Minocycline and tobacco craving in smokers with schizophrenia

NCT 02968602

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Protocol number: HP-00072110

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Nicotine dependence is high in schizophrenia; nearly three times more prevalent than the general population [1, 2]. In smokers with schizophrenia (SWS), the risk of all-cause mortality is doubled and cardiovascular mortality risk is twelvefold higher than nonsmokers [3, 4]. Many factors influence smoking in SWS [5]; however, predictors of craving and smoking behavior are not well established. Craving is a major contributor to smoking behaviors, and, importantly, is a predictor of relapse risk [6]. Since craving may precede relapse, it can be advantageous as a screening tool for those attempting cessation. In addition, focusing on treatments aimed to reduce craving may lead to better therapeutic targets. A combination of cessation strategies may be associated with reduced relapse, primarily due to craving suppression [7], so targeting craving may offer important gains in improving abstinence. The DSM-5 has included craving as a symptom in diagnosing substance use disorders, which may increase attention to craving research [8-10].

Most addictive drugs, including nicotine, enhance dopamine release in the mesolimbic “reward” pathway [11]. Nicotine then mediates reinforcement from smoking via dopaminergic transmission, which is significant in schizophrenia as dopamine-mediated responses to rewarding stimuli may be abnormal [12]. This phenomenon, thought to be related to reward circuitry deficits, may be further complicated by the dopaminergic antagonism of antipsychotics. Together, these factors may affect craving and/or smoking behavior in SWS. Since persons with schizophrenia are not only heavier smokers, but also less likely to quit smoking, additional research is needed to develop specific information leading to interventions for this population.

The proposed 2-week randomized double-blind comparison of adjunctive minocycline vs. placebo in tobacco smokers with schizophrenia is designed to assess potential effects of minocycline on craving and/or smoking behaviors in smokers with schizophrenia. This protocol is funded as a K23 grant for the principal investigator to develop data towards an R01 application to benefit smokers with schizophrenia.

Primary Study Aim:

To determine if treatment with minocycline will result in decreased craving for cigarettes

in SWS compared to placebo.

Hypothesis: Treatment with minocycline will result in decreased craving for cigarettes in SWS compared to placebo. Decreased craving will have effects on cigarette use and/or intensity of smoking in SWS, as defined by changes in cigarette intake, expired carbon monoxide (CO), and urine cotinine.

A published pilot study[13] in smokers without serious mental illness suggests that minocycline exhibits anti-craving effects. This proposed two week pilot study will measure the safety and efficacy of minocycline on craving intensity in SWS in response to triggers for craving presented in a VR environment. Measures of cigarette use (intake of cigarettes daily, CO, urine cotinine) will be used to analyze the relationship between cigarette craving and use.

Secondary Study Aim:

To determine the relationship between changes in craving, cigarette smoking intensity, and responses to individualized triggers of craving in SWS.

Hypothesis: Specific environmental triggers to craving will drive responsivity to changes in craving levels for SWS treated with minocycline. Changes in craving will coincide with changes in smoking intensity.

Data from this protocol will identify relationships between triggers, craving, changes in craving and its relationship to smoking intensity in SWS treated with minocycline or placebo. Data regarding the temporal association of craving and smoking intensity changes impart valuable information for further study, even if minocycline shows little evidence of effectiveness.

RESEARCH STRATEGY

Significance

Up to 60% -70% of persons with schizophrenia smoke cigarettes [1, 2] compared to roughly 20% of adults in the US general population. This issue is of great importance to public health, since persons with schizophrenia are three times more likely to initiate smoking, are more likely to be heavy smokers, and five times less likely to quit than people in the general population[14-17]. SWS are often highly nicotine dependent, with greater carbon monoxide boost and puffs per cigarette [14, 18, 19]. Smoking is known to cause physical health problems, and the risk of smoking-related fatalities is greater in schizophrenia than the general population[20]. Although cessation is an important issue in all populations, it is of increased relevance to clarify the factors involved with smoking in SWS and determine appropriate interventions for reduction and cessation.

Motivation to quit smoking is lower in SWS compared with normal controls [21]. There are many reasons why people smoke (environment, stress reduction, boredom, habit) and craving is a major issue that may incite relapse in smokers attempting cessation[22, 23]. Multiple cues or triggers may induce craving in smokers, and these may differ among populations and among individuals. Preliminary studies suggest that SWS may have differences in reasons for smoking and in the experience of craving. In addition, recent evidence indicates that most addictive drugs, including nicotine, enhance dopamine release in the mesolimbic “reward” pathway in the brain[11]. Growing evidence suggests that persons with schizophrenia have abnormal dopamine-mediated responses to rewarding stimuli, presumably due to deficits in function of mesolimbic reward circuitry[12].

Studies of cue induced craving reactions in smokers exposed to smoking paraphernalia under lab conditions have found that environmental cues (sight or smell of cigarettes) may trigger

strong craving urges even after the attenuation of nicotine withdrawal[24]. Environmental smoking cues have been shown to play an important role in the maintenance of nicotine addiction and in relapse to smoking. Studies have demonstrated greater increases in self-reported craving and changes in autonomic functioning when smokers were exposed to smoking cues compared with neutral cues[25]. Differences in craving experiences appear to exist between persons with severe mental illness and the general population. This potential issue is being addressed within the Principal Investigator's protocols funded by a K23 grant from NIDA. Thus, SWS may require specific interventions designed to address unique factors contributing to their craving.

Minocycline is a synthetic, FDA approved derivative of tetracycline with a more tolerable side effect profile than other agents in this class. Minocycline has been of increasing interest to the field of psychiatry in recent years, and several trials have investigated the efficacy of minocycline to reduce symptoms of schizophrenia [26-30]. All published studies showed favorable safety and tolerability profiles for minocycline use in this population.

