School of Experimental Psychology
12a Priory Road
BRISTOL BS8 1TU
United Kingdom

+44.117.9546847 t.
+44.117.9288588 f.
m.grabski@bristol.ac.uk
www.bristol.ac.uk

STUDY PROTOCOL

Are cognitive deficits associated with tobacco abstinence mediated by nicotine withdrawal?

Meryem Grabski, Valerie Curran, David Nutt, Stephen Husbands, Stuart Ferguson, Marcus Munafò

Background

Smokers report cognitive deficits following tobacco withdrawal, which may contribute to the withdrawal syndrome and subsequent relapse. However, it is unclear whether these deficits are primarily due to the effects of nicotine withdrawal or other mechanisms such as expectancies about the effects of abstaining or the loss of sensorimotor stimulation.

In a systematic review of the literature we identified several cognitive tasks sensitive to a period of acute abstinence (Grabski et al., under review). We now wish to determine whether these effects are indeed mediated via nicotine withdrawal. We will do this by comparing task performance of abstinent smokers receiving nicotine replacement therapy (NRT) with abstinent smokers receiving placebo NRT. We expect that performance of abstinent smokers receiving NRT will be different (and more similar to performance of satiated smokers) than performance of abstinent smokers receiving placebo NRT.

Since nicotine withdrawal is known to be dynamic and complex, and fluctuates over time, it is not well captured by a single assessment (Hughes 2007, Bedi, Preston et al. 2011, Adams and Munafo 2013). We will use ecological momentary assessment to measure craving of participants via an Android mobile device at several time points over the course of a single day, as participants are in their natural environment.

Study Objective and Hypotheses

We propose to examine the effects of nicotine delivered via NRT on cognition and craving in acutely abstinent smokers. The principal research question to be addressed is:

Are changes in cognitive performance and craving levels due to acute tobacco abstinence mediated by nicotine withdrawal?

We hypothesize that abstinent smokers receiving active NRT will display less impairment in cognitive task performance than abstinent smokers who receive placebo NRT. We also hypothesise that craving assessed via EMA will be higher in abstinent smokers receiving placebo than in abstinent smokers receiving NRT.

Study Design

In this laboratory-based within-subjects study participants will attend two sessions. Before both sessions, participants will be asked to abstain from smoking overnight and until the end of the testing day. For one session, participants will be asked to apply an active nicotine patch 30 minutes before the behavioural assessment and use a nicotine nasal spray *ad libitum*. For the other session participants will be asked to apply a placebo patch and will receive placebo nasal spray. Participants will be blinded as to which treatment they receive. The order of the sessions will be counterbalanced across participants.

On each testing day, the behavioural assessments will be completed in the morning, with the order of the cognitive computer-tasks counterbalanced across participants and fixed within participants. After testing, participants will be provided with an Android mobile device to measure craving for another six hours, using a specifically programmed app. The assessment of craving can be done remotely, so participants can leave the testing site. After six hours participants will be asked to return the mobile device and the nasal spray to the researcher, have their abstinence confirmed via breath test and their patch removed.

Study Site

School of Experimental Psychology, University of Bristol, 12a Priory Road, Bristol BS8 1TU, United Kingdom.

Participants and Recruitment

We will recruit individuals aged 18 to 60 years, who are regular smokers from the staff and students of the University of Bristol and from the general population.

Participants will be recruited by existing email lists, posters, flyer advertisement, and by word of mouth. After contacting the research coordinator and reading the study information sheet, participants will complete an initial online screening form. Those who meet the inclusion and exclusion criteria will be contacted by the research co-ordinator to arrange a testing session. Participants will be reimbursed £80 for their

time and expenses.

Inclusion criteria

- 18-60 years old.
- smoke at least 5 cigarettes per day.
- smoke first cigarette within one hour of waking.
- smoking for at least 6 months.
- English as first language (or equivalent level of fluency).
- visual acuity within normal limits.
- willingness to abstain from smoking for about 20 hrs on two occasions, once with using nicotine patch and nasal spray and once using placebo nicotine patch and nasal spray without being made aware of the order of the conditions at any point of the study.
- if female: willingness to take a pregnancy test before each study session.

