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Effects of Nicotine on a Translational Model of Working Memory

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Effects of Nicotine on a Translational Model of Working Memory

by

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DEDICATION

To my parents, Trudy and David MacQueen, who taught me the wisdom of inquiry. To my sister, Kendell Timmers, who showed me the value of dedication. To my beloved Tory, my constant inspiration.

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Over the course of my education I have been privileged with the guidance of exceptional mentors and the support of compassionate peers. It will be a great challenge to give back as much as I have received.

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ABSTRACT

Cognitive research with human non-smokers has demonstrated that nicotine generally enhances performance on tasks of attention but, working memory does not appear to be affected. In contrast, nicotine has been shown to produce robust enhancements of working memory in non-human animals. To address this disparity, the present study investigated the effects of nicotine (2mg, 4mg nicotine gum, and placebo) on the performance of 30 non-smokers (15 male) completing a working memory task developed for rodents (the odor span task, OST). Nicotine has been reported to enhance OST performance in rodents and the present study sought to determine whether the effect is generalizable to human performance. In addition to completing the OST, participants completed a cognitive battery of clinical and experimental tasks assessing working memory and attention. This allowed for a direct comparison of OST performance to other commonly used measures of human cognition. Findings showed that nicotine was associated with dose dependent enhancements in sustained attention, as evidenced by increased hit accuracy on the rapid visual information processing (RVIP) task. However, nicotine failed to produce main effects on OST performance or on alternative measures of working memory (digit span, spatial span, letter-number sequencing, 2-back) or attention (digits forward, 0-back). Interestingly, enhancement of RVIP performance occurred concomitant to significant reductions in self-reported attention/concentration. Human OST performance was significantly related to N-back performance and, as in rodents, OST accuracy declined with increasing memory load. Given the similarity of human and rodent OST performance and the strong association observed

between OST and visual 0-back accuracy, the OST may be particular useful for preclinical studies of conditions characterized by inattention.

INTRODUCTION

The health impact of tobacco smoking is staggering, causing over 5 million deaths per year world-wide and accounting for nearly $100 billion in healthcare costs each year in the US alone (CDC; 2002, 2008). While national prevention and cessation efforts have succeeded in reducing the prevalence of smoking in the US over recent years, more than a fifth of the adult population continues to smoke (SAMHSA, 2013) despite quit attempts reported by 52.4% of adult smokers each year (CDC, 2011). Improving the effectiveness of prevention and cessation efforts relies on a thorough understanding of the reinforcing properties of smoking. Motivations for smoking behavior are diverse and likely evolve across the transition from adoption to dependent smoking. For example, prior to nicotine exposure, initiation of smoking is likely dependent on learned associations between smoking and desired outcomes and attributes (Baker, Brandon, & Chassin, 2004). After initiation however, smoking behavior may be reinforced by the direct pharmacological action of constituents in tobacco smoke as well as by alleviation of the withdrawal syndrome that can occur after discontinuation of extended use. Alterations in cognitive processing after acute exposure to nicotine, the primary psychoactive constituent in tobacco smoke, has been proposed as a mechanism through which smoking is reinforced in some individuals (Evans & Drobes, 2009). However, the effects of nicotine on cognitive processing in humans remain ill refined.

The relationship between cognition and nicotine dependence in humans is most clearly evidenced by the impairments that occur during the course of nicotine withdrawal. Difficulty

concentrating is a frequently reported symptom of nicotine withdrawal (Hughes, Higgins, & Bickel, 1994) and significant impairments in attention as assessed by the rapid visual information processing task (RVIP) can be observed within 30 minutes of nicotine deprivation (Hendricks, Ditre, Drobes, & Brandon, 2006). Furthermore, administration of nicotine during withdrawal has been shown to normalize sensory abilities, motor abilities, selective attention, divided attention, sustained attention, and certain forms of memory (Heishman, Taylor, & Henningfield, 1994). Similarly, nicotine has been shown to improve cognitive functioning in individuals diagnosed with disorders characterized by cognitive deficits (e.g., schizophrenia and attention deficit hyperactivity disorder; Dalack, Healy, & Meador-Woodruff, 1998).

Cognitive enhancement may explain the high prevalence of smoking observed for individuals diagnosed with these conditions (approximately three fold that of the general population). In addition to reinforcing continued smoking in nicotine dependent individuals and in those with pronounced cognitive deficits, nicotine may serve to facilitate the adoption of regular smoking behavior in non-dependent individuals through cognitive enhancement. It has been reported that nicotine can enhance cognitive abilities in non-smokers (Heishman et al., 1994) and the accumulation of evidence over the past decade has made it possible to conduct a meta-analysis these effects.

Acute Effects of Nicotine

Across 41 studies on the effects of nicotine on non-smokers, non-deprived smokers, and minimally deprived smokers (less than 2 hours), Heishman, Kleykamp, and Singleton (2010) have reported significant enhancements in fine motor ability (finger tapping, handwriting and pegboard tasks), sustained attention/alerting attention accuracy and reaction time (e.g. RVIP and continuous performance task; CPT), orienting attention reaction time (e.g. target detection and

letter search), short-term episodic memory (e.g. word recall and word recognition), and working memory reaction time (e.g. N-back and digit recall tasks). Significant effects were not found for orienting attention accuracy (e.g. target detection and letter search), long-term episodic memory accuracy (e.g. word recall and recognition), or working memory accuracy (e.g. N-back and digit recall tasks). That this profile of acute nicotine effects does not include enhancements in working memory accuracy appears to conflict with studies conducted in non-human mammals that have demonstrated robust enhancements in working memory performance after administration of nicotine and selective nicotinic agonists (Levin, McClernon, & Rezvani, 2006). Most notably, nicotine has been shown to enhance delayed match to sample (DMTS) performance in monkeys (Buccafusco, Beach, & Terry, 2009), and radial arm maze performance in rodents (Levin, Bradley, Addy, & Sigurani, 2002; Levin, Briggs, Christopher, & Rose, 1992; Levin, Icenogle, & Farzad, 2005; Levin, Kim, & Meray, 1996; Levin & Torry, 1996).

The discrepancy between animal and human findings may represent a species difference in nicotine effects, methodological differences in the doses tested, or a disparity between the behavioral tasks used to assess the construct of working memory. If there is indeed a prominent species difference in nicotine effects on cognitive processing, thorough characterization of the behavioral and neurobiological discrepancies will need to be addressed if animal models are to inform human cognition. Cross-species methodological issues could be attenuated through the development and use of translational behavioral tasks, thereby allowing for more sensitive comparisons of dose effects. Additionally, such tasks would provide a framework for testing cognition across species and bridge definitional gaps in theories of cognition.

Assessing Working Memory in Animals and Humans

Animal models of working memory are designed to assess processes analogous to those identified in human subjects. Towards this end, procedures such as delayed match-to-sample (DMTS) and delayed non-match-to-sample (DNMTS) tasks have been used with a variety of species to demonstrate patterns of forgetting (loss of stimulus control) across delays that are comparable to patterns observed in humans (Wright, 2007). Variants of DMTS/DNMTS procedures, along with tasks such as the radial arm maze and the within-session Morris swim task, have been used specifically as models of working memory (for a review see Dudchenko, 2004). Operational definitions of working memory procedures for non-humans typically require that stimulus information only be presented during a single learning trial and only be useful for controlling behavior during a single trial or session (Bannerman, Rawlins, & Good, 2006; Dudchenko, 2004; Olton, Becker, & Handelmann, 1979). For example, in match and non-match-to-sample tasks (MTS, NMTS), an animal is presented with a sample stimulus (e.g. a green light) and is subsequently presented with the sample stimulus (a green light) concurrently with a comparison stimulus (a red light). In a MTS task, only responses to the sample stimulus (the green light) are reinforced while in a NMTS task only responses to the comparison stimulus (the red light) are reinforced. Thus, in a given trial of either task, the subject must remember the stimulus initially presented to provide an accurate response when presented with a choice. Subjects are tested across many trials in which the sample stimulus is alternated. Thus, the stimulus information presented during each trial only informs accurate responding during that same trial.

Radial arm maze tasks are also used to assess this form of one trial learning. In this task, rodents are placed in the center of an open field apparatus that has a number of arms extending

from a central platform. Each of these arms is baited with a food reward and the rat is able to explore the apparatus and consume the rewards. Any time the subject re-enters an arm which it has already explored an error is scored. In optimal performance of the task the rat enters each arm only once. During a session (referred to as a trial in some designs), the subject must remember which arms it has visited; errors are interpreted as a failure of memory. The subject can be tested repeatedly as memory for arm entry is only useful for performance during the testing session (though in certain testing arrangements response strategies can develop across sessions).

In humans, working memory is currently described in terms of short term memory stores of limited capacity that require controlled attention (Baddeley, 2003; Saults & Cowan, 2007). Though the capacity limits of working memory in humans have been disputed (Baddeley, 2003; Cowan, 2001), assessment of capacity is the primary way in which working memory is assessed clinically. The Weschler Adult Intelligence Scale (WAIS-III) and the Weschler Memory Scale (WMS-III) both include several tasks assessing working memory capacity (Psychological Corporation, 2002). These subscales include digit span (DS), spatial span (SS), letter-number sequencing (LNS) and arithmetic. All but the arithmetic subscale, can be considered "span tasks" in which individuals provide a recall of strings of stimuli (letters, numbers, or spatial positions). Simple span tasks require the individual to report the string of stimuli in the order it was presented (DS forward, SS forward) while complex span tasks require the individual to reorder the stimulus string (DS Backward, SS Backward, and LNS). Deficits selective to complex but not simple span tasks are inferred to result from impairments of working memory apart from more general impairments of attention.

The use of capacity tasks have been critical to the shaping of current theories of cognition (Richardson, 2007). In turn, theory has led to the development of novel working memory assessments such as the N-back task. Like span tasks, the N-back task requires individuals to remember strings of stimuli of varying lengths. Stimuli are presented sequentially and individuals must respond to each presentation with a response indicating whether the stimulus matches the stimulus which was presented 1, 2, or 3 positions backs (referred to as the 1-back, 2-back, or 3-back tasks). There is also a 0-back task in which individuals simply indicate if each stimulus matches a target stimulus which is identified at the beginning of testing. The 0-back task can be considered a more general assessment of attention. While the N-back task is conceptually similar to span tasks, measures derived from the N-back fail to correlate or correlate weakly with typical span measures suggesting the N-back may capture a unique facet of working memory (Hill et al., 2009; Kane, Conway, Miura, & Colflesh, 2007; Miller, Price, Okun, Montijo, & Bowers, 2009).

Translational Working Memory Tasks

While both human and non-human working memory tasks assess forms of one-trial learning, there is a dearth of procedures available for studying short-term memory capacity in rodents. As such, it is difficult to infer how deficits or enhancements in MTS/NMTS or radial arm maze performance map onto the capacity sensitive measures used in humans. A notable exception is the olfactory span task (OST) for rodents (Dudchenko, Wood, & Eichenbaum, 2000) which incorporates manipulations of memory load into a single-session learning paradigm. In trial 1 of the procedure, rats are presented with a single olfactory stimulus (a cup of scented sand) in an arena. Responses to this stimulus (digging) are reinforced through the retrieval of a food reward buried within the scented sand. On trial 2, a second stimulus cup scented with a different

odor is baited with a food reward and placed in a random position in the arena along with the stimulus presented during the first trial (not baited). The rat is free to respond to either of the scented stimuli present, but only responses to the novel odor produce a food reward. On the third trial, the two previously presented odors are moved to new positions and a third odor is introduced. Once again, only responses to the novel odor are reinforced.

The procedure is continued in this fashion with the introduction of a novel odor on each trial until up to 24 stimuli are present. Thus the procedure can be viewed as a non-match-to-sample task in which each stimulus serves as a sample during its initial presentation and as a comparison stimulus in each additional trial. The task might be best described as an *incrementing* non-match-to-sample task as the number of stimuli that can serve as comparisons increases on each successive trial. Performance on the OST is measured in overall accuracy and span during each session. Dudchenko and colleagues found that the span lengths produced by individual rats varied greatly across testing days, but that the average median span of subjects was relatively consistent (M=8.38 ±.49). More importantly, average percent correct performance across sessions at each span length of the procedure decreased significantly as span increased showing an inverse relationship between accuracy and the number of stimuli to be remembered (memory load).