Proposed Treatment Rationale:

Minocycline and possible effects on substance use and craving: Minocycline may affect craving, perhaps due to inhibition of nitric oxide (NO) formation, as NO acts as a second messenger for glutamate and dopamine receptors[13, 31]. NO also facilitates the effects of nicotine in the reward circuit, and blockade of NO has been demonstrated to eliminate nicotine abstinence symptoms in rats[32, 33]. A small study has demonstrated that minocycline reduces cigarette craving in human subjects without severe mental illness[13]. Although no published data exists with the use of minocycline as an anticraving medication in SWS, the small study described above in the non-mentally ill population has demonstrated the potential use of this medication. The results, which showed decreased craving, provides a rationale for use SWS, who seem particularly vulnerable to craving with potential for exaggerated craving responsivity. With preliminary data suggesting a strong craving response in SWS, and the knowledge that SWS are less motivated to stop smoking, developing a stronger understanding of craving and testing a novel anti-craving intervention will contribute valuable information for future larger-scale research. In a previous study of adjunctive minocycline use in persons with schizophrenia, we were able to pilot the use of this technology in 7 SWS through double blind 10 week pilot trial of minocycline or placebo where we assessed symptom changes as well as tobacco craving measures. Although the numbers of participants were too low to adequately address questions of statistical significance, we demonstrated that SWS were able to tolerate the medication and recruitment and execution of study is feasible. With preliminary data suggesting a strong craving response in SWS, and the knowledge that SWS are less motivated to stop smoking, developing a stronger understanding of craving and testing a novel anti-craving intervention will contribute valuable information for future larger-scale research. The study results from this protocol will provide preliminary data necessary to plan a larger study that may be funded through an R01 application. This study is exempt from requiring IND application per 21CFR312.2(b).

Methods

Study Overview

We will conduct a two-week randomized, double-blind, placebo-controlled, parallel group pilot study to investigate the effects of minocycline vs. placebo on craving and indicators of smoking intensity in SWS. Participants will be initiated at minocycline 50 mg PO BID (or matching placebo) for week 1 and then the dose will be increased to 100 mg PO BID (or matching placebo) for week 2. Participants will complete cigarette cue-elicited craving platforms and related assessments at baseline, and after 1 and 2 weeks of minocycline or placebo treatment, as well as measurements of cigarette use, expired CO, and urine cotinine at baseline and after 1 and 2 weeks of treatment. Prior to the study, all participants must meet inclusion and exclusion criteria including a physical exam and bloodwork.

Innovative virtual reality (VR) technology will be used to elicit cue-elicited craving in SWS, measured by self report and physiological responses. Use of multisensory VR to elicit craving is a novel approach in SWS with potential to more consistently elicit craving than traditional methods of studying cue induced craving by exposure to neutral of smoking associated objects. The VR platform is valuable in order to study social, environmental, and attentional cues that contribute to craving in SWS, and to further explore how environmental factors influence physiological and behavioral aspects of craving. VR environments are particularly useful in this setting as the exposure to cues will be standardized across subjects and very little variation may occur. SWS report a variety of triggers to craving, including environmental, social, and emotional factors. Laboratory experiments (including those with VR) designed to elicit craving are more likely to trigger environmental and socially cued craving. Therefore, it is important to determine whether SWS who identify emotional factors as primary craving triggers will respond to cue-elicited craving induced in the laboratory. This is an important aspect, as a generalizable method of craving elicitation is required to assess the outcome in craving prevention studies. I have led the MPRC Virtual Reality laboratory for the past four years and have served as PI for 3 separate studies using virtual reality in persons with schizophrenia, including piloting the use of a tobacco smoking cue-elicited craving platform. We have established safety and tolerability of virtual reality platforms for smokers with schizophrenia (Wehring et al., in preparation).

Inclusion/Exclusion Criteria

Yes	No	Inclusion Criteria
		DSM-IV diagnosis of schizophrenia or schizoaffective disorder
		Male or Female
		Age: 18 to 65 years
		Caucasian or Non-Caucasian
		Smoke at least 10 cigarettes daily or using nicotine-containing electronic cigarette/vaping 10 times daily with urine cotinine above inclusion threshold of ≥100 ng/ml.
		Urine cotinine level ≥ 100 ng/ml (NicAlert® reading ≥ 3)
		Agrees to wear a head mounted display (HMD) for up to 45 minutes
		Able to complete the Evaluation to Sign Consent (ESC) with minimum score of 80%

Yes	No	Exclusion Criteria
		History of organic brain disease
		DSM-IV diagnosis of Alcohol or Substance Dependence within the last six months (except nicotine)
		DSM-IV diagnosis of Alcohol or Substance Abuse within the last one month (except nicotine)
		Pregnancy or lactation
		Severe liver dysfunction (LFT 3X upper limit of normal)
		Previous known hypersensitivity to tetracyclines
		Current treatment with tetracycline or derivative
		Treatment with oral contraceptives (unless a second form of birth control is used and documented)
		Treatment with cholestryramine or colestipol
		Treatment with Urinary alkalinizers (e.g., sodium lactate, potassium citrate)
		Treatment with warfarin
		Treatment with bupropion, varenicline, or nicotine replacement products in the month prior to study inclusion
		Less than two months treatment of adjunctive medications AND less than one month on same dose: beta blockers, antidepressants, mood stabilizers, antianxiety medications.
		Medical condition whose pathology or treatment would significantly increase the risk associated with the proposed protocol.
		History of head injury, seizures, or stroke
		Positive urine toxicology screen for substances of non-therapuetic use prior to craving assessments
		Plan to implement smoking cessation within next three months