Exclusion criteria

- currently taking any psychoactive medication
- actively trying to give up smoking
- history of substance/alcohol misuse or dependence (other than nicotine or cannabis)
- if female: pregnant, breast feeding or trying to conceive
- having extensive dermatitis/other skin disorder that precludes patch use
- having had acute coronary syndrome or a stroke within the past three weeks
- allergic to any component of the nicotine replacement therapy patch or nasal spray

Sample size determination

We will require 70 participants to achieve 80% power and an alpha-level of 0.5, in order to detect the smallest effect size indicated in our recent meta-analysis (d = 0.34 for the delay-discounting task) (Grabski et al., under review). The smallest effect size available was used in order to provide the most conservative sample size estimate. For the cognitive task with the largest effect size (d = 0.59 for the n-back task), a sample size of 25 participants would suffice (Mendrek et al., 2006).

Withdrawal of participants

Participants will be informed that they are able to withdraw from the study at any time. In the case of an adverse event, that is any unforeseen or serious reaction to the procedure or the NRT products, participants will be given full reimbursement of £80. In the case of the participant not tolerating the NRT product by displaying any of the harmless side effects described below, participants will reimbursed £35 if this occurs in the first session, and £80 if this occurs in the second session. In the case of the researcher being unable to track the eyes of the participant they will be reimbursed a monetary amount that is commensurate with the time they have spent in the laboratory (e.g., stopping half way through the first session would result in a reimbursement of 25%).

Randomisation

The order of sessions (placebo, NRT) will be counterbalanced. The order of the cognitive tasks during the laboratory session will be randomized across participants, and fixed within participants.

Measures and Materials

- Working memory performance (number of commission errors) as measured by the n-back task
- Cognitive bias (dwell time and reaction times) as measured by an eye-tracking dot probe task
- Reward choice (area under the curve; AUC) as measured by the delay-discounting task
- Craving measured via ecological momentary assessment using a mobile device

Questionnaires

Questionnaire measures will comprise the Fagerström Test of Nicotine Dependence (FTND) (Heatherton, Kozlowski et al. 1991), the Readiness to Quit Ladder (Biener and Abrams 1991), the Minnesota Nicotine Withdrawal Scale-self report (Hughes and Hatsukami 1998), and the Questionnaire of Smoking Urges – Brief (Tiffany and Drobes 1991).

Delay-discounting task

Participants are made aware at the start of the task that the amounts of money will be hypothetical and no real money will be gained in this task. Participants are asked to make 91 choices between a standard hypothetical amount of money (£100) available after one of five delays (0, 7, 30, 90, or 180 days) and one of 23 alternative hypothetical amounts available immediately ("Which would you prefer: £100 in 180

days or £30 now?"). Question order is randomized.

N-back task

Participants are required to decide whether each stimulus in a sequence matches the one that appeared n items ago. A 2-back and 3-back condition is used. Eight phonologically-distinct letters will be used as stimuli (*B, F, H, K, M, Q, R,* or *X*). Response time and accuracy of responses will be recorded. Each condition (2-back and 3-back) will be presented in 4 blocks, which will each consist of 48 trials and 2 additional practice blocks of 20 trials. Each trial begins with a central fixation cross, presented for 500 ms, followed by the stimulus in that location for 500 ms and a 2000 ms inter-stimulus interval. Participants will be asked to make a "yes"/"no" response via button press as quickly and accurately as possible.

Go/no-go task

Participants are required to make a response (press button) when a designated "go" cue is presented and withhold responding to a designated "no-go" cue. At the start of each trial a fixation cross is displayed for 500 ms. The cues will be shapes (arrow or star) presented in the centre of a screen for 1000 ms. A practice phase of 6 trials will be implemented, where participants will receive feedback on their performance. The first 20 trials will be go-trials to build a pre-potent response and the remaining 90 trials will be made up of 30 no-go trials and 60 go-trials, presented in randomized order.

Dot-probe task

Participants are presented with two stimuli (photographs), one of which is neutral and one of which is related to smoking. Each trial starts with a central fixation cross shown for 1000 ms, which is followed by a side by side presentation of a pair of the stimuli for 2000 ms. 120 main trials will be presented, of which 80 will be critical trials, including a neutral and a smoking related picture and 40 will be filler trials including neutral pictures only. After presentation of the pictures a dot will appear on one side of the screen, in the former location of one of the two pictures. Participants are instructed to indicate the location of the dot as fast as possible via button press. Eye-movements towards these locations on the screen will be recorded from the start of the fixation cross until a button press has been made. There will be 14 practice trials before the start of the main trials.

