, 35(November 2017), pp. 94–103. doi: 10.1016/j.dcn.2017.12.006.
Dedovic, K. *et al.* (2005) 'The Montreal Imaging Stress Task : using functional imaging to investigate the ...', *Psychiatry & Neuroscience*, 30(5), pp. 319–325.
Ditzen, B. *et al.* (2007) 'Effects of different kinds of couple interaction on cortisol and heart rate responses to stress in women', *Psychoneuroendocrinology*, 32(5), pp. 565–574. doi: 10.1016/j.psyneuen.2007.03.011.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

Eckstein, M. *et al.* (2020) 'Calming Effects of Touch in Human, Animal, and Robotic Interaction—Scientific State-of-the-Art and Technical Advances', *Frontiers in Psychiatry*, 11(November), pp. 1–17. doi: 10.3389/fpsyt.2020.555058.
Eriksson Hagberg, E. *et al.* (2019) 'Spatio-temporal profile of brain activity during gentle touch investigated with magnetoencephalography', *NeuroImage*, 201(November 2018). doi: 10.1016/j.neuroimage.2019.116024.
Fox, H. C. *et al.* (2007) 'Stress-induced and alcohol cue-induced craving in recently abstinent alcohol-dependent individuals', *Alcoholism: Clinical and Experimental Research*, 31(3), pp. 395–403. doi: 10.1111/j.1530-0277.2006.00320.x.
Garland, E. L., Ingmar, F. H. A. and Howard, M. O. (2012) 'Cue-Elicited Heart Rate Variability and Attentional Bias Predict Alcohol Relapse Following Treatment', *Psychopharmacology*, 222(1), pp. 17–26. doi: 10.1007/s00213-011-2618-4.Cue-Elicited.
Ghiasi, S. *et al.* (2020) 'Assessing Autonomic Function from Electrodermal Activity and Heart Rate Variability During Cold-Pressor Test and Emotional Challenge', *Scientific Reports*. Springer US, 10(1), pp. 1–14. doi: 10.1038/s41598-020-62225-2.
Gilpin, N. W. and Koob, G. F. (2008) 'Neurobiology of alcohol dependence: Focus on motivational mechanisms', *Alcohol Research and Health*. National Institute on Alcohol Abuse and Alcoholism, pp. 185–195.
Girven, K. S. *et al.* (2020) 'Glutamatergic input from the insula to the ventral bed nucleus of the stria terminalis controls reward-related behavior', *Addiction Biology*, (December 2019), pp. 1–11. doi: 10.1111/adb.12961.
Haaranen, M., Scuppa, G., *et al.* (2020) 'Anterior insula stimulation suppresses appetitive behavior while inducing forebrain activation in alcohol-preferring rats', *Translational Psychiatry*. Springer US, 10(1). doi: 10.1038/s41398-020-0833-7.
Haaranen, M., Schäfer, A., *et al.* (2020) 'Chemogenetic Stimulation and Silencing of the Insula, Amygdala, Nucleus Accumbens, and Their Connections Differentially Modulate Alcohol Drinking in Rats', *Frontiers in Behavioral Neuroscience*, 14(November), pp. 1–12. doi: 10.3389/fnbeh.2020.580849.
Heinz, A. *et al.* (2010) 'Alcohol craving and relapse prediction: Imaging studies', in *Advances in the Neuroscience of Addiction*. CRC Press, pp. 137–161. doi: 10.1201/9781420007350.
Hellhammer, D. H., Wüst, S. and Kudielka, B. M. (2009) 'Salivary cortisol as a biomarker in stress research', *Psychoneuroendocrinology*, 34(2), pp. 163–171. doi: 10.1016/j.psyneuen.2008.10.026.
Junghanns, K. *et al.* (2005) 'Attenuated salivary cortisol secretion under cue exposure is associated with early relapse', *Alcohol and Alcoholism*, 40(1), pp. 80–85. doi: 10.1093/alcalc/agh107.
Kane, S. (2019) *Sample Size Calculator*, *Sample Size Calculator. ClinCalc: https://clincalc.com/stats/samplesize.aspx. Updated July 24, 2019. Accessed March 7, 2022.* Available at: https://clincalc.com/stats/samplesize.aspx (Accessed: 7 March 2022).