Schedule of events

Schedule of Events					
Encounter # →	1	2	3	4	5
Week of Study/Study Visit →	Screening	Baseline	Wk 1 (med initiate)	Wk 2 (dose increase)	EOS assess
Procedure ↓					
Informed consent	R				
Demographic Forms	R				
Patient Past Psychiatric and Medication History Form	R				
SCID-Interview	R				
Medical History	R				
Physical Exam and height	R				
SMAC incl gluc, lipids, CBC, UA, BUN, LFT	R (or base)				

Cotinine and biomarkers for smoking (blood)	R (or base)				R
Urine Pregnancy Test (female subjects)	R	R		R	
Expired CO	R	R	R	R	R
DAUS	R				
Cigarette use form	R	R	R	R	R
Fagerstrom Test for Nicotine Dependence		R			R
Practice VR session		R			
**Craving paradigm (assessments include Cigarette Craving Visual Analogue Scale, Tobacco Craving Questionnaire Short Form, Questionnaire for Smoking Urges- Short Form, Triggers to Smoke, Wisconsin Withdrawal Scale, and, physiological measures including blood pressure, heart rate, temperature, skin conductance, urine toxicology		R		R	R
Brief Psychiatric Rating Scale	R	R	R	R	R
Scale for the Assessment of Negative Symptoms	R	R	R	R	R
Side Effect Checklist	R	R	R	R	R
Monitoring for Skin Pigmentation			R	R	R
Pill Count				R	R
Urine toxicology	R or base	R or screen			

**separate schedule as below

Craving paradigm schedule of assessments (performed at base, med initiation study visit, after first week of drug study visit, and after 2nd week of drug/EOS visit)

	Pre Session	Post Cue 1	Post Cue 2	Post Cue 3	Post Cue 4	Post Session
TCQ-SF	X					X
QSU-Brief	X					X
SEC	X					X
CCVAS	X	X	X	X	X	X
Physiological measures (NOT IN DB)		X	X	X	X	
Triggers to Smoke	X					
Wisconsin Withdrawal Scale	X					
BPRS	(will be done at beginning of study visit)					X

Assessments for primary outcome measures:

Primary Assessments
Cigarette Craving Visual Analogue Scale (CCVAS), Questionnaire of Smoking Urges-Brief (QSU-Brief) and Tobacco Craving Questionnaire-Short Form (TCQ-SF): During the Cue trials the Cigarette Craving Visual Analogue Scale (CCVAS) will be administered to measure cigarette craving. The CCVAS will be projected into the VR environment and participants will respond via hand controller.

Secondary Assessments
Secondary assessments include The Wisconsin Inventory of Smoking Dependence Motives (WISDM-68), Wisconsin Smoking Withdrawal Scale (WSWS), Fagerstrom Test for Nicotine Dependence (FTND), the Drug and Alcohol Use Survey (DAUS), Smoking History Form, questions regarding cigarette and cue preferences, which will be given during the training/adaptation session, and the Mood form which will be administered at baseline and following the cue session, the Brief Psychiatric Rating Scale (BPRS), Schedule for the Assessment of Negative Symptoms (SANS), and Side Effect Checklist (SEC), which will be administered prior to the VR session. The physiologic monitoring of heart rate, blood pressure, and skin conductance) will occur continuously during the session. The Triggers for Smoking (TFS) form will be used to identify self-asserted triggers for smokers, divided by emotional, social, and environmental factors.

Fagerstrom Test for Nicotine Dependence: The FTND is a measure of nicotine dependence[34].

Drug and Alcohol Use Survey: The DAUS will be used to determine usage of substances of abuse.

Tobacco Craving Questionnaire-Short Form: The TCQ-SF is a reliable and valid 12-item self-report instrument that assesses tobacco craving in four dimensions: emotionality, expectancy, compulsivity, and purposefulness[35].

Questionnaire of Smoking Urges-Brief (QSU-Brief): This is a 10-item assessment used to measure craving to smoke and used in studies of smokers with schizophrenia[36].

Wisconsin Smoking Withdrawal Scale (WSWS): The WSWS is a 28-item self-report scale with a highly reliable 4-item craving subscale[37].

Wisconsin Inventory of Smoking Dependence Motives (WISDM-68): The WISDM-68 is a scale with 13 subscales that assesses motives of smoking dependence (Piper et al., in press)

Cigarette Craving Visual Analogue Scale (CCVAS): This is a 4-item scale used to assess, on a Likert Scale (0-100), the amount of craving for cigarettes, attention paid to cigarette sight, attention paid to cigarette scent, and the amount of thought devoted to cigarettes at given

moment in time.

Assessments for Other Psychiatric Symptoms and Safety:

Brief Psychiatric Rating Scale (BPRS): The BPRS is an 18-item scale which measures positive symptoms, general psychopathology and affective symptoms. The BPRS will be used to assess for symptom exacerbation during the experimental session.

The Schedule for the Assessment of Negative Symptoms (SANS): Scale for the Assessment of Negative Symptoms: the SANS total score, minus the global items, inappropriate affect, poverty of content of speech, and attention items, will be used to measure negative symptoms.

Side Effect Checklist (SEC): The SEC will be completed at all study visits, and before and after the experimental sessions. Adverse effects will be defined as follows: a score of 4 on any item, unless the item is 4 at baseline, or an increase of 2 on any one item from baseline score. This will include side effects specific to minocycline use.

