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Impact of Nicotine Messaging on Beliefs and Behavior

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Study Protocol

The current study tested the impact of multiple exposures to a brief nicotine corrective messaging intervention and nicotine content of study cigarettes on nicotine beliefs and subsequent use of tobacco and nicotine products in a 28-day lab-based study of 74 adults who smoke cigarettes daily. Data were collected at the University of Pennsylvania Center for Interdisciplinary Research on Nicotine Addiction. A parallel population-based study of exposure to nicotine corrective messaging was conducted in adult smokers and non-smokers followed for 12 weeks (NCT04805515).

The lab-based study was a prospective, randomized factorial trial of nicotine corrective messaging (NCM vs. control) and nicotine content of cigarettes (normal vs. reduced) in current daily adult cigarette smokers (minimum 5 daily cigarettes for the past year, carbon monoxide (CO) at least 5 parts per million at Week 0, and no current use of other tobacco or nicotine containing products) for a 35-day protocol. Recruitment occurred through community and campus bulletin boards, local newspapers, radio and television ads, and previous participants who agreed to be re-contacted for non-cessation or non-treatment studies. At the baseline visit (Week 0), eligible, consenting participants provided a CO sample, complete demographic and smoking history measures, and measures of nicotine beliefs and intentions/use of nicotine and tobacco products (see Study Measures). They also provided breath and urine samples for biomarker assessment and continued to smoke their usual brand cigarettes to assess their baseline cigarette smoking behavior. Participants were instructed to continue smoking their own brand cigarettes and collect used filters as a verified count of daily smoking using re-sealable bags provided, while also recording their smoking in a calendar. Participants returned on Day 7 (Week 1; end of baseline), returned used cigarette filters, and were randomized to one of four groups: 1) Nicotine Corrective Messaging (NCM) + RNC cigarettes, 2) NCM + normal nicotine content (NNC) cigarettes, 3) Control + RNC cigarettes, or 4) Control + NNC cigarettes. Participants receiving normal nicotine content or reduced nicotine content cigarettes were explicitly told which product they have been given (i.e., unblinded).

The Nicotine Corrective Messaging (NCM) intervention condition was based on messages tested in our team's pilot study.[1] It included six original messages and two new messages addressing nicotine in cigarette and e-cigarette products that were adapted from several evidence-based sources to be more accessible to a lay audience. The sources consisted of FDA's 2017 comprehensive plan for tobacco and nicotine regulation,[2] FDA's 2013 modifications to labeling of NRT products for over-the-counter human use,[3] the 2014 U.S. Surgeon General's Report on the Health Consequences of Smoking,[4] reports on carcinogens from the International Agency for Research on Cancer,[5-7] and the NASEM report on the "Public Health Consequences of E-cigarettes.[8] Participants in the NCM condition were exposed to all eight messages in the same order at each exposure.

Participants in the NCM condition received their first exposure to the study messages on Day 7 in the lab. Eye tracking was assessed during a five-second exposure to each educational message, equating to a minimum 40-second exposure. At the end of the session, all participants left with study-provided cigarettes to last until the next visit. Participants continued to collect used filters and record daily number of study cigarettes, non-study cigarettes, and other nicotine or tobacco products used each day for the duration of the study. At lab visits on Days 14 (Week 2), 21 (Week 3), and 28 (Week 4), daily nicotine/tobacco use calendars and used filters were collected at the start of the visit. At the Week 2 visit, all participants completed the first post-exposure measures of nicotine beliefs and intentions/use of nicotine and tobacco products. In visits at Weeks 2-4, participants smoked their study product with CO collected at the start and each smoking session videotaped to extract smoking

behavior. Participants in the NCM conditions were then be exposed to the nicotine corrective messages in the lab and eye-tracking data collected. At the end of the session, all participants left with study-provided cigarettes to last until the next visit, collect used cigarette filters, and asked to track their nicotine and tobacco use via their calendars. The Week 5 visit (Day 35) began with the collection of daily nicotine/tobacco use calendars and used filters and include the final assessment of nicotine beliefs and intentions/use of nicotine and tobacco products in all participants. Participants also provided breath and urine samples for biomarker assessment. Upon completion of assessments, participants in the control conditions (control + NNC cigarettes, control + RNC cigarettes) were exposed to the nicotine corrective messages and all participants were directed to resources on quitting smoking.