In recent work with the OST in rats, several controls have been implemented that enhance the interpretation of OST performance and make the task more suitable for pharmacological investigations (MacQueen, Bullard, & Galizio, 2011). In the original design of the OST, the capacity manipulation (number of odors to remember) is inherently confounded with the number of choices presented, such that decreased accuracy at increasing span lengths could be inferred to represent the effect of increasing comparisons. By limiting stimulus

presentation to a maximum of 5 odors on each trial (the novel sample and four comparison odors chosen randomly from the set of odors previously presented), chance performance is equated at 20% for each trial beyond the fourth. Use of this procedural adaptation has demonstrated that in rats, reductions in accuracy relate to the number of stimuli to remember even when the number of comparisons presented is held constant (MacQueen et al., 2011). Additionally, a control task (a repeated olfactory simple discrimination task) was implemented within-session to detect changes in motivation and olfactory discrimination during performance of the span task. Use of this control allowed for the detection of drug-induced deficits that were selective only to odor span accuracy (sparing performance control accuracy).

The OST has demonstrated considerable promise for investigating the neurobiological determinants of memory capacity and is sensitive to cholinergic manipulations. In rats, performance of the odor span task is transiently disrupted by lesioning of the basal forebrain cholinergic system (Turchi & Sarter, 2000). The procedure has also been successfully adapted for testing mice (Young, Kerr, et al., 2007) and performance decrements have been observed in α7 nicotinic cholinergic receptor knockout (Young, Crawford, et al., 2007) and in human amyloid over-expressing mice (Young, Sharkey, & Finlayson, 2009). Notably, direct facilitation of OST performance after administration of nicotinic agonists has been recently reported in rats (Rushforth et al. 2010). Taken together, these findings suggest that nicotinic receptors play an integral role in OST performance in non-human animals, and suggest that nicotine administration enhances performance.

Importantly, the odor span task has been adapted for use with humans (Levy, et al., 2003). Thus, the OST exemplifies the type of translational behavioral task that can be used to assess short term memory capacity in both humans and non-human animals. By using identical

procedures this paradigm mitigates methodological and operationalization issues, thus allowing for cross-species comparisons of dose-response effects. Such tasks allow for stronger inferences to be drawn from animal models.

To clarify what cognitive processes are augmented by nicotine and to arrive at a better understanding of the neurocognitive processes assessed by the OST, the present study sought to evaluate the effects of nicotine on a human adaptation of the OST as well as well-validated clinical and experimental tasks of attention and working memory. To avoid the confounds associated with distinguishing withdrawal reversal from a more general enhancement of cognition, only non-smokers with a limited history of nicotine exposure were recruited. Prior studies investigating the effects of nicotine on attention and memory in non-smokers and ex-smokers suggested that nicotine was most likely to enhance accuracy on sustained attention (also described as alerting attention) tasks and reaction time on tasks of sustained attention/alerting attention, and working memory. Thus, it was expected that nicotine would produce robust enhancement of accuracy on a task of sustained attention (RVIP), limited enhancement of accuracy on the attention related components of clinical working memory tasks (Digit Span forward, Spatial Span forward, 0-back accuracy), enhacement of reaction time on experimental sustained attention and working memory tasks (RVIP, 0-back and 2-back), while producing no effects on the accuracy of working memory performance derived from clinical or experimental tasks (Digit Span backward or total, Spatial Span backward or total, Letter-number Sequencing, and 2-back accuracy).

The present study sought to expand upon prior findings with non-smokers by testing participants on a modified version of the OST which incorporated novel control procedures described in preclinical investigations. The primary aim of including the OST was to validate

the task in humans and determine if the nicotine facilitation of OST performance evidenced in rats generalizes to human performance. Validation of the OST occured through several methods. First, the human OST procedure previously reported by Levy et al. (2003) was modified such that chance performance and capacity were not confounded. This allowed for an assessment of the effects of capacity on human OST accuracy and a comparison with the effects observed in rodents. The modified version also included a performance control component designed to assess motivation to respond accurately and the reliability of odor discrimination during performance of the task. Given the novelty of the OST, a primary goal was to examine convergent and divergent validity of the OST with well validated tasks of attention and working memory.

Though the OST has been most frequently described as a working memory task (Dudchenko et al., 2000; Young, Kerr, et al., 2007), it has been suggested that OST performance deficits may actually represent attentional deficits (Young, Crawford, et al., 2007). Thus, we expected odor span measures to demonstrate convergent validity with the accuracy measures of attention related tasks such as RVIP, 0-back, and the forward components of clinical working memory tasks. In contrast, we did not expect odor span measures to be significantly related to accuracy on complex working memory tasks such as 2-back accuracy, Letter-number sequencing, or the backward and total measures of clinical span tasks. As a control measure, we expected that simple odor discrimination would show divergent validity with odor span performance and all measures of attention or working memory.

Given the substantial preclinical data implicating involvement of the cholinergic system in OST perfromance (Rushforth, Allison, Wonnacott, & Shoaib, 2010; Rushforth, Steckler, & Shoaib, 2011; Turchi & Sarter, 2000; Young, Crawford, et al., 2007; Young, Kerr, et al., 2007), it was expected that nicotine would enhance OST performance amongst human non-smokers as

has been observed in rats (Rushforth et al., 2010). Demonstrating consistent pharmacological effects across species and determining neurocognitive correlates of human OST performance represent critical steps in realizing the translational utility of the OST.

METHODS

Experimental Design

Participants completed 3 experimental sessions over the course of three weeks with each session scheduled to start within 2 hours of the start time used for each participant's other sessions. During each experimental session participants received 2mg, or 4mg nicotine gum or a placebo gum. Dose order was counterbalanced across participants in this double-blind placebo controlled within-subjects design. Dependent measures included cognitive task performance and self-report measures of cognition.

Participants

Power analyses (Cohen, 1988) suggested that 28 participants would be necessary to achieve a power level of .80 in detecting a significant medium sized effect ($f = .25$) within subjects at an α level of .05. Based upon the test-retest reliability of the clinical working memory measures included in the study (.71-.83) the correlation of repeated measures was assumed to be approximately .75. Because the nicotine manipulation was expected to affect the performance of some individuals more than others, we predicted the correlation of measures to be somewhat lower in our study. As such, a correlation of measures estimate of .50 was used to calculate power. To ensure sensitivity and to provide complete counterbalancing of dose order across participants, recruitment continued until 30 participants completed all three experimental sessions. Participants between the ages of 18 and 54 were recruited from the Tampa area through internet, newspaper and radio advertisements as well as through the Tobacco Research

and Intervention Program (TRIP) participant database. Participants received an initial screening over the telephone. Those who appeared to meet inclusion criteria were informed that the study involved one assessment/screening session and three experimental sessions for which they would be compensated at a rate of $20/hr. and a bonus of $20 at the end of the final session.

Individuals who appeared eligible and interested in study participation during the phone screening were scheduled for an assessment session. During the assessment session, informed consent was obtained and subjects were evaluated through standardized interviews, questionnaires, and biochemical analysis to ensure the inclusion criteria were met.

Inclusion/Exclusion Criteria

Participants were screened to ensure they were between the ages of 18 and 54 and able to read and understand the consent form and questionnaires. Participants were required to be non-smokers who reported no more than 5 occasions of nicotine product use (e.g., cigarettes, cigars, chewing tobacco) during their lifetime with no use of nicotine products within the past year. Recent smoking and drinking was determined from analysis of breath samples collected at the beginning of each assessment and experimental session. Any participant providing a breath sample with a carbon monoxide level of greater than 3ppm at any point was excluded from the study. This cutoff has been shown to identify those who have smoked within the past day with 71.5% sensitivity and 84.8% specificity (Javors, Hatch, & Lamb, 2005). Any participant submitting a breath sample with a detectable alcohol content was not allowed to participate in the scheduled experimental session but permitted to reschedule. Any participant who submitted two alcohol positive breath samples was excluded from the study. Any evidence of recent illicit substance use identified by urinalysis also resulted in study exclusion.

Participants were also excluded if they had any history of heart disease, high blood pressure, any blood circulation disorder, phenylketonuria, asthma, food allergies, other serious medical condition (e.g. cancer, kidney disease) as well as any dental conditions that would prohibit gum chewing. Participants were screened for psychiatric illness by an advanced clinical psychology doctoral student, utilizing the Structured Clinical Interview for DSM-IV (First, Spitzer, Gibbon, & Williams, 1994). Participants meeting criteria for a current mood or psychotic disorder, substance dependence or panic disorder were also excluded. For female participants, pregnancy was assessed through urinalysis and participants who were currently pregnant or expected that they could become pregnant during the course of the study were excluded.

Procedures

Assessment Session

At the start of the assessment session participants were greeted by a member of the research team who described the study in detail and responded to questions. Potential participants were informed of the potential risks, benefits, purpose, compensation associated with participation and their HIPAA privacy rights before consent was sought. Research staff presented the consent document verbally and allowed the participant to review the consent materials before administering a written consent form quiz to the participant. Both the consent form and the associated quiz were written at a sixth grade level and the experimenter provided clarification as necessary.

The consent quiz was composed of 5 multiple choice questions on the material covered in the consent form. If a participant failed to answer a question correctly, they were directed to re-read the section of the consent form containing the relevant information and answer again. No

participant failed to answer any question on the quiz correctly on the second attempt. As several of the measures used in the proposed study have only been developed in English, our sample was limited to English speaking participants. Those demonstrating an adequate understanding of consent materials were given the opportunity to consent. Participants were informed that their participation was voluntary and could be withdrawn at any time. After signing the consent form, participants received a copy of the consent document (including HIPAA authorization) for their personal records.

After the consent procedure, the participants submitted two breath samples for the purpose of estimating carbon monoxide and breath alcohol content. A urine test was administered to test for drug use. It was possible to detect cannabis usage within 14 to 30 days, tricyclic antidepressants usage within 10 days, barbiturate and PCP usage 3 to 8 days, benzodiazepine usage within 2 to 14 days, amphetamine/methamphetamine usage within 2 to 6 days, and cocaine/opiate usage within 2 to 5 days. Urine samples submitted by women were additionally tested for pregnancy. Participants were excluded for pregnancy because the proposed study required nicotine administration and nicotine products present well-known risks to developing fetuses.

A demographic and health related information questionnaire was then administered to obtain demographic information for the purposes of describing the study sample and to verify inclusion criteria. Variables assessed included age, race, socioeconomic status, employment, education, marital status, height, weight, health status, current medications and drug use history. The participant was then administered the SCID (First et al., 1994) by a trained clinician to screen for psychiatric conditions. When study participation criteria were met, the participant was scheduled for 3 experimental sessions to occur no less than five days apart, beginning between

8:00 AM and 5:00 PM. For each participant each session was scheduled at approximately the same start time (within two hours of other sessions). If the assessment session concluded between this time period, the participant was given the option to move immediately into the first experimental session. Assessment sessions lasted approximately an hour and all participants were compensated at a rate of $20/hr regardless of whether study participation criteria were met.

Experimental Sessions

Upon arrival to the facility, participants were required to submit two breath samples to ensure that breath carbon monoxide and breath alcohol content criteria were met. Each experimental session began with the administration of gum delivering 2mg or 4mg nicotine, or placebo gum delivering no active substance (gum preparation and administration described below). After dose administration, participants completed a series of self-report questionnaires which assessed their current physiological and emotional state. Completion of these measures took approximately 10 minutes. Research staff then administered a modified version of the human OST (adapted from Levy et al., 2003). Because the present study sought to investigate performance of the OST as a primary aim, this task was the first cognitive task administered during each experimental session. The human OST required about 40 minutes. Following completion of the OST, research staff then administered the digit span, letter number sequencing, and the spatial span tasks of the WMS-III (Wechsler, 1997b) the rapid visual information processing task (RVIP) and the N-back task in a quasi-random order (three possible orderings). An example timeline of the experimental session is presented below (Figure 1). After completion of the final cognitive task, participants were compensated for their time ($20/hr) and given an appointment card to serve as a reminder for their next appointment. Each experimental session lasted approximately an hour and 45 minutes.