Clinical Global Impression Scale (CGI) [38]: the CGI severity of illness and change scores items will assess severity of illness and global changes**.**

Laboratory Measures: Participants will undergo blood draw at baseline to ensure physical health to be included in the study. Each will have a CBC, and laboratory assessments for liver enzymes and renal function (BUN/creatinine). We will also measure fasting glucose, total cholesterol, HDL, and LDL. Blood will also be taken to quantify smoking status (baseline and end of study) and measure potential for biomarkers for propensity to craving responsivity (baseline only). An estimated total of 13.5 teaspoons of blood will be taken, total, for this study. In addition, urine will be collected during screen/baseline and at study visits when craving assessments are performed to measure urine cotinine as well as screen for substances of abuse.

Study Procedures and Dosing:
Minocycline Dosing:

Minocycline (Dynacin® or generic) will be available in 50 and 100 mg capsules (not pellet filled dosage form; i.e. Minocin®). There will be matched placebo-minocycline capsules for each minocycline capsule strength. Study medication will be dispensed on a weekly basis. They will be given two extra days of medication in case of a missed appointment. Inpatient subjects will receive their study medication daily from the nurses in the Spring Grove Hospital Center Treatment Research Program. During the first week subjects will receive one 50 mg capsule BID (minocycline 100 mg total or matching placebo) and during week 2, subjects will receive 1-100 mg capsule BID (minocycline 200 mg total or matching placebo). This dose is based on the dose from published double blind studies in persons with schizophrenia which showed excellent safety and tolerability profiles, including one 6-month study in which only 4 patients had side effects (11%) and were discontinued from the study [39] including our 10 week clinical trial in schizophrenia patients[28]. Many tetracycline preparations have chelation with calcium and iron. This is not a significant concern with minocycline; it can be given without regard to meals. Standard dosing will be requested to occur at approximately 9:00 am and 9:00 pm. Patients will

be instructed to avoid OTC medications such as antacids or Pepto-Bismol that may impede absorption of minocycline within 2 hours of dosing.

Concomitant Medications:
Subjects may remain on other medications not excluded from the protocol. In order to decrease the likelihood that any observed change in tobacco craving or smoking habits during the study is related to one of these adjunctive treatments, a subject must have been on the adjunctive medication(s) for at least 2 months and on their current dose(s) of adjunctive medication(s) for at least 1 month prior to study participation. The dose of adjunctive medication will remain fixed throughout the conduct of the study, except for required side effect-related adjustments (e.g., in anticholinergic or β-blocker agents). Although not expected for this short-term, non-symptom-related study, if at any time throughout the study the subject's condition worsens (as defined below), the subject may be withdrawn from the study.

Procedures/Methods:
This study will utilize the cue-elicited VR platform and a standard box mechanism cue-reactivity platform to examine the effects of smoking versus neutral cues on measures of cue-elicited reactivity in smokers treated with minocycline or placebo. In the randomized box mechanism cue elicited craving platform participants open a box which contains either a pencil and sharpener (neutral) or a lighter and cigarette (smoking cue) and handle these objects. Cigarette cue-elicited craving platforms, and measures of cigarette intake, will be performed at baseline, the end of week 1, and the end of week 2.

In addition to the measures used in Aim 1, cigarette intake will be measured by self-report, urine cotinine levels, and expired carbon monoxide levels will be collected. Additional safety measures include monitoring for side effects of minocycline (see below risks of minocycline). During the VR Cue trials (as used in Aim 1) the CCVAS will be administered at baseline, after each cue session, and after the experimental session. The TCQ-SF/QSU Brief will be administered to measure cigarette craving at baseline and after the session. During the experiment participants will be monitored for physiological reactivity (heart rate, blood pressure, and skin conductance response) to cues of smoking as well as neutral cues measured. During the standard cue-elicited craving platform, participants will be administered a CCVAS at baseline after a cigarette, 15 minutes after the cigarette, and after each cue (neutral, smoking) as well as after the experimental session, and will be monitored for physiological reactivity to cues of smoking and neutral cues. Participants will be exposed to each and will repeat the session after 1 and 2 weeks of treatment. Mixed model analysis of covariance will be used to estimate the difference in magnitude of cue-induced craving and physiological responses in the minocycline versus placebo groups at Week 2, adjusting for corresponding cue-induced responses at baseline (pre-drug) session. Smoking intensity will be measured via cigarette intake, urine cotinine, and expired CO levels at baseline, Week 1, and Week 2.

Study Procedures for Virtual Reality Cue-elicited craving sessions:

General procedures. The general procedures for this study are modeled after the design utilized by Bordnick and colleagues (2005) in their study of virtual reality cue reactivity in cigarette smokers, in addition to pilot studies performed by this PI and research group in smokers with schizophrenia. Participants will be allowed to smoke ad libitum before study sessions. Women will have a urine pregnancy test done before each session. If a pregnancy

test is positive, the participant will be dismissed from the study because smoking is toxic to fetal development. Pregnant women will be offered information regarding smoking cessation.

Training/adaptation session. A training session will take place prior to the baseline study session so participants can be familiarized with the equipment and assesed from potential side effects of VR immersion (headache, dizziness). An adaptation session will follow during which participants will be familiarized with the HMD and hand controllers. Subjects will be informed to immediately report any nausea, dizziness, headache, or other physical or psychological discomfort durng the session. Subjects will be asked the standardized question “Do you feel comfortable with the use of the virtual reality equipment?”. Subjects expressing discomfort will discuss this with the study personnel and will be offered relaxation tapes. Participants who continue to express discomfort after relaxation tapes and further discussion will be removed from the study.

Experimental sessions. There will be a baseline and endpoint experimental session for the craving endpoints in the primary aim. Pre-gelled electrodes will be placed at this time for physiological and skin conductance measurements. All participants will smoke one cigarette of their choice and then take a 20-30 minute break prior to the virtual reality program. Participants will complete the CCVAS after each cue exposure using a game pad immediately upon completion of the experimental session; physiological measures will be recorded continuously. Participants will complete the TCQ-SF, QSU-Brief, CCVAS, Mood form, and SEC at the end of the cue session.