Study Measures

	Aim 1: Population study	Aim 2: Lab study
Intervention/Exposure		
Nicotine messaging vs. control	Wave 1	Week 1
Normal nicotine vs. RNC cigarette		Week 1
Heatmapping	Waves 1-4	
Perceived message effectiveness	Wave 4	Week 5
Message credibility	Wave 4	Week 5
Eye-tracking		Weeks 1-4
Biomarkers		Weeks 0, 5
Outcomes		
Nicotine beliefs	Waves 1, 2, 4	Weeks 0, 2, 5
Intention to use nicotine/tobacco products	Waves 1, 2, 4	Weeks 0, 2, 5
Nicotine/tobacco use and behavior	Waves 1, 2, 4	Weeks 0, 2, 5
Subjective rating of study cigarette		Weeks 1-5
Manipulation check	Waves 1, 2, 4	Weeks 0, 2, 5
Moderators		
Sociodemographics	Wave 1	Week 0
Literacy	Wave 1	Week 0
Cancer risk beliefs	Wave 1	Week 0
Cancer risk behaviors	Wave 1	Week 0
Fagerstrom test for nicotine dependence	Wave 1	Week 0
Other key constructs		
Attitudes about nicotine	Waves 1, 2, 4	Weeks 0, 2, 5
Nicotine-related norms	Waves 1, 2, 4	Weeks 0, 2, 5
Behavioral control	Waves 1, 2, 4	Weeks 0, 2, 5
Stages of change	Wave 1, 4	Week 0, 5
Policy support	Wave 4	Week 5

Statistical Analysis Plan

Sample Size. Power for Aim 2 was calculated for 160 adult current daily cigarette smokers using an ANCOVA framework to detect differences between any two study conditions; with n=40 in each of the four conditions in the trial, we will have 80% power to detect medium effect sizes (Cohen's f = 0.33) in continuous outcomes (i.e., nicotine beliefs, behavior, subjective ratings), assuming three covariates in the model (i.e., age, gender, cigarettes per day at baseline). In addition, using a two-way analysis of variance framework, 40 participants in each study condition provides 85% power to detect a statistically significant moderation of these same outcomes by the nicotine content of the study cigarettes, assuming that the responses by those in the NCM + NNC condition differ from those in the other three conditions by 1 standard deviation. Sample size for the eye tracking analyses is based on our earlier work that recruited 200 participants to a 2 x 2 factorial design and used the same outcomes (i.e., dwell time, time to first area of interest).[9]

Data Preparation. We will conduct data screening steps similar to Aim 1 including assessing outliers, missing data presence/patterns, and normality/equality of variance assumptions. Non-normal data will be transformed as needed. In all analyses, the assumptions underlying the application of all the statistical methods that are used will be examined, principally using standardized residuals, influence diagnostics, and graphical displays. Where needed, appropriate transformations will be applied to ensure that data meet model assumptions. Descriptive analyses will characterize participants overall and by study condition. Aim 2 eye tracking data will be cleaned and pre-processed using standard procedures from our previous work. Descriptive analyses will characterize participants overall and by study conditions. Bivariate tests will assess for differences in participant characteristics by conditions and examine if any baseline variables that are imbalanced between study groups are associated with primary and secondary outcomes (p <.10). Any such variables will be accounted for in analyses as covariates.

Analytic Approach.

Aim 2: Test the impact of NCM (messaging vs. control) and nicotine content of cigarettes (normal vs. reduced) on nicotine beliefs and subsequent use of tobacco and nicotine products using a 2 x 2 factorial design in a sample of 160 adult current smokers followed for 4 weeks.

Hypothesis 2a: Adult smokers in the NCM + normal nicotine content (NNC) cigarette condition will report the fewest false beliefs about nicotine compared to those in the NCM + RNC and control conditions (Control + RNC cigarettes, Control + NNC cigarettes); those in the Control + NNC cigarette condition will report the highest study cigarette use.

Hypothesis 2b: Lower subjective ratings of study cigarettes (e.g., strength) will predict greater false beliefs about RNC cigarettes.

Analyses for Aims 1 and 2 employ common measures and a common analytic framework to test the impact of nicotine education on nicotine beliefs and behavior. Primary analyses will use an intention-to-treat approach, employing a general linear mixed model to accommodate missing data.[10,11] Outcomes for **Aim 2** focus on continuous outcomes (*primary outcome*: nicotine beliefs; *secondary outcomes*: frequency and intensity of nicotine/tobacco use), with the primary hypotheses (**Hypotheses 2a**) focused on the effect of the two interventions (nicotine education and nicotine content of study cigarettes) on these outcomes. Preliminary analyses will examine differences in these outcomes across study conditions at the first post-exposure time point (Week 2) and at the final timepoint (Week 5) using ANCOVA controlling for covariates that are differentially distributed between study groups. These outcomes as well as measures of tobacco use (study cigarette use, topography patterns, CO) will then be analyzed using the general linear model approach to repeated measures analysis. Of primary interest in Aim 2, we will evaluate

whether using study cigarettes with a known nicotine content (i.e, normal or reduced) moderates the effect of NCM on our primary and secondary outcomes by incorporating an interaction between NCM condition (NCM/control) and study cigarette condition (NNC/RNC) through the following steps: 1) bivariate analyses, to determine if outcomes vary by these variables; 2) testing whether study cigarette condition moderates ($p < .05$) experimental effects in the models above; if so, performing post-hoc statistical testing to examine the direction and magnitude of the moderation; 3) if no evidence of moderation exists, including study cigarette condition as a covariate.