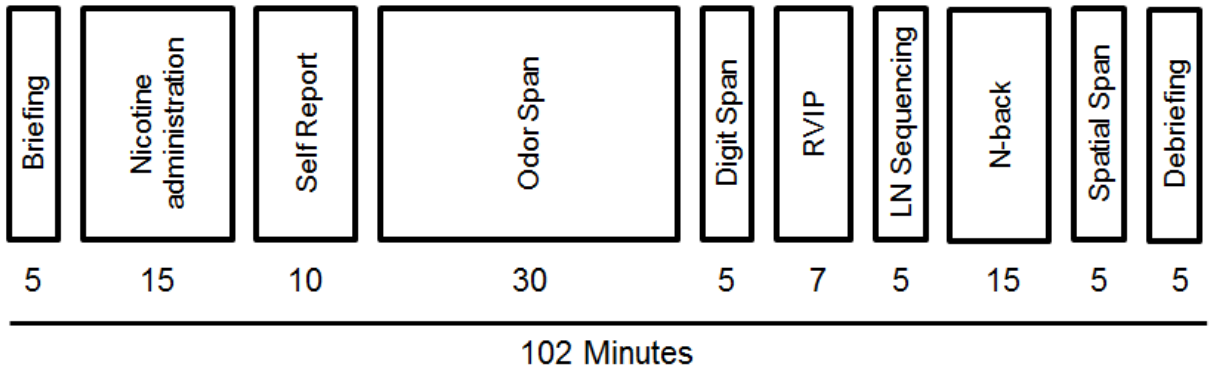

Figure 1. *A timeline of experimental sessions. All values are expressed in minutes.*

Nicotine Administration

Nicotine and placebo gum were prepared daily by the experimenter and provided to research staff, who were blind to the dose received. A placebo procedure described by Kleykamp et al. (2005) was used to prevent the detection of active doses through differences in odor, texture, taste and sensation between nicotine and placebo gum. Nicotine gum (Nicorette® Freshmint™, Pfizer Health AB, Helsingborg, Sweden) or a similar dragée style gum (Dentyne Ice® Peppermint, Kraft Foods Inc., Northfields, Illinois) was wrapped with Wrigley's sugar-free peppermint gum and received two drops (0.1 mL) of Tabasco sauce. To ensure consistent chewing patterns across participants, a procedure was used in which the participant was prompted to chew their gum for 15 minutes at 3 second intervals by a computer generated tone (as in Houtsmuller, Fant, Eissenberg, Henningfield, & Stitzer, 2002; Kleykamp et al., 2005; Nemeth-Coslett & Henningfield, 1986).

Compliance to the chewing procedure was monitored by research staff. This dosing procedure has been demonstrated to produce peak blood plasma levels of 4.6 ng/mL for the 2 mg dose and 8.5 ng/ml for the 4mg dose (Hindmarch, Kerr, & Sherwood, 1990). Time to peak blood

plasma concentration (Tmax) after chewing nicotine gum has been estimated at about 45 minutes to 1 hour for the 2mg and 4mg doses and plasma concentrations remain substantially elevated (approximately 75% of peak plasma) at 180 minutes (Dautzenberg, Nides, Kienzler, & Callens, 2007; Shiffman et al., 2009). Thus a single administration should have been sufficient to sustain nicotine levels for the duration of experimental sessions.

Measures

Current State Measures

Wisconsin Smoking Withdrawal Scale (WSWS). The WSWS (Welsch et al., 1999) concentration subscale was completed to provide a self-report measure of attention and concentration. This measure supplemented objective experimental measures of attention and working memory.

Positive Affect and Negative Affect Scale (PANAS). The 20-item PANAS (Watson, Clark, & Tellegen, 1988) was completed after nicotine administration during each experimental session to assess current affective state. The PANAS was scored to produce positive affect and negative affect scales which are internally reliable, and have been extensively validated (Watson, Wiese, Vaidya, & Tellegen, 1999).

Feeling State Questionnaire (FSQ). Six items from the FSQ (D. G. Gilbert et al., 2008) (irritable, attentive, jittery, nauseous, sick, and dizzy) were administered to assess effects of nicotine commonly reported by non-smokers.

Working Memory and Attention Tasks

The OST and clinical assessments were administered to participants in an experimental session room by trained members of the research team. All three of the clinical assessments were administered according to the protocol specified by the WMS-III administration and scoring

manual (Psychological Corporation, 2002). The N-back and RVIP task were completed on a personal computer in the session room running E-prime software (Psychology Software Tools, Inc.).

Olfactory Span Task (OST). The OST used in the present study was adapted from procedures used by Levy et al. (2003) and was consistent with the olfactory span task originally developed for rats (Dudchenko et al., 2000). The task was administered using 22 household spices (High Quality Organics; Reno, NV). Spices were individually stored and presented in opaque plastic test tubes (Lake Charles Manufacturing; Lake Charles, LA) with plastic stoppers. Each test tube was approximately 1/3 filled with ground spice. Prior to the first experimental session, participants were randomly assigned to a set of 2 performance control odors which would remain constant across all three sessions and 1 of 3 orders in which the 3 stimulus sets of 20 odors used in the odor span task would be presented across sessions. Each stimulus set was constructed with the constraint that no stimulus could serve as a comparison more than 3 times during the task.

The task began with the experimenter introducing the participant to the performance control stimuli. A test tube rack holding the two performance control test tubes was placed on the table in front of the participant. The participant was instructed to sample both of the odors from left to right by removing the rubber stopper, placing their nose approximately one inch above the test tube, and breathing in gently before replacing the rubber stopper and moving to the next stimuli. After the participant sampled the second stimuli, the experimenter instructed the participant to remember the last odor they had sampled. The experimenter then reordered the control stimuli behind a cardboard shield. The stimuli were presented again and the participant was asked to sample the stimuli and indicate which tube contained the target odor. Participants

were given feedback on each choice. The performance control stimuli were reordered and presented for ten consecutive trials. The performance control procedure was conducted to ensure that participants were able to adequately discriminate between odorants during testing.

Next the experimenter instructed the participant that on each new trial they would be asked to either identify the odor they were asked to remember or to identify an odor that they had not yet sampled. Each session contained 20 trials in which they were to detect the novel odor (span component) and 4 trials in which they were to identify their performance control odor. The span component began with the presentation of a single odor from the experimental odor set. The participant was asked to sample the odor and report whether they had sampled this odor during the session. This stimulus was then moved to a random position on the test tube rack and a second (novel) odor was added to the rack. The participant was then instructed to sample both odors and to report which odor was new. On each subsequent trial, a novel odor was presented with a comparison odor chosen quasi-randomly from odors that were presented as novel samples in previous trials.

After every fourth trial of the OST, the performance control odors were presented and the participant was asked to report which odor they had been asked to remember at the beginning of the session. This was done to determine whether odor discrimination and motivation to perform had been maintained across trials of the session. For each trial, the experimenter recorded each response as a hit, or a false alarm, and overall performance was reported in terms of span, longest span and percent correct accuracy. Accuracy within each session was computed for each participant for both the span component and the performance control component. Accuracy at each span length (memory load) was computed by averaging accuracy at each span length (trial number) across participants.

Digit Span. To administer the digit span task, the experimenter read strings of digits to the participant at a rate of 1 digit per second and the participant was instructed to repeat the string of digits back to the experimenter. Administration included a digit forward component, where participants were instructed to repeat each string in the order presented by the experimenter, and a digit backward component where strings were to be repeated in reverse order. Digit string lengths incremented from 2 to 9 digits during the course of the digit forward component, and from 2 to 8 digits during the digit backward component. Participants were given 2 trials at each string length in both the forward and backward components, and each component was discontinued when the participant failed both trials of a digit string length. Measures derived from digit span performance included the number of correct trials within each component, longest string recited in each component, and total correct trials. The digit span task has sound psychometric properties including good test-retest reliability ($R = .83$) across approximately one month for individuals within the age range of the present study (Psychological Corporation, 2002).

Spatial Span. The spatial span task was administered in a manner analogous to the digit span with the exception that instead of verbally presenting strings of digits, the experimenter sequentially pointed to blocks which were identical in appearance but were located on discrete spatial positions of a board. Participants were then required to reiterate the string of spatial locations by sequentially pointing to each block in the string. As in the digit span task, the spatial span task included both a forward and backward component during which string lengths incremented with two trials at each string length.

As with digit span, spatial span yielded measures including the number of correct trials in each component (forward and backward), the longest string length completed in each

component, and total correct trials. The spatial span has sound psychometric properties and good test-retest reliability (*R* = *.72*) across approximately one month (Psychological Corporation, 2002).

Letter-number Sequencing. To administer the letter-number sequencing task, the experimenter read strings of digits and letters to the participant who was instructed to repeat the numbers first in ascending order, followed by the letters in alphabetical order. String lengths incremented from 2 to 9 digits and letters across the course of the task with 2 trials at each string length. The task was discontinued when the participant failed to correctly recite two consecutive strings of the same length. The primary outcome measures were total correct trials and the longest letter-number sequence recited correctly. The letter-number sequencing task has sound psychometric properties and good test-retest reliability (*R* = *.71*) across approximately one month (Psychological Corporation, 2002).

N-back (0-back and 2-back). The N-back task is a computerized assessment of working memory designed to assess performance across increasing memory loads. Participants were presented with sequences of uppercase letters on a computer screen and were required to respond to each stimulus according instructions specific to each component of the task. During the 0-back (non-mnemonic) component of the task, participants were instructed to press the leftmost button of the response box when the presented stimulus matched a pre-specified target stimulus (i.e., an "X") and to press the rightmost button of the response box when the presented stimulus was any other letter than the target.

During the 2-back (mnemonic) component, participants were instructed to press the leftmost button of the response box when the presented stimulus matched the stimulus that had been presented 2 stimuli prior, and to press the rightmost button when the stimulus did not

match. Participants were instructed to keep both of their hands on the response box for the duration of the task and to enter responses as quickly as possible with their corresponding index finger (left or right). Practice trials were administered to verify understanding the task prior to performing each of the N-back components, which were administered in a fixed order (0-back then 2-back).

There were a total 180 trials for each component (0-back and 2-back) divided into three blocks of 60 trials. The 180 trials for each component included 54 targets and 126 non-targets, equally distributed across the three blocks in that condition. The stimuli were presented one at a time in the center of the monitor for 250 milliseconds with an intertrial interval of 2 seconds. Each of ten uppercase letters (height = 3 cm) were presented on 10% of trials, or 18 times per condition (6 times per block).

Rapid Visual Information Processing Task (RVIP). The RVIP (Wesnes & Warburton, 1983) is a neurocognitive measure of sustained attention/vigilance. Participants were presented with a string of digits on computer monitor at a rate of 100 digits per minute and were instructed to respond by pressing the leftmost button of a response box when three consecutive even or odd digits were presented. Eight target stimuli appeared during each minute, separated by between 5 and 30 foils. Participants were instructed to keep both of their hands on the response box for the duration of the task and to enter responses as quickly as possible with their left index finger. Responses within 1,500 milliseconds of a target were scored as a hit while all other responses were scored as a false alarm. The task took approximately 4 minutes to complete and yielded measures of hits, false alarms, and hit reaction time.

Primary Data Analyses

Two-way repeated measures analysis of variance (ANOVA) tests were conducted to test within-subject effects of nicotine dose by dose order on the performance of cognitive tasks and self-reported attentional control. For the analysis of each task the threshold of significance ($p < .05$) was adjusted to account for the number of measures obtained from that task. For variables in which the assumption of sphericity was violated a Greenhouse-Geiser correction was made to degrees of freedom.