VR Cue-reactivity Assessment (VRCRA). Participants will be asked to put on the VR helmet and make adjustments for comfort. The VR helmet is equipped with two-way audio and with a monitored video display. Participants will hold a hand controller in their dominant hand. The VR-NCRAS program will be started and participants will enter a relaxation phase for 5 minutes. During this time, subjects will be immersed in a dark room while listening to music. After 5 minutes, the VR-NCRAS will move each along a preprogrammed path to the next stimulus. At the start of the VRCRA session, participants will complete the CCVAS.

Each participant will then experience a neutral cue room, two smoking cue rooms (one inanimate smoking cue room and the other a social interaction party scene room involving smoking) ending with a final neutral cue room. Subjects will experience cues in order of Neutral- Smoking- Smoking- Neutral. The order of smoking cue presentation will be the inanimate smoking room followed by the social interaction smoking room. Each room provides 3 minutes of exposure that will include timed pauses in front of stimuli that allow viewing of each relevant stimulus or social interaction. After each cue room, participants enter a questionnaire room and rate their craving intensity on a visual analog scale (VAS) by using the game pad to select a position along a line anchored on the left by “not at all “ and on the right by “more than ever”. The CCVAS is integrated into VR to help maintain immersion in the VR experience. The entire VR NCRAS session will be computer guided. The experimental session of VAS will take 26 minutes. The VR-NCRAS neutral cues consist of nature based video scenes with accompanying audio arranged in a room. At preprogrammed interval, participants will be guided to each nature scene. The VR NCRAS smoking cues include a room that contains smoking paraphernalia (i.e., burning cigarettes, cigarette packs, a bar and percolating coffee pot) and a separate room that consists of a party setting. The party setting allows participants to interact

with people in VR who are smoking, drinking and who offer a cigarette to the participant. The experimenter controls the VR social interactions using a keyboard interface. Each social interaction will occur between participants and video of people inserted into the three dimensional world. Responses from the VAS, as well as physiological and skin conductance measures, will be captured by computer and downloaded to the study database. Upon completion of the VRCRA session, participants will be debriefed as described below. The schedule of the VRCRA session is depicted in Table 1.

Table 1. Virtual Reality Cue Exposure laboratory schedule

Time (minutes)	Activity
Arrival 00:00 – 10:00	Equipment setup Placement of electrodes Relax
10:00 – 11:00	Begin VRCE session Baseline Assessment
11:01 – 14:00	Neutral Cue Room exposure
14:01 – 15:00	Assessment 1
15:01 – 18:00	Smoking Cue Room 1 exposure Smoking paraphernalia
18:01 – 19:00	Assessment 2
19:01 – 20:00	Smoking Cue Room 2 exposure Social Interaction
20:01 – 21:00	Assessment 3
21:01 – 24:00	Neutral Cue Room exposure
24:01 – 25:00	Assessment 4
25:01	End of VRCE Session

Virtual Reality Equipment: A virtual reality nicotine cue reactivity assessment system (VR-NCRAS) already developed and used in studies of nicotine dependent subjects (without mental illness) will be used for the experimental session. Disposable pre-gelled skin conductance electrodes will be attached to the index and ring finger of the nondominant hand. Participants will be seated in a nonreclining chair. The skin conductance electrodes will be hooked up to Biopac monitoring equipment and monitoring of skin conductance response will begin. The Biopac Physiologic monitor will be used to continuously monitor blood pressure, heart rate, and skin conductance via arm cuff, and data will be collected and downloaded via computer to the study database. Participants will wear the HMD (NVIS Sx high resolution display head set) and will be provided with a game pad. Using a custom interface the researcher will select the experimental path, brand representation/type of cigarettes, presence of alcoholic beverages and gender of person offering cigarettes. An olfactory system is also included to release scent based upon cues in the virtual environment (smell of cigarette smoke, food, etc.).

Debriefing

At the conclusion of the last experimental session, subjects will be allowed to smoke one cigarette. Following this break, the PI, MAI, or other qualified research staff with bachelor level or higher degrees and significant clinical experience and training will meet with participants to

answer any questions they might have about the study. This will also serve as an opportunity to intervene with respect to the participant's tobacco dependence. The investigator will counsel the participant on the risks of continued smoking and the benefits of quitting and give them a referral list of smoking treatment programs. Subjects will be assessed for any increase in psychotic symptoms. If the BPRS Positive symptom scale increases by one point or greater, or subject reports distress or discomfort, relaxation tapes will be offered along with further interaction with the investigator. Subjects will be reassessed at the conclusion of the relaxation tape for need to further intervene. If the participant expresses ongoing distress, the participant will be offered additional relaxation tapes and discussion for a period of at least twenty minutes. If the participant still expresses distress, he or she must be seen by the PI or coinvestigator for further evaluation and intervention.