Ecological momentary assessment

After completion of the laboratory testing session participants will be given an Android mobile device with ecological momentary assessment software installed on it. The software will randomly alert participants over the next six hours to respond to the following questions:

- Able to focus? (yes, no)
- Alert? (yes, no)

- Angry/frustrated? (yes, no)
- Bored (yes, no)
- Calm/relaxed? (yes, no)
- Difficulty concentrating? (yes, no)
- Enthusiastic? (yes, no)
- Happy? (yes, no)
- Irritable? (yes, no)
- Miserable? (yes, no)
- Nervous/tense? (yes, no)
- Quiet/sleepy? (yes, no)
- Restless? (yes, no)
- Sad? (yes, no)
- Cigarette craving? (yes, no)

Participants will furthermore be asked to indicate the highest craving they experienced since the last prompt.

Procedures

Participants will be scheduled to arrive in the laboratory between 8.00 a.m. and 11.00 a.m. Two appointments will be scheduled, at least one week apart. Upon arrival at the first session, participants will be given the opportunity to read the information sheet and ask any questions about the study. The researcher will verbally confirm the schedule of the study and will remind them that they can stop the study at any time without having to give a reason. The participant will complete two copies of the informed consent form, one of which they are able to take away and the other will be filed in the study master file.

The following procedures will be used on both occasions if not indicated otherwise. If the participant is female a pregnancy test will be administered. Participants' breath CO level will be measured and participants will then complete the questionnaires. Following this, the nicotine (or placebo) patch will be administered by the researcher on the participant's arm. The participant will then be instructed to use the nicotine (or placebo) nasal spray once and will be asked to wait for 30 minutes so the nicotine can take its effect. They will then be asked to complete the four cognitive tasks.

After completion of the behavioural assessments, participants will be provided with a mobile device and instructed how to use the mobile device application in order to assess craving. They will be asked to remotely indicate craving levels on the device whenever they are prompted to do so. Craving will be assessed at random intervals for six hours. Participants will be instructed to bring the device as well as the nasal spray back to the testing site at the end of this period, where continued abstinence will be tested via breath test and participant will complete the Questionnaire of Smoking Urges- Brief (Tiffany & Drobes, 1991) once more. The patch will then be removed by the researcher. Participants will be debriefed and reimbursed for their time and expenses, after the completion of the second test day.

Drug Administration

The strength of the active nicotine patch will be 14 mg. Both active and placebo patches will be applied by the researcher 30 minutes before the start of the testing session. The strength of the active nicotine nasal spray will be 10 mg/ml. The participant will be instructed how to use the nasal spray by the researcher. Each nasal spray canister will contain 1 ml of nicotine nasal spray or placebo nasal spray. All NRT will be locked in a storage cupboard and be kept in a locked room.

Statistical Plan

Delay-discounting task

Indifference points will be derived and will be used to calculate area under the curve (AUC), using the following equation: (x2 – x1)[9y1 = y20/2], where x2 and x1 represent successive delays to receiving the standard and y1 and y2 the indifference point values associated with these delays. Smaller AUC values indicate greater discounting and impulsive choice (Mitchell 1999, Ashare and Hawk Jr 2012). The principal statistical analysis will be a one-way ANOVA of AUC values, with nicotine (active, placebo) as a within-subjects factor.

N-back task

The principal statistical analysis will be a one-way ANOVA of hit rates, with nicotine (active, placebo) as a within-subjects factor.

Go/no-go task

The principal statistical analysis will be a one-way ANOVA of commission error data, with nicotine (active, placebo) as a within-subjects factor.

Dot-probe task

The principal statistical analysis will be a 2 by 2 repeated measures ANOVA of eye movement dwell time data, with picture type (smoking, neutral) and nicotine (active, placebo) as within-subjects factors.

Ecological momentary assessment

Cigarette craving will be analysed using Generalized Estimation Equations in order to determine differences between the active and placebo NRT conditions.