Significant main effects were explored post-hoc, using simple effects analysis with a Bonferroni correction for multiple comparisons. Digit span and spatial span each yielded four raw scores (total and longest span forward, total and longest span backwards) while letter-number sequencing task yielded only total score and longest span. N-back and RVIP measures consisted of percent correct accuracy on target stimuli, and distractor (non-target) stimuli. Additionally, RVIP reaction time for hits and N-back reaction time to targets and distractors were computed. Measures derived from performance of the OST included span (number of correct consecutive trials minus one), longest span (the longest string of consecutive correct choices at any point after the first trial) and accuracy, which was computed for both span and odor discrimination components.

Validity of the OST was assessed in several ways. Bivariate correlation was used to determine whether human accuracy on the OST is capacity dependent as is observed in rats (MacQueen et al., 2011). To conduct this analysis, mean accuracy for each trial of the span component of the OST was computed across participants from data obtained during the placebo administration session. Accuracy of the OST was then submitted to a correlational analysis with the span length at which each estimate was determined. Capacity dependence was to be

identified by a significant negative correlation coefficient. Additionally, convergent validity of the OST with other measures of working memory and attention were assessed through a series of bivariate correlations in which relevant dependent variables derived from the cognitive measures were correlated with total session OST accuracy, span, longest span as well as odor discrimination training accuracy odor discrimination performance.

RESULTS

Participant Characteristics

Thirty participants (14 male), ranging from 18 to 48 years of age (*M*=25.48, *Std. Dev.* = 7.65), completed all three experimental sessions. As presented in Table 1 (see page 27), two-thirds of the sample identified as Caucasian, with the remainder identifying as African American, or Asian. Nearly one third of the sample identified as Hispanic or Latino. One participant identifying as Hispanic or Latino did not report on race, and one participant identifying as African American did not report on ethnicity. All but one participant had completed high school and 90 percent of the sample had completed some college or earned a degree. On average, participants had completed 14.97 years of education at the time of study enrollment.

Subjective Effects of Nicotine

Feeling State Questionnaire (FSQ)

Responses to the six items of the FSQ were submitted to a mixed two-way analysis of variance (ANOVA) evaluating the effects of nicotine dose and the between subject factor of dose order. As depicted in Figure 2, participants reported feeling significantly more nauseous [$F(1.50,40.41) = 10.775$, $p = .001$], sick [$F(1.45,39.04) = 12.514$, $p < .001$], and dizzy [$F(1.52,41.02) = 21.358$, $p < .001$] after receiving gum containing nicotine. In each case, simple effects analysis revealed that subjective report of these sensations was significantly greater after receiving the high dose when compared with the low or placebo doses (bonferroni corrected $p < .05$). The effect of nicotine trended toward significance with regard to reports of irritability

[F(2,54) = 3.476, p = .038], and attentiveness [F(2,54) = 4.178, p = .021], but did not reach the family-wise error adjusted significance threshold (p < .008). No effect of nicotine was observed on reports of feeling "jittery" [F(2,54) = 1.620, p = .207]. No effects of dose order, or nicotine dose by dose order interactions were detected for any of the FSQ items (all ps > .05).

Table 1. *Demographic Characteristics*

	n	*%*
Gender		
Male	16	53.3
Female	14	46.7
Ethnicity		
Hispanic or Latino	9	30.0
Not Hispanic of Latino	20	66.7
Not reported	1	3.3
Race		
Caucasian	20	66.7
African American	7	23.3
Asian	2	6.7
Not reported	1	3.3
Education Level		
Some High School	1	3.3
Completed High School	2	6.7
Some College	20	66.7
Completed College	4	13.3
Some Graduate Work	1	3.3
A Graduate Degree	2	6.7

Positive Affect/Negative Affect Scale (PANAS)

Review of PANAS responses revealed that a single participant failed to provide responses to the second column of PANAS items after receiving the high dose of nicotine gum. As a result, this participant was excluded from analyses of PANAS measures (which included

the remaining 29 participants for whom complete data was available). Positive affect and negative affect scales derived from responses on the PANAS were submitted to a mixed two-way ANOVA of nicotine dose by dose order. No effect of nicotine, dose order, or their interaction was detected with regard to positive affect (*p*s > .05). For negative affect, the main effect of nicotine dose increasing negative affect trended towards significance [$F(1.50,42.18) = 4.146$, $p = .033$], but did not reach the adjusted significance threshold ($p < .025$). No effect was observed for dose order or its interaction with nicotine dose.

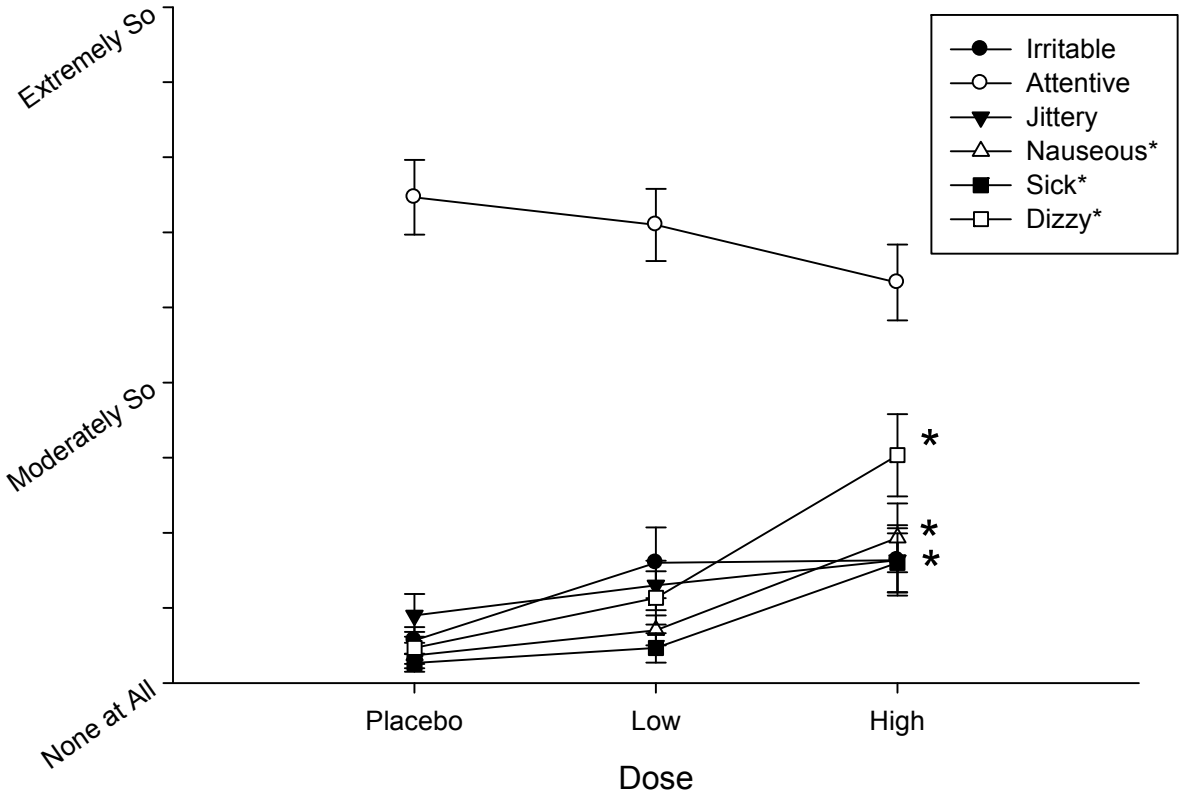

Figure 2. *Effects of nicotine dose on the Feeling State Questionnaire items. Asterisks indicate a significant difference relative to placebo.*

Wisconsin Smoking Withdrawal Scale

As presented in Figure 3, nicotine dose produced significant effects on the endorsement of the three WSWS items, "my level of concentration is excellent" [$F(2,54) = 6.233$, $p = .004$], "it is hard to pay attention to things" [$F(1.38,37.12) = 5.538$, $p = .015$], and "It's difficult to think clearly" [$F(1.52,41.07) = 6.473$, $p = .007$]. Simple effects analysis revealed that the high nicotine dose significantly reduced endorsement of excellent concentration, and significantly increased endorsement of difficulty thinking and paying attention relative to placebo (bonferroni adjusted $ps < .05$). Dose order produced a significant effect on endorsement of difficulty thinking [$F(2,27) = 4.853$, $p = .016$], but not on endorsement of difficulty paying attention, or excellent concentration ($ps > .05$). Participants assigned to receive the high nicotine dose during the first session (followed by placebo and then the low dose in subsequent sessions), reported significantly greater difficulty thinking when compared with participants who received the low dose followed by the high dose and placebo (bonferroni adjusted $p < .05$). A nicotine dose by dose order interaction was not detected for any of the three WSWS items.

Effects of nicotine on attention and working memory

Odor Span Task

Two-way mixed ANOVAs of nicotine dose by dose order (3 orderings) revealed no significant main effect of dose or dose order on any of the odor span measures (Odor span accuracy, span, longest span, simple discrimination training, and simple discrimination performance; all p's $>.05$). Odor span performance collapsed across dose order conditions is illustrated in Figure 4 (see page 31).

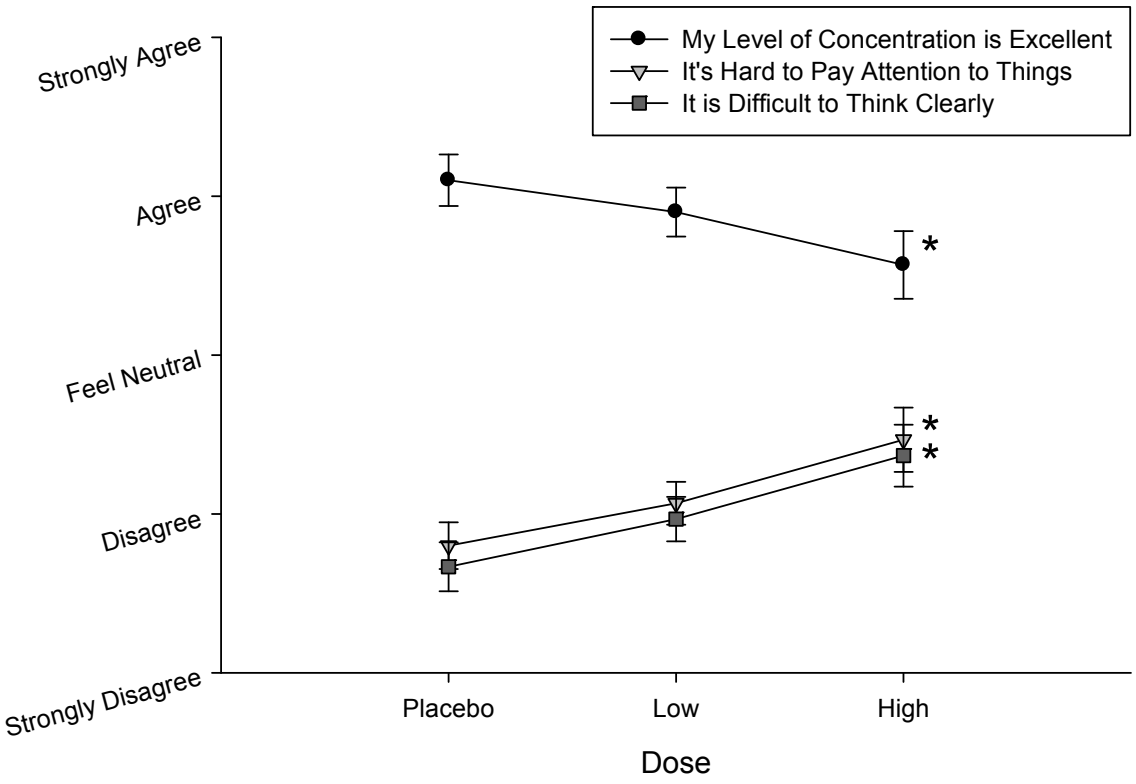

Figure 3. *Effects of nicotine dose on selected Wisconsin Smoking Withdrawal Scale items. Asterisks indicate a significant difference relative to placebo.*

A nicotine dose x dose order interaction was detected for odor span accuracy [$F(4,54) = 2.967$, $p = .027$], span [$F(4,54) = 3.910$, $p = .007$], and longest span [$F(4,54) = 3.910$, $p = .007$]. However, the interaction observed with odor span accuracy did not meet the family-wise error adjusted significance threshold ($p < .01$). Nicotine dose by dose order interactions for span and longest span were characterized with one-way ANOVAs of nicotine dose within each of the three dose order groups. No effect of dose on span or longest span was detected which reached the family-wise error adjustment significance level ($p < .01$) in any of the three dose order groups on span or longest span.