Data Analysis:

All data analysis will be conducted on an intention to treat basis including all available data from patients with at least one post baseline measure, using methods (e.g. mixed models for incomplete repeated measures) that allow analysis of data for participants with missing visits. Exploratory analyses will be conducted using mixed models for repeated measures analysis of covariance to explore the effects of minocycline versus placebo on subjective dimensions and physiological craving responses to smoking cues presented in a VR environment. For each craving measure we will fit the model: follow-up craving measurement = baseline craving measurement + minocycline week 1 + placebo week 1 + minocycline week 2 + placebo week 2, where the variables "minocycline week 1," etc. are yes/no indicators marking the week the measure was collected and the participants treatment group. The model will be fitted without an intercept to make the parameters identifiable, and I will estimate the following contrasts of the parameter estimates: minocycline week 1 - placebo week 1, minocycline week 2 - placebo week 2, ½[(minocycline week 1 - placebo week 1) + (minocycline week 2 - placebo week 32)] and (minocycline week 1 - placebo week 1) - (minocycline week 2 - placebo week 2). These define, respectively, acute (1 week) effect of minocycline on craving, longer term (week 2) effect of minocycline on craving, average (over time) effect of minocycline on craving (treatment main effect) and change from week 1 to week 2 in effect of minocycline on craving (treatment x week interaction), which may reflect minocycline dose escalation after week 1. For each craving response, we will calculate these effect estimates, together with 95% confidence intervals, to assist in planning future studies of minocycline as an adjunct treatment to supplement smoking cessation interventions. For the secondary aim, descriptive statistics (mean, median, s.d.) will be calculated by treatment group on the magnitude of craving change from the start of the study in participants reporting each trigger type experience during at weeks 1 and 2, both at pre-VR baseline each week and when presented with environmental or social cues during the VR sessions. Even if minocycline has no effect on craving, data regarding the relationship of craving and smoking intensity across time will impart valuable information for further study. Baseline and end of study data in all subjects (n=30) will be used to estimate Spearman correlations between measures of smoking intensity and craving; Fisher's z-transformation will be used to calculate confidence limits on these correlations. There will be power>0.8 to detect correlations ≥r=0.5. Both cross-sectional correlations between craving and smoking at baseline and correlations in changes in craving level and smoking will be examined. As noted above, treatment effects on concurrent treatment-related changes in craving and smoking intensity will be estimated. In order to get 30 subjects that will complete the study procedures, 60 participants

will sign consent documents.

Power for primary outcome: As is common in pilot studies, there will be adequate power to detect only strong effects; power will depend on the strength of the within-subject correlation, r, between baseline and follow-up measures. For example, to detect a treatment differences at week 2, with n=13 per group (≈15% dropout), we would have power=0.80 to detect an effect size of d=0.92 for r=0.6, or d=0.82 for r=0.7. Smoking intensity will be measured via cigarette intake (self report), urine cotinine, and expired carbon monoxide (CO) levels at baseline, Week 1, and Week 2; treatment-related changes in these measures will be estimated using models similar to those outlined above.

Recruitment

Patients with schizophrenia will be recruited by MPRC through their patient recruiting network including the Treatment Research Program and the Outpatient Research Program and affiliated sites.

ResearchMatch.org will be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University as an IRB-approved data repository.

Medication Adherence

Patients receiving >75% of their assigned medication will be considered adherent. Outpatient adherence will be monitored through weekly pill counts and subject interviews. Medications will be dispensed on a weekly basis and will only be dispensed after adherence is assessed and all other assessments are completed. Inpatient adherence will be monitored through inpatient medication records. If a patient is observed to have an adherence problem, then this will be discussed with the patient and a plan formulated to bring the patient back into adherence.

Randomization and Blinding

Treatment assignment will be random with equal numbers of subjects assigned to each treatment group. Subjects will be randomly assigned to minocycline or placebo-minocycline using a permuted block randomization system with separate randomization sequences for inpatients and outpatients. Treatment assignment order is random within each block, and an equal number of subjects are assigned to each treatment within a block. The block sizes will vary in random sequence and randomization will be stratified for inpatient/outpatient status. Thus, it will be difficult to ascertain the next treatment assignment, even if a subject becomes unblinded, while the imbalance of numbers between the treatment groups is kept within tight limits. Randomization requests are sent to the study statistician, who sends a coded treatment assignment to an unblinded pharmacist. Separate emergency unblinding envelopes for each subject will be kept in a locked cabinet at each dispensing pharmacy. All raters, investigators and other staff will be blind to treatment assignment except for the pharmacist. The pharmacist does not participate in study assessments and conveys no information about treatment assignment to subjects or staff except in a medical emergency.

Sources of Materials

All records of the research will be kept in locked files with subjects' evaluation study materials

identified by code only. A separate file will hold the code key. Patients will not be personally identified in any publications or reports of the study. All blood specimens drawn for research purposes will be identified at the laboratory by code only. Whenever possible, existing clinical laboratory data will be used as a source of information for the study, which will minimize testing done on subjects. Any data used will be recopied to research files with the subject identified by code only. The highest standards of subject confidentiality will be kept and no subjects will have identifiable information available. Computerized records of data are kept in password-only accessible computer in a locked room. Appropriate firewalls and protections of computerized data are maintained to ensure that entry by those other than research personnel is not possible.

The principal investigator or designated research staff will conduct the final consent interview with all subjects. All participating research staff will undergo documented HIPAA training and all study records and data will be monitored for HIPPA compliance throughout the study. As previously stated, all laboratory data and records will be identified only by code numbers and kept in a locked cabinet. The PI and Medically Accountable Physician will be available to handle any emergencies or adverse reactions. When medically indicated, the patient will be sent to University Hospital for emergency and/or continued care. All adverse effects will be reviewed by the Data and Safety Monitoring Board (see below) and all Serious Adverse Effects will be reported to the IRB as per their specific requirements (see below).