Ethical Considerations and Informed Consent

Ethics approval has been obtained from the Faculty of Science Research Ethics Committee at the University of Bristol. The study will be conducted according to the revised Declaration of Helsinki (2013) and the 1996 ICH Guidelines for Good Clinical Practice E6 (R1). The investigator will explain the nature, purpose and risks of the study to the participant. The participant will receive the information sheet in

advance of the study session. There will be no time restriction on how long participants take to respond, with the exception that participants who respond after all study places have been filled will not be offered a place on the study. Therefore, participants will be given sufficient time to read the information and consider any implications, and to raise any questions with the investigators prior to making a decision to participate. On arrival at the study session participants will be given the opportunity to read the information sheet again and ask the investigator any questions. Written consent will then be obtained. Participants will be informed that they are free to withdraw at any time.

Safety

There are no expected hazards. Discomfort due to cigarette abstinence might be experienced. This will not exceed the discomfort experienced in any regular smoking-cessation attempt for this amount of time.

The study medication (NRT) is a licensed smoking cessation aid and available over the counter. It has been associated with some side effects. The more common side effects of both the patch and the nasal spray are upset stomach, headache or dizziness. The nicotine patch may cause skin irritations on the site of the patch application. The nasal spray can lead to watery eyes, sneezing and coughing. Generally these side effects may cause discomfort but are not considered dangerous. Furthermore as nicotine is rapidly cleared from body any side effects will subside quickly once nicotine is removed.

Participants will be given information on the possible side effects of the drug, will be encouraged to report any effects that they feel and will be able to withdraw from the study at any time. Contra-indications are hypersensivity to the drug. The dose we are administering is standard therapeutic dose prescribed for smoking cessation, and therefore is considered safe in healthy volunteers.

Adverse Event Reporting

Adverse events or adverse reactions will be documented at the end of the relevant session using an adverse event report, and will be recorded in the CRFs. The adverse event reports will be anonymised by unique study identifier and stored in the master file. Adverse events or adverse reactions will be followed up until resolved if possible. At the end of the study a safety report will be complied and sent to the Principal Investigator (PI) listing all adverse events and adverse reactions. All procedures related to adverse events will follow the University of Bristol adverse events policies and procedures.

Data Management

All aspects of the Data Protection Act will be adhered to. Consent forms will be retained by the School of Experimental Psychology for a period of 10 years after study completion. In the event that a participant revokes authorisation to collect or use personal health information, the investigator retains the ability to use all information collected prior to the revocation of participant authorisation.

Anonymised study data

The case report forms (CRFs) and electronic data will be anonymised by a unique numeric identifier. CRFs will be stored in a locked office. All data requested on the CRF will be recorded. All missing data will be explained. If any entry errors are made, a single straight line will be drawn through the incorrect entry and the correct data entered above it; to correct such an error. All such changes will be initialled and dated. Once data from CRFs have been inputted into a data spreadsheet they will undergo a reliability check (20% check by independent researcher). If an error rate greater than 1% is obtained the data will be re-inputted in full and assessed again. After the data have been positively assessed, the CRFs will be destroyed in the School's confidential waste facility.

Original computer data files will be backed up on a secured University of Bristol network drive. At the end of the study, electronic study data (including finalised data sheet) will be transferred to a designated University of Bristol Research Data Storage Facility for long-term archiving. Study data will be kept for a minimum of 15 years. At the appropriate time the data sheet will be locked and made open using the University of Bristol Research Data Repository.

Screening documents and participant contact details

Screening documents, participant contact details and participant identifier logs will be stored separately in a study master folder and kept confidential. These will be kept in the study master folder for one year after study completion or until data are made open (whichever comes first), after which these documents will be destroyed. Failed screening documents will be shredded immediately using School's confidential waste facility.

Revoked data

If a participant decides that they do not want their data used after their participation they have the right to request that the data are withdrawn. They can request this up to one year after study completion or until the data are made open (whichever comes first).

Quality Control and Quality Assurance

The investigators will be responsible for data quality. After approximately 10% of data collection has been completed, the study will undergo an in-house quality assessment. During this monitoring process all CRFs and study documents will be assessed as well as the investigators laboratory management and participant engagement, and corrected where necessary.