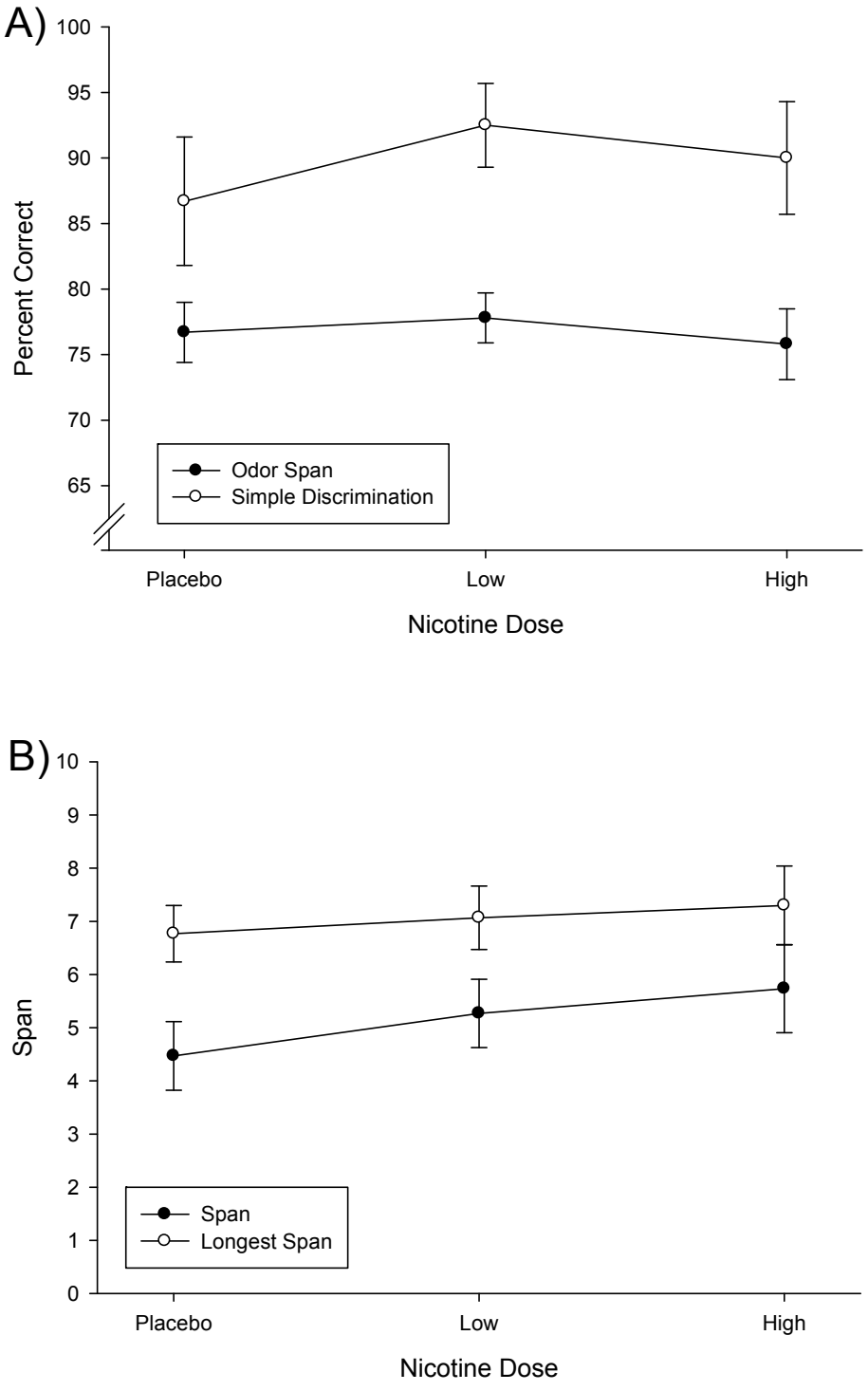

Figure 4. *Effect of nicotine dose on odor span measures: A) Odor span and simple discrimination accuracy B) Span and longest span measures.*

Digit Span

Two-way mixed ANOVAs of nicotine dose by dose order revealed no significant main effects of dose or dose order, and no significant interactions between these variables on digit span measures (forward, backward, total, longest forward span, and longest backward span; all *ps* > .05).

Spatial Span

Nicotine dose by dose order ANOVAs revealed no significant main effects of either nicotine dose or dose order on spatial span measures (forward, backward, and total; *ps* > .05). However, significant nicotine dose by dose order interactions were detected on both the spatial span backward [F(4,54) = 3.812, $p = .008$], and total [F(4,54) = 4.302, $p = .004$] measures. Interactions were characterized with one-way repeated measures ANOVAs of nicotine dose for each dose order group. These analyses revealed no significant effect of nicotine dose on spatial span backward within any of the three dose order groups (all *p*s > .05). With regard to the spatial span total measure there was a significant effect of dose amongst participants assigned to receive the low dose, followed by the high dose and placebo in subsequent sessions [F(1,18) = 6.695, $p = .007$]. Simple effects analysis revealed that performance after receiving the low dose (but not high dose) was significantly reduced relative to placebo (bonferroni corrected $p < .05$). No effects of nicotine dose were detected in the placebo first or high dose first dose order conditions.

Letter-number sequencing (LNS)

Two-way mixed ANOVAs of nicotine dose by dose order revealed no significant main effects of dose or dose order, and no significant interactions between these variables on LNS total or longest sequence (*ps* > .05).

0-back

Nicotine dose by dose order analyses exposed no significant main effects of nicotine dose or dose order and no significant interaction between these variables on any of the 0-back measures (target accuracy, distractor accuracy, target reaction time and distractor reaction time, all *p*s > .05).

2-back

Review of 2-back performance revealed that during a single session, one participant responded only to target stimuli and failed to respond to distractor stimuli. As a result, this participant was excluded from analyses of 2-back performance. Amongst the remaining 29 participants who completed the 2-back task during all three experimental sessions, mixed two-way ANOVAs of nicotine dose and dose order found no significant main effect of either variable on any 2-back performance measure (target accuracy, distractor accuracy, target response time, or distractor response time; all *p*s > .05). However, significant nicotine dose by dose order interactions were detected for both the target reaction time [$F(4,52) = 4.652$, $p = .003$] and distractor reaction time [$F(4,52) = 3.862$, $p = .008$] measures. These interactions were characterized with one-way ANOVAs evaluating the effect of nicotine dose within each dose order condition.

A significant effect of nicotine dose on target reaction time was detected for participants assigned to receive the high nicotine dose during the first session [$F(2,18) = 5.788$, $p = .011$]. Participants within this group were significantly slower in responding to target stimuli after receiving the high dose gum relative to placebo (bonferroni adjusted $p < .05$). No effect of nicotine dose on target reaction time was detected amongst participants in either of the other two dose order conditions. With regard to distractor reaction time, a significant effect of nicotine

dose was detected amongst participants who received the placebo dose first [$F(1.58,14.19) = 7.941$, $p = .007$]. Simple effects analysis revealed that participants within this group produced significantly reduced reaction time to distractor stimuli after receiving the high dose relative to placebo (bonferroni adjusted $p < .05$). No effect of nicotine dose on distractor stimuli reaction time was detected for participants in either of the other two dose order conditions (ps > .05).

Rapid Visual Information Processing (RVIP)

Two-way mixed ANOVA of nicotine dose by dose order revealed a significant effect of nicotine dose on RVIP target accuracy [$F(2,54) = 4.586$, $p = .014$]. As presented in Figure 5, RVIP target accuracy was significantly greater after participants received the high dose nicotine gum when compared with placebo (bonferroni corrected $p < .05$). No effect of dose order or a nicotine dose by dose order interaction was detected for any of the RVIP measures (target accuracy, false alarm rate, and hit reaction time; all ps > .05). Nicotine dose produced no significant main effects on either RVIP false alarm rate or hit reaction time.

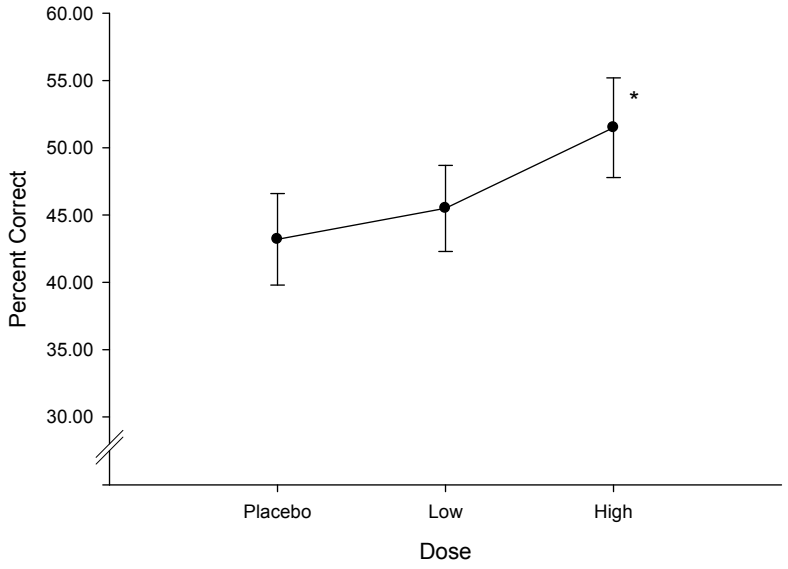

Figure 5. *Effects of nicotine dose on RVIP target accuracy. Asterisks indicate a significant difference relative to placebo*.

Validation of the Odor Span Task

During the placebo gum session, two thirds of participants provided perfect performances (10/10 correct responses) during odor discrimination training (m = 9.37, std = 1.16). During completion of the OST (after placebo), 80% provided perfect performance on all four odor discrimination trials (m = 86.67%, std = 26.86%) while a single participant failed to perform above chance. Span ranged from 0 to 16 (m = 4.47, std = 3.53), longest span ranged from 3 to 16 (m = 6.77, std = 2.91), and average accuracy was 76.67% (std = 12.34%) with a single participant failing to perform above chance. To evaluate the effect of memory load on accuracy, accuracy was computed across participants at each trial number (span length/memory load). As reported above, nicotine produced no significant effects on odor span accuracy. As such, accuracy for all three sessions (placebo, low dose, and high dose) were averaged for each participant to produce a more reliable estimate of accuracy at each span length.

A bivariate correlation analysis was then conducted to assess the relationship between accuracy and memory load. Accuracy at trial 1 was excluded from this analysis as participants were only presented with a single odor during this trial and thus accuracy was 100%. As depicted in Figure 6, a significant negative correlation (r = -.528, $p < .05$) was detected between accuracy and the number of odors to remember reflecting a capacity dependent effect on odor span accuracy. Figure 6 depicts accuracy on the four odor discrimination trials, which were conducted after every 5th trial of the OST, collapsed across doses. A bivariate correlation analysis was not appropriate for this data given the relatively few number of odor discrimination trials conducted during the OST. However, as illustrated in Figure 7, a one-way repeated measures ANOVA of odor discrimination found no effect of trial number on accuracy, $F(3,87)$ = .809, $p > .05$.