Kaplan, R. F. *et al.* (1985) 'Reactivity to alcohol-related cues: Physiological and subjective responses in alcoholics and nonproblem drinkers', *Journal of Studies on Alcohol*. J Stud Alcohol, 46(4), pp. 267–272. doi: 10.15288/jsa.1985.46.267.
King, C. E. and Becker, H. C. (2019) 'Oxytocin attenuates stress-induced reinstatement of alcohol seeking behavior in male and female mice', *Psychopharmacology*.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

Psychopharmacology, 236(9), pp. 2613–2622. doi: 10.1007/s00213-019-05233-z.
Koob, G. F. (2013) 'Addiction is a reward deficit and stress surfeit disorder', *Frontiers in Psychiatry*, 4(AUG), pp. 1–18. doi: 10.3389/fpsyt.2013.00072.
Koob, G. F. and Volkow, N. D. (2016) 'Neurobiology of addiction: a neurocircuitry analysis', *The Lancet Psychiatry*. doi: 10.1016/S2215-0366(16)00104-8.
Korpi, E. R. *et al.* (2015) 'Mechanisms of action and persistent neuroplasticity by drugs of abuse', *Pharmacological Reviews*, 67(4), pp. 872–1004. doi: 10.1124/pr.115.010967.
Kvamme, T. L. *et al.* (2018) 'Distraction towards contextual alcohol cues and craving are associated with levels of alcohol use among youth', *BMC Psychiatry*. BMC Psychiatry, 18(1), pp. 1–10. doi: 10.1186/s12888-018-1919-0.
Kvamme, T. L. *et al.* (2019) 'Pupillary reactivity to alcohol cues as a predictive biomarker of alcohol relapse following treatment in a pilot study', *Psychopharmacology*. Psychopharmacology, 236(4), pp. 1233–1243. doi: 10.1007/s00213-018-5131-1.
Laeng, B., Sirois, S. and Gredebäck, G. (2012) 'Pupillometry: A window to the preconscious?', *Perspectives on Psychological Science*, 7(1), pp. 18–27. doi: 10.1177/1745691611427305.
Littel, M. *et al.* (2012) 'Electrophysiological indices of biased cognitive processing of substance-related cues: A meta-analysis', *Neuroscience and Biobehavioral Reviews*. Elsevier Ltd, 36(8), pp. 1803–1816. doi: 10.1016/j.neubiorev.2012.05.001.
Löken, L. S. *et al.* (2009) 'Coding of pleasant touch by unmyelinated afferents in humans', *Nature Neuroscience*, 12(5), pp. 547–548. doi: 10.1038/nn.2312.
Maruyama, K. *et al.* (2012) 'Tactile skin stimulation increases dopamine release in the nucleus accumbens in rats', *Journal of Physiological Sciences*, 62(3), pp. 259–266. doi: 10.1007/s12576-012-0205-z.
Matheus-Roth, C. *et al.* (2016) 'Occipital event-related potentials to addiction-related stimuli in detoxified patients with alcohol dependence, and their association with three-month relapse', *BMC Psychiatry*. BMC Psychiatry, 16(1), pp. 1–12. doi: 10.1186/s12888-016-0782-0.
Namkoong, K. *et al.* (2004) 'Increased P3 amplitudes induced by alcohol-related pictures in patients with alcohol dependence', *Alcoholism: Clinical and Experimental Research*, 28(9), pp. 1317–1323. doi: 10.1097/01.ALC.0000139828.78099.69.
Naqvi, N. H. *et al.* (2014) 'The insula: A critical neural substrate for craving and drug seeking under conflict and risk', *Annals of the New York Academy of Sciences*, 1316(1), pp. 53–70. doi: 10.1111/nyas.12415.
Nummenmaa, L. *et al.* (2016) 'Social touch modulates endogenous μ-opioid system activity in humans', *NeuroImage*. Elsevier Inc., 138, pp. 242–247. doi: 10.1016/j.neuroimage.2016.05.063.
Olausson, H. *et al.* (2002) 'Unmyelinated tactile afferents signal touch and project to insular cortex', *Nature Neuroscience*, 5(9), pp. 900–904. doi: 10.1038/nn896.