Post-study checks will be conducted on data entry by an independent researcher. This researcher will re-enter 20% of hardcopy participant data. A threshold of 1% will be used, whereby error rates greater than 1% will require the data to be re-entered. A 100% check will be made on allocation to test condition.

Insurance

This study will be sponsored by the University of Bristol. The University has Clinical Research Insurance to cover the liability of the University to research participants. In the event that something goes wrong and a participant is harmed during the research study there are no special compensation arrangements. If a participant is harmed and this is due to someone's negligence then they may have grounds for a legal action for compensation against Bristol University [or the NHS Trust] or one of the other parties to the research, but they may have to pay their own legal costs.

Publication Policy

The findings from this research study may be published in an appropriate scientific journal (and made available open access), and/or presented at an appropriate meeting. Study data will be collected and held by the study investigators. The data will be made available for sharing via a University of Bristol online data repository.

Study Personnel

Meryem Grabski
School of Experimental Psychology
University of Bristol
12a Priory Rd
Bristol BS8 1TU
Tel: +44 (0) 117 33 10495
Email: m.grabski@bristol.ac.uk

Valerie Curran
Clinical Psychopharmacology Unit
University College London
Gower Street
London WC1E 6BT
Tel: +44 (20) 7679 1898
Email: v.curran@ucl.ac.uk

David Nutt
Department of Medicine
Imperial College
Du Cane Road
London W12 0NN
Email: d.nutt@imperial.ac.uk

Stephen Husbands
Department of Pharmacy and Pharmacology
University of Bath
Claverton Down
Bath BA2 7AY

Tel: +44 (0) 1225 383103
Email: s.m.husbands@bath.ac.uk

Stuart Ferguson
School of Medicine
University of Tasmania
Private Bag 34
Hobart TAS 7000
Australia
Tel: +61 3 6226 4295
Email: stuart.ferguson@utas.edu.au

Marcus Munafò
School of Experimental Psychology
University of Bristol
12a Priory Rd
Bristol BS8 1TU
Tel: +44 (0)117 954 6841
Email: marcus.munafo@bristol.ac.uk

Funding Source

This research is part of an ESRC-funded PhD project with co-funding from Rusan Pharma Ltd, who are also providing and manufacturing the nicotine patches and matching placebo as well as the nicotine nasal spray and matching placebo.

Conflicts of Interest

This research is partially funded by the pharmaceutical company Rusan Pharma Ltd. However, Rusan do not control the intellectual content, data collection, publication policy etc.

References

Adams, S. and M. R. Munafo (2013). "Will peak provoked craving prove superior to cuereactivity?" Addiction **108**(6): 1027-1028.
Ashare, R. L. and L. W. Hawk Jr (2012). "Effects of smoking abstinence on impulsive behavior among smokers high and low in ADHD-like symptoms." Psychopharmacology **219**(2): 537-547.
Bedi, G., K. L. Preston, D. H. Epstein, S. J. Heishman, G. F. Marrone, Y. Shaham and H. de Wit (2011). "Incubation of cue-induced cigarette craving during abstinence in human smokers." Biological psychiatry **69**(7): 708-711.
Biener, L. and D. B. Abrams (1991). "The Contemplation Ladder: validation of a measure of readiness to consider smoking cessation." Health Psychology **10**(5): 360.
Heatherton, T. F., L. T. Kozlowski, R. C. Frecker and K.-O. Fagerström (1991). "The Fagerström test for nicotine dependence: a revision of the Fagerström Tolerance Questionnaire." British journal of addiction **86**(9): 1119-1127.
Hughes, J. and D. Hatsukami (1998). "Errors in using tobacco withdrawal scale." Tobacco Control **7**(1): 92.
Hughes, J. R. (2007). "Effects of abstinence from tobacco: Valid symptoms and time

course." Nicotine & Tobacco Research **9**(3): 315-327.
Mitchell, S. H. (1999). "Measures of impulsivity in cigarette smokers and non-smokers." Psychopharmacology **146**(4): 455-464.
Tiffany, S. T. and D. J. Drobes (1991). "The development and initial validation of a questionnaire on smoking urges." British journal of addiction **86**(11): 1467-1476.