Bivariate correlations were also used assess the association between measures derived from the odor span task after receiving placebo gum. An adjusted significance threshold of $p < .005$ was used to control for the number of associations considered (10). As noted a single participant was found to perform below chance levels on odor discrimination and the odor span during the placebo gum session. This same participant was also found to be an outlier (two standard deviations above or below the mean) on several other measures of performance (0-back target reaction time, 0-back distractor reaction time, 0-back hit rate, 0-back false alarms). Given exceptionally poor performance on several variables, this participant was excluded from all correlational analyses so as not to inflate the significance of any observed relationships. All correlational analyses were conducted with the remaining 29 participants.

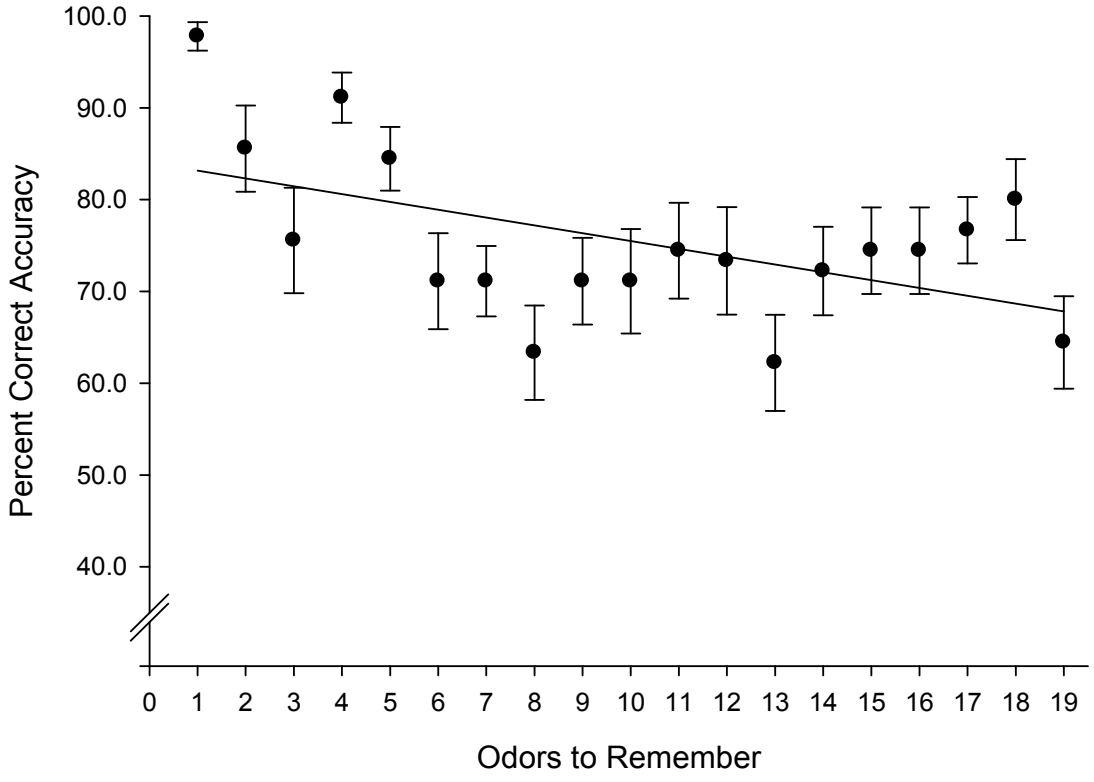

Figure 6. *Average accuracy on odor span trials collapsed across dose conditions.*

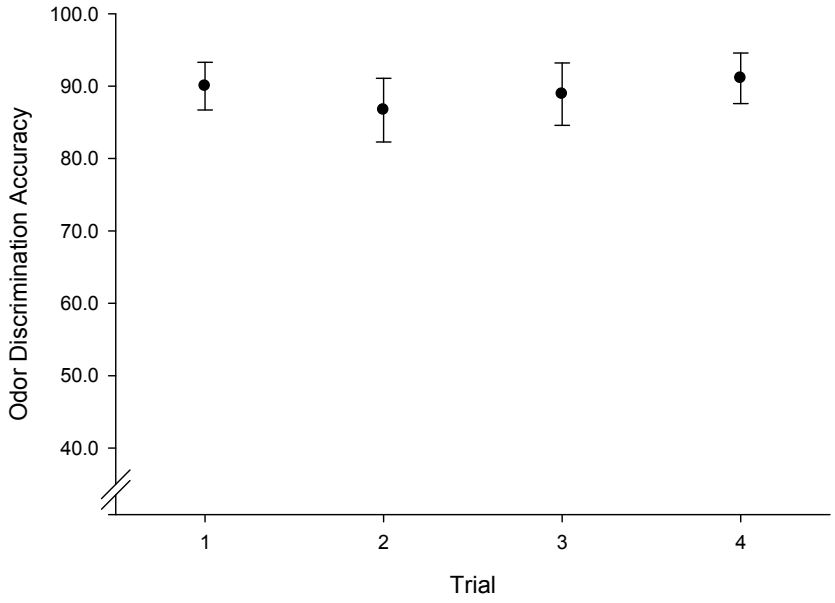

Figure 7. *Average odor discrimination trial accuracy averaged across dose conditions.*

It was expected that measures intended to evaluate the mnemonic components of the OST (percent correct accuracy, span, and longest span) would be modestly to strongly related, while measures of olfactory discrimination (simple discrimination training and simple discrimination accuracy) would relate with each other but not mnemonic indexes. Consistent with these hypotheses, simple discrimination training and simple discrimination accuracy demonstrated a strong significant positive association ($r = .714$, $p < .001$). The relationship between simple discrimination practice trials and span trended towards significance ($r = .441$, $p = .017$) but did not reach the adjusted significance threshold. Neither simple discrimination training nor accuracy provided a significant association with any of the other mnemonic odor span measures (all ps $> .05$). As presented in Table 2, each of the three mnemonic odor span indices demonstrated a significant association with the other two mnemonic measures (rs ranging from .565 - .711; all ps $\leq .001$).

Table 2. *Correlations Between Odor Span Measures*

Measure	1	2	3	4	5
Training and Control Measures					
1. Odor Discrimination Training	-				
2. Odor Discrimination Accuracy	**.714***	-			
Mnemonic Measures					
3. Percent Correct Accuracy	.353	.369†	-		
4. Span	.441†	.254	**.565***	-	
5. Longest Span	.181	.198	**.711***	**.658***	-

† $p < .05$, * $p < .005$. Adjusting for multiple comparisons within each odor span measure, associations meeting a threshold of $p < .005$ were deemed significant.

Bivariate correlations were also used to assess the association between odor span measures and measures derived from other tasks of attention and working memory (e.g., digit span, spatial span, letter-number sequencing, RVIP, 0-back, and 2-back) after placebo gum administration. As each odor span measure was compared with 21 measures derived from the alternative cognitive tasks, the threshold used to identify significant associations ($p < .002$) was determined by adjusting for the number of comparisons made with each odor span measure. Table 3 presents the strength of association of odor span performance with other cognitive measures. As expected, neither of the simple olfactory discrimination measures derived from the odor span task (simple discrimination training and accuracy) produced a significant association with any of the measures from the 6 cognitive tasks (all *p*s > .05).

As depicted in Figure 8, odor span accuracy demonstrated a moderate significant positive association with 0-back hit rate ($r = .589$, $p = .001$) . Similar positive trends were observed between span and 0-back hit rate ($r = .374$, $p = .046$) and longest span and 0-back hit rate ($r = .444$, $p = .016$) though these effects did not reach the adjusted significance threshold. Trends were also observed between longest span and 2-back hit rate ($r = .414$, $p = .026$). Both span and OST accuracy produced trend level associations with 2-back target reaction time ($r = .374$, $p =$

.045 for both associations). The span measure additionally showed a trend level positive association with 2-back distractor reaction time (r = .396, p = .033) however, none of the relationships between span or longest span and the N-back measures reached the adjusted significance threshold.

Table 3. *Correlations between odor span and cognitive measures*

	SD Practice	SD Accuracy	Span	Longest Span	OS Accuracy
Digit Span					
Forward	-.247	-.060	-.103	.225	-.239
Backward	.031	.156	.154	.306	.128
Total	-.136	.042	.014	.292	-.082
Longest Forward	-.075	.104	-.190	.001	-.322
Longest Backward	.084	.149	.203	.354	.240
Spatial Span					
Forward	-.156	-.050	-.165	-.051	-.297
Backward	.028	-.111	-.066	.041	.024
Total	-.083	-.096	-.151	-.021	-.156
Letter-Number Sequencing					
Longest Sequence	.045	.177	.005	.098	.051
Total	-.292	-.130	-.125	.205	-.066
0-Back					
Hit Rate	.070	.146	**.374†**	**.444†**	**.589***
False Alarm Rate	-.137	-.230	-.262	-.328	-.317
Target Reaction Time	-.042	.056	-.245	.313	.167
Distractor Reaction Time	-.099	.027	-.259	.281	.157
2-Back					
Hit Rate	-.231	-.010	.086	**.414†**	.337
False Alarm Rate	.253	.085	.045	-.344	-.149
Target Reaction Time	.179	.171	**.374†**	.313	**.374†**
Distractor Reaction Time	.144	.116	**.396†**	.281	.294
RVIP					
Hit Rate	.002	.147	-.047	.067	.242
False Alarm Rate	-.045	.154	-.111	-.172	-.129
Hit Reaction Time	.202	.203	-.029	-.159	-.122

*† $p < .05$, * $p < .002$. Adjusting for multiple comparisons within each odor span measure, associations meeting a threshold of $p < .002$ were deemed statistically significant.*

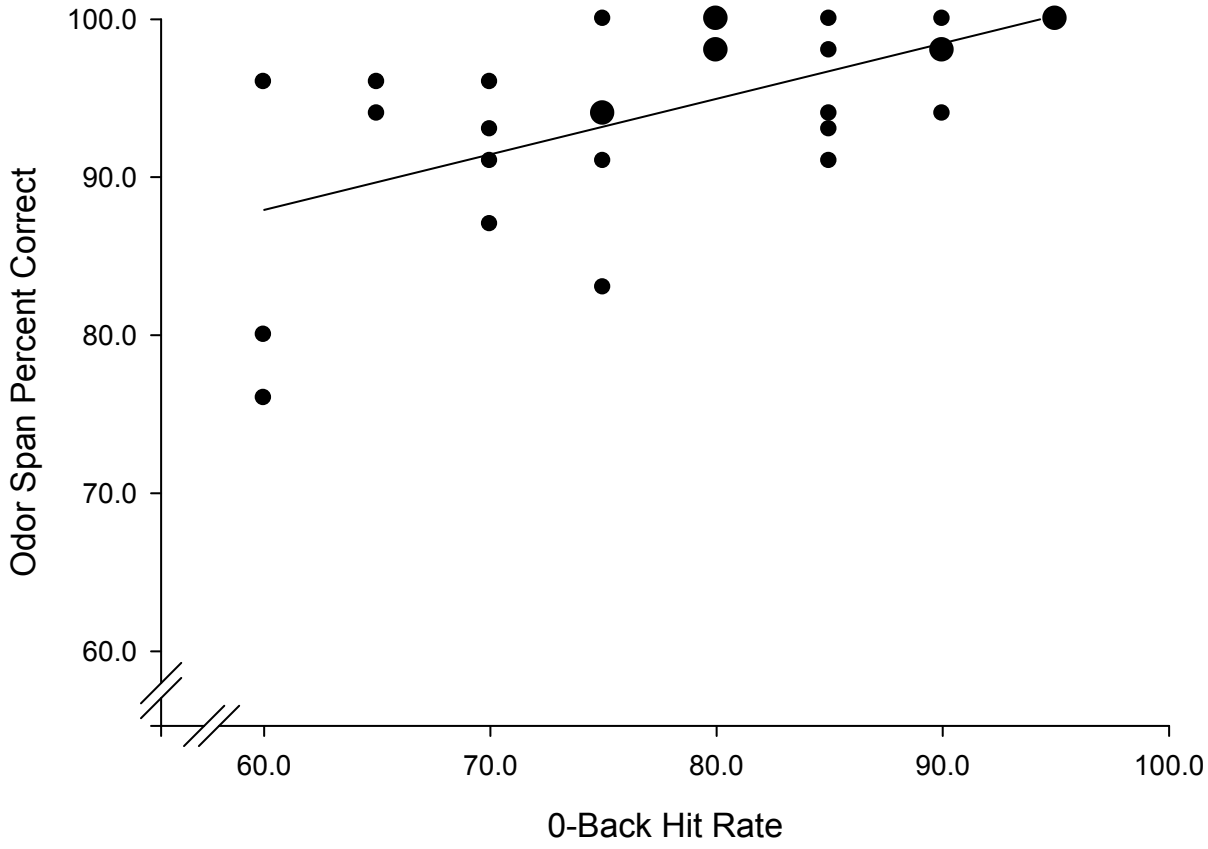

Figure 8. *Relationship between OST accuracy and 0-back hit rate during placebo sessions.*