Data will be entered into a Microsoft Access database. All forms are identified within this database by a unique participant ID number, participant initials, and the protocol ID number and acronym, together with the protocol phase and visit. The protocol data collection schedule is kept in a data base table and is used to monitor the progress of patients through the protocol, missing assessments, and other protocol deviations during the study. Data entry screens incorporate range checks or lists of valid responses for each item. Forms with missing or invalid data in key identifying fields are referred back to raters for correction before entry. Patient confidentiality is maintained by restricting study data access to specified study personnel. Most authorized personnel will have read-only access, and write/edit access will be restricted to data entry and data management staff assigned to the study. An electronic audit trail records all changes to the data base once data have been entered. The database resides on a central server, and all server data are backed up several times weekly. Access to the server from outside the MPRC is restricted by a firewall. Norton Anti-Virus software, updated automatically whenever new virus data files are provided, is installed on the server and all PCs linked to the server. Development of the data management system for this study will be facilitated by the existence of this data base structure, which already contains tables and data entry screens for many of the assessments used in this study.

Potential Risks

Risks of the study include: transient increase in craving for cigarettes, skin irritation at site of physiological monitoring leads, bruising at the site of blood draw, boredom or uncomfortable with questions or assessments, side effects of virtual reality (dizziness, headache, nausea, motion sickness) and privacy of information concerns.

Potential risks from the minocycline may include GI side effects (nausea, vomiting, diarrhea, loose stools, anorexia, and flatulence), dizziness, drowsiness, headache, fatigue and tinnitus. Side effects will be assessed weekly. Photosensitivity has occurred with tetracyclines, however

photosensitivity with minocycline is usually not reported. All subjects in the study will be asked to wear sunscreen when going outside during the two weeks of intervention. Vaginal candidiasis may occur with antibiotic treatment. A maculopapular, morbilliform or erythematous rash has occurred in rare instances. This will also be assessed at each visit. Increases in blood urea nitrogen (BUN) and liver enzyme elevations (LFT) have also been reported. The most frequently occurring side effects are nausea and dizziness. Antibiotic resistance to tetracycline derivatives has been identified however tetracyclines have been widely used due to their low toxicity and broad spectrum of activity. This class of medications is used in the long term for acne vulgaris. Rare but serious side effects include: esophageal ulcers, exfoliative dermatitis, or acute renal failure. In the rate event an infection arises during the course of minocycline, antibiotics other than tetracyclines or penicillins (possible drug interaction) will be prescribed.

Skin pigmentation is an infrequent reported side effect of minocycline and the subjects in the study will be examined by study clinician weekly for any notice of skin changes. The patient will be asked to watch for any discoloration of the skin particularly in areas of scar tissue. There are 3 types of skin pigmentation that may occur. Type 1, is the most common, is reversible and consists of blue-black macule in areas of scarring or inflammation. Type 2 consists of well circumscribed blue-grey pigmentation on the shins and forearms, also reversible. Type 3 is rare and composed of a diffuse muddy brown discoloration accentuated in sun-exposed skin. It may persist indefinitely. The reported incidence of type 1 pigmentation ranges from 2.4-14.8% based on long-term studies. Types 2 and 3 are known to be associated with long term administration (6 months – 4 years) and high cumulative doses (>100 grams)[40]. Many long term studies have recently been completed (12-24 months) for various disorders and they all report rates of discontinuation due to skin pigmentation to be very low (i.e., <5%)[41-45]. In our recently completed 10-week study of minocycline compared to placebo for adjunctive treatment of schizophrenia symptoms, four (14%) participants assigned minocycline and 2 (9%) assigned placebo were noted to have skin pigmentation or skin discoloration during the study (p=0.54)[28]. The potential for skin pigmentation will be taken seriously and proactively and will include dermatologic consultation if needed. Skin will be examined weekly, however, we anticipate few or none to have any pigmentation during this short-term (2 week) exposure, and most likely type 1(reversible, mild and present on scar tissue). The likelihood of serious adverse events occurring is very low. It is not expected that other unexpected frequent side effects would occur. Females of childbearing age will agree to the use of medical approved birth control, which includes condoms, diaphragms and intrauterine device. Pregnant and lactating females will be excluded. Oral contraceptives are excluded, unless there is a second documented form of birth control, as minocycline may decrease the efficacy.

Protection Against Risks

Informed Consent

All participants must be judged capable of signing informed consent and must give their consent to participate in the study. Patients will be given a signed copy of the consent form and a copy will be placed in their research file. We pay particular attention to the consent process in the population of persons with schizophrenia. The Evaluation to Sign Consent (ESC), which tests understanding of study procedures, study risks and right to refuse consent and/or withdraw without affecting normal treatment, prior to signing consent. The consent procedure is usually a process. Dr. Carpenter, former Director of our Center has had several grants in research ethics and we have found previously that the ESC is a valid and reliable instrument to assess

understanding of the study procedures and risks. All research staff are trained in great detail on the ESC and consent process.

Clinical Worsening and Side Effects
If clinical worsening would occur, although unexpected in this short-term, non-symptom-intervention study, the subject will be discontinued from the study. Worsening is in the opinion of the treating psychiatrist and PI and may be defined as the following: worsening of symptoms (relative to the baseline BPRS, an increase of 3 or more points on a positive symptom item OR an increase of 2 or more on the CGI global severity OR the subject is judged to be entering an exacerbation of his/her illness by the treating clinician).

If adverse events occur, clinical judgment will be used in following standardized steps for possible rechallenge, with results recorded as part of physician judgment of the probability that an event is linked to the experimental treatment (drug or placebo). Once a participant is discontinued from the study, completion ratings will be performed if possible.

These studies are not expected to be associated with clinical worsening. The PI as well as the medically accountable investigator (MAI) will be available to handle any emergencies or adverse reactions. When medically indicated, the patient will be sent to University Hospital for emergency and/or continued care. If clinical worsening would occur a participant will be discontinued from the study. If adverse events occur, clinical judgment will be used in following standardized steps for continuing study participation, with the results recorded as part of medically accountable physician judgment of the probability that an event is linked to the experiment. Once a participant is discontinued from the study for whatever reason, completion ratings will be performed if possible.