Olausson, H. *et al.* (2016) *Affective touch and the neurophysiology of CT afferents*, *Affective Touch and the Neurophysiology of CT Afferents*. doi: 10.1007/978-1-4939-6418-5.
Pawling, R. *et al.* (2017a) 'C-tactile afferent stimulating touch carries a positive affective value', *PLoS ONE*, 12(3), pp. 1–16. doi: 10.1371/journal.pone.0173457.
Pawling, R. *et al.* (2017b) 'C-tactile afferent stimulating touch carries a positive affective value', *PLoS ONE*, 12(3), pp. 1–15. doi: 10.1371/journal.pone.0173457.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.

Petit, G. *et al.* (2015) 'Reduced processing of alcohol cues predicts abstinence in recently detoxified alcoholic patients in a three-month follow up period: An ERP study', *Behavioural Brain Research*. Elsevier B.V., 282, pp. 84–94. doi: 10.1016/j.bbr.2014.12.057.
Posada-Quintero, H. F. and Chon, K. H. (2020) 'Innovations in electrodermal activity data collection and signal processing: A systematic review', *Sensors (Switzerland)*, 20(2). doi: 10.3390/s20020479.
Schuurmans, A. A. T. *et al.* (2020) 'Validity of the Empatica E4 Wristband to Measure Heart Rate Variability (HRV) Parameters: a Comparison to Electrocardiography (ECG)', *Journal of Medical Systems*. Journal of Medical Systems, 44(11). doi: 10.1007/s10916-020-01648-w.
Shaffer, F. and Ginsberg, J. P. (2017) 'An Overview of Heart Rate Variability Metrics and Norms', *Frontiers in Public Health*, 5(September), pp. 1–17. doi: 10.3389/fpubh.2017.00258.
Shu, L. *et al.* (2018) 'A review of emotion recognition using physiological signals', *Sensors (Switzerland)*, 18(7). doi: 10.3390/s18072074.
Sinha, R. *et al.* (2009) 'Enhanced negative emotion and alcohol craving, and altered physiological responses following stress and cue exposure in alcohol dependent individuals', *Neuropsychopharmacology*, 34(5), pp. 1198–1208. doi: 10.1038/npp.2008.78.
Sinha, R. (2009) 'Modeling stress and drug craving in the laboratory: Implications for addiction treatment development', *Addiction Biology*, 14(1), pp. 84–98. doi: 10.1111/j.1369-1600.2008.00134.x.
Sinha, R. (2013a) 'Modeling Relapse Situations in the Human Laboratory', *Curr Top Behav Neurosci.*, (13), pp. 379–402. doi: 10.1007/7854.
Sinha, R. (2013b) 'The clinical neurobiology of drug craving', *Current Opinion in Neurobiology*. Elsevier Ltd, 23(4), pp. 649–654. doi: 10.1016/j.conb.2013.05.001.
Sobell, L. C. *et al.* (1996) 'The reliability of the Alcohol Timeline Followback when administered by telephone and by computer', *Drug and Alcohol Dependence*, 42(1), pp. 49–54. doi: 10.1016/0376-8716(96)01263-X.
Triscoli, C. *et al.* (2017) 'Heart rate variability is enhanced by long-lasting pleasant touch at CT-optimized velocity', *Biological Psychology*. Elsevier, 128(December 2016), pp. 71–81. doi: 10.1016/j.biopsycho.2017.07.007.
Uvnäs-Moberg, K., Handlin, L. and Petersson, M. (2014) 'Self-soothing behaviors with particular reference to oxytocin release induced by non-noxious sensory stimulation', *Frontiers in Psychology*, 5(OCT), pp. 1–16. doi: 10.3389/fpsyg.2014.01529.
Walker, S. C. *et al.* (2017) 'C-tactile afferents: Cutaneous mediators of oxytocin release during affiliative tactile interactions?', *Neuropeptides*, 64, pp. 27–38. doi: 10.1016/j.npep.2017.01.001.
Walker, S. C. *et al.* (2020) 'C-low threshold mechanoafferent targeted dynamic touch modulates stress resilience in rats exposed to chronic mild stress', *European Journal of Neuroscience*, (May), pp. 1–14. doi: 10.1111/ejn.14951.

This protocol and all information relating to it is confidential and proprietary property of Aalto University and may not be reproduced, published, or disclosed to others without written authorization of the authors.