DISCUSSION

A primary goal of the present study was to assess the effects nicotine on cognition, as it has been suggested that cognitive enhancement may be amongst the factors which motivate and reinforce tobacco smoking (Evans & Drobes, 2009). Amongst non-smoking participants, nicotine dose-dependently improved RVIP accuracy, indicating direct enhancement of attention. This corroborates earlier findings of improvement of "fast hits" (hits occurring in ≤450 ms) on the RVIP task in non-smokers receiving subcutaneous injections of 0.3 and 0.6 mg of nicotine (Foulds et al., 1996) and a trend towards improved RVIP hits in non-smokers after nicotine delivered by an inhaler (File, Fluck, & Leahy, 2001). Results of another recent study also confirm improvement in RVIP accuracy after administration of nicotine gum (4mg) in non-smokers (Knott et al., 2011). However, in contrast with Foulds et al. (1996) and Knott et al., (2011) significant reductions in RVIP hit reaction time were not detected presently.

The RVIP is a target detection task which has been described as a measure of sustained attention or vigilance (Wesnes & Warburton, 1983). Sustained attention refers to behavioral performance in detecting stimulus events which occur infrequently and unpredictably over extended periods of time (Sarter, Givens, & Bruno, 2001). In addition to variants of the RVIP, alternative continuous performance tasks have been used to assess the effects of nicotine on sustained attention, revealing robust enhancements amongst smokers in deprivation and more limited evidence of enhancement in non-smokers (Heishman et al., 2010; Heishman et al., 1994). Performance on sustained attention tasks is critically mediated by cholinergic projections

stemming from the basal forebrain which serve to alter the processing of sensory stimuli in distributed areas of cortex as part of a right hemisphere lateralized fronto-parietal network (Sarter et al., 2001). It has been suggested that the effect of nicotine on sustained attention is produced primarily by augmentation of the basal forebrain cholinergic system, which is also influenced by manipulations of GABAergic and glutamatergic signaling (Sarter et al., 2001).

Interestingly, the enhancement of RVIP performance reported presently occurred in the context of self-reported impairments in attention and concentration. That participants reported increased difficulty paying attention and increased difficulty with thinking and concentration after receiving the high dose nicotine gum suggests that the enhancement of RVIP accuracy is not easily accounted for by participant expectancies regarding the cognitive effects of nicotine. Further, the lack of an effect of nicotine on RVIP reaction time counters the suggestion RVIP effects were produced by enhancement of sensorimotor abilities. Analyses evaluating the interactive effect of nicotine dose with the order in which doses were received, produced no evidence that the effect of nicotine on RVIP performance was moderated by dose order.

The present study also included other measures which have been suggested to index aspects of attention, most notably the 0-back component of the N-back task. The 0-back is essentially a target identification task, requiring participants to respond “yes” or “no” as to whether each stimulus that is presented matches a predefined target. In contrast to the RVIP, which requires participants to respond only when three even or odd digits are presented in a row (thus accurate responding requires continuously updated memory of the last two stimuli presented), the 0-back task has no mnemonic demand. As such, one might expect more pronounced effects on the 0-back (when compared with the RVIP) if the cognitive effects of nicotine are primarily constrained to attentional processes. However, several factors need to be

considered when interpreting the observed enhancement of RVIP in the absence of effects on 0-back accuracy. In the present study, RVIP hit rate (target accuracy) was approximately 43% (median = 42%) during sessions in which participants received placebo gum, compared with approximately 93% (median = 95%) for 0-back hit rate. As such, a ceiling effect may have limited the ability to observe an enhancement effect of nicotine on 0-back accuracy. Although the 0-back task required attention to be sustained over a longer period of time (nearly 7 minutes compared with 4 minutes for RVIP), the speed at which stimuli were presented (1 stimulus every 600ms for RVIP compared with 2250ms for 0-back), and the infrequency with which a response was required (only after targets for RVIP compared with every trial for 0-back) are likely critical aspects of the RVIP which create greater demands upon sustained attention, yielding greater difficulty than 0-back.

Compared with the RVIP, the effect of nicotine on N-back accuracy has been studied more extensively in non-smokers, using a variety of methods for the administration of nicotine (See Heishman et al., 2010). In a prior investigation which used an identical placebo controlled nicotine gum administration procedure with never-smokers, nicotine failed to produce effects on visualspatial, or phonological 2-back or 3-back accuracy (Kleykamp et al., 2005). Across a range of delivery methods, nicotine generally has not produced significant effects in N-back accuracy amongst non-smokers (Heishman et al., 2010). However, a notable exception is found in a study reported by Kumari et al. (2003) in which subcutaneous doses of nicotine (12 µg/kg body weight) were delivered to 11 male non-smokers who subsequently completed an N-back task (0-back, 1-back, 2-back, and 3-back) while undergoing functional magnetic resonance imaging (fMRI). Compared with performance after subcutaneous delivery of saline, nicotine improved accuracy across the N-back conditions tested. Given that nicotine induced

enhancement of N-back accuracy has not typically been observed, even in studies with larger sample sizes (and presumably greater power to detect such effects), certain aspects of the methods used in this study are worth mentioning.

The Kumari et al. (2003) study utilized a modified version of the N-back task, previously described by Callicot et al. (1999), in which both visuospatial and visual/symbolic cues could be used in the service of accurate responding. Only four digits were presented during the task (1-4) and each digit was only ever presented in a distinct spatial position (one of the four corners of the screen). This contrasts with more typical procedures in which all stimuli are presented in the center of the screen. Thus participants could respond based upon the spatial position of the stimulus, the phonological or symbolic content of the stimulus, or a combination of these factors. Additionally, instead of providing a yes/no match response, participants were required to respond to each stimulus by pressing the response button which was associated with the stimulus presented N positions back, depending on the component being tested (i.e., 0-back, 1-back, 2-back or 3-back). Either of these task modifications may have introduced additional neurocognitive demands which are more readily augmented by nicotine.

The inclusion of a spatial element seems unlikely to be critical as a prior study which tested phonological and spatial N-back performance separately found no effect of nicotine on either task in never-smokers (Kleykamp et al., 2005) and no effects of nicotine were found in the spatial working memory task (spatial span) included in the present study. However, it remains conceivable that the ability to use a preferred cue or a combination of cue modalities influences the detection of nicotine effects in non-smokers. In addition to the unique response requirement (identifying the stimulus which occurred N positions back), the N-back task used by Kumari, et al. (2003) also utilized a more rapid stimulus presentation than is typical for N-back

tasks (1 stimulus every 1750 ms). Future investigations in which these parameters of the N-back task (cue modality, response requirement, and stimulus presentation rate) are explicitly manipulated may help clarify domains of cognitive performance enhanced by nicotine in non-smokers.

In the present study, no main effect of nicotine dose was detected on any of the measures derived from common clinical assessments of working memory (i.e., digit span, spatial span, and letter-number sequencing). While these tasks receive widespread use in the clinical practice of neuropsychology, they have been less commonly used to evaluate to the cognitive effects of smoking/nicotine when compared with experimental/computerized tasks such as the RVIP and N-back. In contrast with measures of sustained attention, abstinence amongst smokers has not been associated with impairments in digit span performance (Merritt, Cobb, & Cook, 2012; Merritt, Cobb, Moissinac, & Hirshman, 2010) and no evidence of nicotine-induced enhancement of digit span was detected in a study which included both smokers and non-smokers (Jones, Sahakian, Levy, Warburton, & Gray, 1992). To our knowledge, the present study represents the first test of the effects of nicotine on the spatial span and letter number-sequencing tasks conducted in healthy non-smokers. However, nicotine did not reliably enhance performance of either task.

The findings reported presently are largely consistent with a meta-analysis of prior studies regarding the effects of nicotine on cognition in never-smokers, ex-smokers, and non-deprived smokers (Heishman et al., 2010). That is, we detected significant enhancement of sustained attention accuracy (as measured by RVIP performance), but no effects of nicotine on accuracy across a range of experimental and clinical measures of working memory. The parameters of working memory remain hotly contested and the tasks which are used to assess

working memory performance in clinical settings, such as the Digit Span, Letter-Number Sequencing, Arithmetic, and Spatial Span subtests of the Wechsler intelligence and memory batteries (Wechsler, 1997a, 1997b), differ substantially from tasks more commonly used to assess this construct in cognitive research (i.e., N-back, Operation Span, Listening Span).

A benefit of the Wechsler tests is that they are highly standardized and normative data is readily available. However, it has been suggested that contemporary lab-based working memory tasks are more consistent with defining aspects of the working memory construct, have better predictive ability in discriminating clinical pathology, and are associated with similar neurobiological substrates (Shelton, Elliott, Hill, Calamia, & Gouvier, 2009). Yet, there remains a dearth of information available on the relationship between clinical and experimental tasks of working memory. Further, the lack of standardization of experimental tasks complicates between study comparisons. One study directly comparing the working memory subtests of the Wechsler batteries with experimental tasks (including a word recall variant of the N-back) reported significant but relatively modest associations (*r*s ranging from .23-.54) between clinical and experimental tasks (Shelton et al., 2009). Thus, each task likely captures unique aspects of mnemonic performance that may depend in part on distinct neurocognitive processes.

The odor span task may provide a useful model for comparing mnemonic performance across species and outlining the neurobiological substrates involved. With regard to the present investigation of nicotine effects, human odor span performance was of particular interest in that manipulations of cholinergic signaling have been found to impact odor span performance in rodents (Rushforth et al., 2010; Turchi & Sarter, 2000; Young, Crawford, et al., 2007). However, no main effect of nicotine was detected on any of the measures derived from the human odor span task in the present study. Significant interactions between nicotine dose and

dose order were detected for both the span and longest span measures however, follow-up analyses evaluating the effect of dose within each of the three dose order groups failed to uncover any significant effects of dose. There was however, a trend for increased span at the high dose ($p = .057$) for those who received the low dose first (followed by the high dose and then placebo on subsequent sessions), and for increased span at the low dose ($p = .084$) for those who received the high dose first (followed by placebo, then the low dose). Similarly, while no significant effects of dose were detected in either of the 3 groups with regard to longest span, there was a trend towards increased span at the high dose amongst participants who received the low dose during their first session ($p = .088$). In each of these cases there was a trend towards increased span (or longest span) during the second session in which nicotine was administered, regardless of whether the participant received the low or high dose during the second nicotine session.

Thus, it is conceivable that recent exposure to nicotine is necessary before enhancement of odor span performance can be detected in non-smokers. While not conclusive, it is notable that the means for both span and longest span followed a pattern of dose dependent facilitation. A similar pattern was not observed with regard to overall odor span accuracy. Nicotine effects on overall accuracy has yet to be assessed in rats. A prior study of the effects of nicotine used a procedure in which the task was terminated after the first error was observed and thus, did not yield an overall accuracy measure (Rushforth et al., 2011).