Data storage and participant confidentiality
All records created during research will be kept in locked files with participants' evaluation study materials identified by code only. A separate file will hold the code key. Participants will not be personally identified in any publications or reports of the study. All specimens (blood, saliva, urine) drawn for research purposes will be identified at the laboratory by code. Whenever possible, existing clinical laboratory data will be used as a source of information for the study, which will minimize testing done on subjects. Any data used will be recopied to research files with the subject identified by code only. The highest standards of participant confidentiality will be kept and no participants will have identifiable information available. Computerized records of data are kept in password-only accessible computer in a locked room. Appropriate firewalls and protections of computerized data are maintained to ensure that entry by those other than research personnel is not possible.

Potential Benefits of the Proposed Research to the Subjects and Others
The study may not benefit the participants themselves, or participants in the minocycline arm may experience a decrease in craving for cigarettes. The knowledge obtained by this research will be of great importance to further the study of craving in persons with schizophrenia with the ultimate goal of contributing to the development and assessment of interventions designed to attenuate craving and aid in smoking cessation and abstinence efforts.

Importance of the Knowledge to be Gained

The importance of the knowledge gained outweighs any risks that may occur in this study. There is little information available regarding the assessment of craving in persons with schizophrenia, especially since pilot data have suggested differences in craving between schizophrenia and the general population. The potential to utilize a systematically tested mechanism for cue-elicited craving, and to test a novel medication, minocycline, on craving and effect on cigarette intake, will be valuable for future research. In addition, the collection of pilot data will lead to a more concentrated effort to discover interventions for craving and smoking cessation.

Data and Safety Monitoring Plan

This study will use a Data and Safety Monitoring Board (DSMB). The MPRC DSMB consists of two research/practicing psychiatrists; one community psychiatrist; one internal and one external biostatistician; and one research pharmacist.

The MPRC DSMB will:

1) review proposed protocols and consent forms prior to the start of enrollment to assess how the PI will report adverse events, early withdrawals and terminations
2) evaluate recruitment and rate of enrollment in relation to study targets
3) monitor the occurrence of adverse events, serious adverse events, and early withdrawals or terminations
4) evaluate study outcomes, when available
5) monitor studies throughout their progress

Meetings: Once a protocol has been initially reviewed, the DSMB will determine the frequency of review for each study, at minimum yearly.

Meeting Format: The DSMB will have a standard quarterly meeting. Depending on the need, the DSMB will provide an interim review of a study with a conference call to the PI to facilitate the start of a study. The principal study investigator will prepare a protocol summary, which will be sent to the DSMB Coordinator two weeks prior to the meeting.

Study Monitoring: If as a result of data monitoring or interim analysis, the DSMB determines that a trial (a) has answered the primary study question, (b) will not be able to reach a firm conclusion, (c) is not being conducted according to high scientific or ethical standards, or (d) poses an unreasonable or unnecessary risk to study participants, the DSMB will recommend to the principal investigator that the study be amended, temporarily suspended or terminated as appropriate. If in the Board's judgment, safety concerns need to be conveyed to the MPRC Director and the IRB immediately, they will do so.

Interim Analysis: An interim, unblinded analysis may be conducted in response to unusual patterns of side effects, response, or recruitment difficulties. In these rare circumstances, the study biostatistician will prepare a study data Closed Session report and forward it to the MPRC DSMB Coordinator to send to the DSMB members prior to the meeting. These reports will contain the most up-to-date data permitted by the timeframe necessary for the study biostatistician to prepare and review the analyses.

Communication of DSMB Recommendations: At the conclusion of each DSMB meeting, the Board will provide a verbal report to the principal study investigator indicating areas of concern regarding performance and safety. The DSMB will not communicate any information that could lead to the unblinding of investigators or suggest interim treatment-specific results. The minutes from the DSMB Board will be sent to the principal investigator documenting (a) the date of the review, (b) that all relevant interim safety and efficacy data were reviewed, (c) recommendations concerning the study execution or modifications to the study protocol, and (d) the anticipated date of the next review. The PI must provide a copy of the DSMB minutes to all oversight IRBs or other governing bodies at the time of their regularly scheduled renewal or sooner if modifications are requested by the DSMB. Should the DSMB require additional information from the PI after a review, the PI must provide a formal written response to the DSMB and submit it to the DSMB Coordinator who will forward the document to the Board Members.

Serious Adverse Events: A serious adverse event (SAE) is defined as any untoward medical outcome occurring in the context of the conduct of research, that:

- results in the death of the participant
- is life threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- results in a congenital anomaly/birth defect
- requires intervention to prevent permanent impairment or damage

The PI and Medically Accountable Physician will receive all SAE reports within 24 hours of their occurrence. All serious adverse events (SAEs) will be reported to the DSMB, PI the University of Maryland School of Medicine IRB, and the appropriate NIH program officer. Any SAE that occurs to a subject enrolled in a study in which the MPRC DSMB is the DSMB of record, must be reported to the DSMB within 5 working days; or within 48 hours if it is directly related to the research intervention. The University of Maryland School of Medicine IRB will receive the reports within their required time frame, dependent of the nature of the event. NIDA will receive SAE reports within 72 hours of occurrence in accordance with regulation. If the incidence of any side effect/adverse event is 25% or more or any SAE occurs in excess in either treatment group, then the DSMB will notify the PI. The PI, coinvestigators, and DSMB will determine whether possible protocol modifications are required to minimize the further occurrence of such events.

Payment

Participants will receive $25 each for screening visit and visit 4. Participants will receive $50 each for visits 2, 3, and 5, which will involve more time spent for study interventions Participants will be paid by check or as per MPRC protocol. Checks are disbursed monthly.

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