Our previous work highlights that assessing perceptions of RNC cigarettes that find an association between participants perceiving that their assigned product is low nicotine and reporting lower harm perceptions of the product.[12,13] To address **Hypothesis 2b**, we will also use autoregressive, cross-lagged panel analysis[14] to examine the direction of the relationships between subjective ratings and nicotine beliefs over three timepoints (Weeks 0, 2, and 5) and whether the regression paths vary in magnitude by study condition.

As in Aim 1, our exploratory analyses will focus on three areas: 1) potential moderators of the relationship between intervention condition and the study outcomes; 2) nicotine beliefs as a potential mediator of the relationship between intervention condition and constructs identified in the Theory of Planned Behavior (i.e., nicotine-related attitudes, norms, behavioral control, and nicotine/tobacco intentions and use); and 3) the relationship between visual attention to nicotine education messages and nicotine beliefs in the participants exposed to the NCM intervention.

For the first area (*moderation*), we will conduct exploratory analyses using the same steps described for Hypothesis 2a to determine whether there are potential moderators of the relationship between the study condition (NCM + RNC, NCM + NNC, Control + RNC, Control + NNC) and outcomes, specifically age, gender, baseline cancer beliefs and cancer risk behaviors.

For the second area (*mediation*), we will draw from traditional mediation frameworks and use robust methods to explore the relationships outlined in our theoretical framework and whether changes in nicotine beliefs post-exposure (Weeks 2, 3, 4) influence nicotine-related attitudes, norms, behavioral control, intention, and behavior at the final assessment (Week 5). To complement Aim 1 analyses, we will examine whether nicotine beliefs at Week 2 are associated with these Week 5 outcomes using bivariate statistics and the general linear model-based analysis accounting for any covariates as described above. Where these preliminary steps indicate potential correlation between nicotine beliefs and study outcomes ($p < .05$), we will test for mediation by estimating the indirect effects of nicotine beliefs on follow-up outcomes via the study condition using a bias-corrected bootstrapping method with 1,000 resamples. This approach estimates indirect (i.e., mediation) effects and produces bias-corrected asymmetric 95% CIs correcting for non-normality of the distribution of indirect effects and providing higher power and better control over the Type I error rate versus traditional approaches to test mediation. Asymmetric 95% confidence intervals around indirect effect estimates for nicotine beliefs that do not include zero will be interpreted to indicate significant mediation.

For the third area (*visual attention*), we will identify the regions of interest (ROIs) in each nicotine education message based on responses to the Aim 1/Wave 1 heatmapping task. Using eye-tracking, we will explore whether attention to the ROIs (i.e., dwell time, fixations) is correlated with scores on the nicotine beliefs scales (i.e., nicotine, NRT, e-cigarette, RNC cigarette beliefs) at the same assessment (Weeks 1-4). Given the content of messages in the nicotine education condition, we will be able to assess whether attention to specific ROIs is correlated with specific beliefs (i.e., whether attention to a specific ROI within the “Nicotine does not cause cancer” is correlated with response to the nicotine belief item regarding nicotine causing cancer). We will also be able to explore the prospective relationship between visual attention to the nicotine

education messages (e.g., duration spent viewing messages, attention to ROIs) and nicotine beliefs and whether there are differences in visual attention in the NCM intervention condition over the four weeks of data collection. These data will inform potential refinements to nicotine education messages for future studies.

Missing data: The most effective approach to eliminating biases and inefficiency caused by missing data is to collect complete data. In both aims of our study, we will use several tools available to maintain contact and verify that forms are complete. Use of computerized survey platforms (e.g., Qualtrics) in this study will provide additional mechanisms to improve completeness of survey responses, but even so, some participants may refuse to answer certain questions. We anticipate that one cause of missing data will be item non-response on self-report questionnaires; another will be missing assessments. Finally, missing data can arise through attrition; however, the steps outlined in the proposal are meant to minimize this. The general/generalized linear mixed model that we will be using for analysis for Aim 2 is a maximum likelihood approach to repeated measures analysis of variance that does not rely on imputation, which may give differing results depending upon the exact imputation procedure used. Rather, this approach estimates the parameter values that would maximize the probability of observing the data collected. In the event of missing variables, the likelihood for a given individual is the probability of observing the non-missing variables. Thus, the maximum likelihood approach allows the use of data from subjects for the time period for which data is available, but not for time periods for which the data is missing. This procedure uses information from earlier time periods to estimate the effects of later time periods, while also accounting for the uncertainty of the projection in the computation of standard errors and test statistics. Maximum likelihood estimation is considered superior to imputation methods for the treatment of missing data in clinical trials.[15]

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