The human adaptation of the odor span task used presently built upon the methods described by Levy et al., (2003) by including control procedures previously implemented in the odor span for rats (MacQueen et al., 2011). This included training subjects on an olfactory discrimination of two odors not used in the span procedure which was subsequently tested at four

points during completion of the odor span task. As in rats, humans achieved higher accuracy on simple discrimination trials (86.7%) when compared with odor span trials (76.7%) after placebo and after both nicotine doses. Odor span accuracy was somewhat lower than averages observed in healthy controls (90.6%) in the Levy et al. (2003) study. However, this was somewhat expected as the present design included 20 odors whereas the previous design included only 14. The present study also observed lower mean span (4.47 after placebo) than has been previously reported. Though, as has been observed in rats (April, Bruce, & Galizio, 2013; Galizio, Deal, Hawkey, & April, 2013; MacQueen et al., 2011), participants frequently responded with high rates of accuracy even after an initial error. As such, longest span (the longest string of consecutive correct responses observed after trial 1) was considerably higher (6.77 after placebo) and more consistent with the span lengths typically reported for animals and in the prior human study.

In the original version of the OST for rats, and in the prior human odor span study, the number of odors to be remembered on any given trial was inherently confounded with the number of stimuli presented, and thus chance performance, during that trial. In rats, accuracy has been shown to decrease as the number of comparison stimuli presented is increased (April et al., 2013). This observation complicates evaluation of the association between memory load and performance in designs in which the number of comparisons increments across trials. In the present study, this issue was avoided by presenting no more than two stimuli (one novel odor and one comparison) on any given trial. As a result, chance performance was equated across all trials beyond the first at 50%. As is observed with rats, a negative association between accuracy and the number of odors to be remembered was detected in our human participants. A similar trend was not observed across odor discrimination trials conducted after every fifth OST trial

suggesting that declining accuracy is not easily accounted for by fatigue or progressive impairments in odor discrimination or task motivation. However, it should be noted that with an OST procedure that limits the number of comparison stimuli presented on any given trial there remains a potential confound between memory load and delay. That is, as the task progresses the comparison (non-target) odor that is selected for any trial may be more temporally distant from the initial presentation of that odor as a target than on earlier trials. An advantage of the two choice odor span procedure described previously in rats (April et al., 2013) and used presently in human non-smokers, is that it could facilitate designs in which variations of delay are systematically programmed in addition to capacity (memory load), allowing for a concurrent evaluation of both variables across species.

Though odor span performance appears to be capacity dependent in both humans and rats, the task likely does not index a *limited* capacity memory process of the kind proposed in contemporary models of human working memory. As noted in rats (see April et al., 2013) and observed presently in humans, performance does not fail entirely after initial production of errors as is observed in human span tasks such as the digit span or spatial span tasks. Our data with non-smokers found accuracy to be well above chance at the highest memory load tested (19 odors to be remembered) and rats have performed above chance at a memory load of at least 71 odors (April et al., 2013). A capacity limit for either species has yet to be identified. As such, it has been suggested that odor span may assess function of a near limitless form of recognition memory akin to picture recognition in humans (April et al., 2013).

To evaluate the relevance of the OST to human cognition, the present study evaluated the relationship between the measures derived from the OST as well as their associations with well accepted measures of attention and working memory. As expected, the odor discrimination

training and performance measures were strongly correlated with each other but showed no significant correlation with odor span, longest span, or overall accuracy. Further, simple odor discrimination measures did not correlate significantly with any other measure of human attention or working memory.

In contrast, significant relationships *were* detected between odor span measures and those derived from the N-back task. Most notably, moderate positive correlations were detected between 0-back hit rate and the span, longest span, and overall accuracy measures (*r*s ranging from .374-.589). Overall accuracy showed the strongest association with 0-back hit rate which remained significant after correcting for multiple comparisons. Positive correlations were also detected between each of the odor span measures and aspects of 2-back performance. However, these relationships were mostly constrained to 2-back reaction time and also did not reach the corrected significance threshold. In general, odor span performance appears to be most related to 0-back performance, somewhat associated with 2-back performance, and demonstrated no relationship with other span tasks. Thus, the present data lends support to the suggestion that the odor span task is not a model for the form of limited capacity short-term memory assessed by human working memory tasks.

The relationship between odor span accuracy and hit rate on a visual/symbolic N-back task is intriguing as it suggests that odor span measures may index neurocognitive processes of attention and recognition which are shared across tasks that utilize disparate stimulus modalities. Several psychiatric conditions are characterized by inattention, as evidenced by deficits in N-back performance, including ADHD (Karatekin, Bingham, & White, 2009; Klein, Wendling, Huettner, Ruder, & Peper, 2006; Shallice et al., 2002) and schizophrenia/psychosis (Haatveit et al., 2010; Jansma, Ramsey, van der Wee, & Kahn, 2004; Karatekin et al., 2009). While these

conditions are also associated with deficits in working memory, it has been suggested that inattention may underlie the deficits observed in these groups on more complex working memory tasks (Karatekin et al., 2009).

The OST has been acknowledged as a promising tool for investigating cognitive effects in preclinical models of schizophrenia (Dudchenko, Talpos, Young, & Baxter, 2013) and the present study lends credibility to the translational merits of the task. While other tasks of attention and recognition have been modeled for animals based on procedures used in humans (i.e. go/no-go tasks, serial response tasks), the OST variant described presently is somewhat unique in that it includes control procedures for concurrently assessing effects on stimulus discrimination, and could be used to evaluate both capacity and delay effects within the same task. The availability of a validated task which utilizes olfactory stimuli also provides a means by which the shared and distinct neurocognitive processes involved in attention and recognition tasks can be assessed across stimulus/sensory modalities. Further, the odor span task may be particularly useful for evaluating the neurocognitive pathology of conditions which have been associated with abnormalities or changes in olfaction such as Alzheimer's disease (Devanand et al., 2000; P. E. Gilbert, Barr, & Murphy, 2004; P. E. Gilbert & Murphy, 2004a, 2004b) and PTSD (Croy, Schellong, Joraschky, & Hummel, 2010; Dileo, Brewer, Hopwood, Anderson, & Creamer, 2008).

It is somewhat surprising that nicotine effects were not observed on the OST given the preclinical literature suggesting cholinergic involvement in odor span performance and nicotine induced enhancement of performance (Rushforth et al., 2010; Rushforth et al., 2011; Turchi & Sarter, 2000; Young, Crawford, et al., 2007; Young, Kerr, et al., 2007). However, nicotine also did not enhance N-back performance as has been previously reported in human non-smokers

(Kumari et al., 2003). In sum, the results of the present study are largely consistent with meta-analyses of nicotine effects in non-smokers/ex-smokers in that nicotine enhanced sustained attention (as indexed by RVIP performance) but produced no effects on alternative measures of attention and working memory. A strength of the present study was that the sample was constrained to participants who had a very limited history of nicotine use (less than 6 lifetime uses of nicotine products). However, it is plausible that this inclusion criteria biased the sample towards individuals who experience only limited positive effects of nicotine or are especially sensitive to the aversive effects of nicotine.

Participants did indeed report dose dependent increases in aversive nicotine effects which may have counteracted any cognitive enhancing effects of the drug. Recent nicotine use may be important for allaying these aversive effects and thus for detecting cognitive effects in non-dependent individuals. In the present sample, trend level effects suggested that enhancement of odor span performance was more likely after the second administration of nicotine regardless of whether the high dose or lose dose was delivered. Future studies may avoid sampling individuals who have opted out of initiating regular smoking as a result of their individual reaction to nicotine by restricting their sample to younger participants. Additionally, recruiting non-dependent individuals who have had multiple recent exposures to nicotine, or providing nicotine exposure prior to testing, may reduce the occurrence of aversive nicotine effects which could counter beneficial cognitive effects.

It is also worth noting that the present sample was screened for medical and psychiatric conditions, as well as substance use. This was done for participant safety and to avoid potential confounding cognitive effects of these variables. However, this also serves to limit generalizability of the findings to non-smokers who are free of the aforementioned conditions. It

has been suggested that the cognitive enhancing effects of nicotine may be more pronounced in, or constrained to, individuals who have relative deficits in certain cognitive domains (Evans & Drobes, 2009). To the extent that such deficits are associated with the excluded conditions or general functioning, the sample may also have been biased towards individuals less likely to demonstrate nicotine induced enhancement. As a whole the inclusion/exclusion criteria used in the present study allowed for timely recruitment of a non-smoking sample larger than is typical of experimental studies of nicotine and cognition (See Heishman et al., 2010). This provided additional power for detecting subtle drug effects and allowed for an analysis of the potential moderating effects of dose-order. However, dose order did interact with nicotine dose on the OST (span and longest span), spatial span (backward and total) and 2-back (target and distractor reaction time) performance.

Detrimental effects of the first nicotine dose administered were detected on spatial span for those who received the low dose first and on 2-back target reaction time for those who received the high dose first, while those who received placebo first subsequently showed enhancement of 2-back distractor reaction during the second nicotine administration (high dose). Though not conclusive, considered alongside the dose order effects for odor span this pattern suggests that initial exposures to nicotine may impair performance while subsequent exposures provide enhancement. This highlights the need to counterbalance dose orderings in drug studies which use a repeated measures design (a strength of the present study). Another strength of the study was the use of a double-blind testing procedure in which the experimenter was blind to which dose the participant received and the participant was informed only that they *may* receive nicotine during the study. This served to limit the possibility of drug expectancies influencing participant performance and/or experimenter bias.

The present study was successful in further validating the placebo controlled nicotine gum administration procedure used previously by Kleykamp et al. (2005) to deliver nicotine to never-smokers. As in the Kleykamp et al. study, nicotine delivered in this manner produced significant effects on multiple subjective report measures evaluating common negative side-effects of nicotine (i.e., nausea, sickness, and dizziness). While significant effects in these domains were only detected after administration of the high dose (4mg) of nicotine gum, average ratings for each item followed a dose-dependent pattern suggesting that a lesser level of nicotine was delivered through administration of the low dose (2mg) gum. Though administration of high dose nicotine gum produced a range of aversive effects in never-smokers, it is also worth noting that these effects were generally reported as mild to moderate. Thus, the present study lends further support to the use of the placebo controlled gum procedure as a safe and effective method for administering nicotine to never-smokers.

While the randomized, double-blind, placebo controlled, counterbalanced, repeated measures design provided many strengths, there remains several limitations worth noting. Though the placebo controlled nicotine gum administration procedure provided a safe, non-invasive, and well validated means by which to administer nicotine to non-smokers there is likely considerable variability in the dose delivered to individual participants through this method. As in Kleykamp et al. (2005), a standardized chewing procedure was implemented to reduce variability; however, no biological measure was collected to quantify the dose delivered to participants after administration. Assessment of plasma nicotine levels would have allowed for a more precise analysis of nicotine effects on cognitive performance. However, this measure was not obtained due to concerns regarding the invasiveness of collection, participant reactivity to the procedure, as well as cost and time constraints. Other limitations stem from the use of the

repeated measures design. Though repeated testing can enhance statistical power by controlling for between subject variance it also introduces the potential for practice effects as participants completed each task repeatedly. On average, experimental sessions were spaced approximately 9 days apart (no less than 6 days for any participant). However, it remains conceivable that an initial exposure to these tasks could significantly improve performance during subsequent testing. This may be particularly relevant for the non-clinical tasks (odor span, RVIP, and N-back) for which normative test-retest data are not available.

In sum, the present study provides further validation for a placebo controlled nicotine gum administration procedure for never-smokers and evidence of dose dependent enhancement of sustained attention amongst this group. Effects on sustained attention were detected despite dose dependent impairments in self-reported attention and concentration. Indications of broader cognitive enhancement were obtained amongst participants during their second experimental exposure to nicotine. Additionally, the odor span task was successfully adapted for humans with the inclusion of control procedures described in preclinical studies. Human odor span performance demonstrated capacity effects, as are observed in rats, and was positively associated with accuracy on a visual/symbolic 0-back task. Thus, the odor span task may be best conceptualized as an attention or recognition memory task which is sensitive to capacity effects. As such, it may be particularly useful in preclinical models of disorders characterized by impaired attention or selective olfactory deficits.

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