

Visuospatial Memory Deficits Emerging During Nicotine Withdrawal in Adolescents with Prenatal Exposure to Active Maternal Smoking

Leslie K Jacobsen^{*,1,2,3}, Theodore A Slotkin⁴, Michael Westerveld^{5,6}, W Einar Mencl³ and Kenneth R Pugh^{2,3}

¹Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA; ²Department of Pediatrics, Yale University School of Medicine, New Haven, CT, USA; ³Haskins Laboratories, New Haven, CT, USA; ⁴Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, USA; ⁵Department of Neurosurgery, Yale University School of Medicine, New Haven, CT, USA;

⁶Department of Child Study, Yale University School of Medicine, New Haven, CT, USA

Active maternal smoking during pregnancy elevates the risk of cognitive deficits and tobacco smoking among offspring. Preclinical work has shown that combined prenatal and adolescent exposure to nicotine produces more pronounced hippocampal changes and greater deficits in cholinergic activity upon nicotine withdrawal than does prenatal or adolescent exposure to nicotine alone. Few prior studies have examined the potential modifying effects of gestational exposure to active maternal smoking on cognitive or brain functional response to tobacco smoking or nicotine withdrawal in adolescents. We examined visuospatial and verbal memory in 35 adolescent tobacco smokers with prenatal exposure to active maternal smoking and 26 adolescent tobacco smokers with no prenatal exposure to maternal smoking who were similar in age, educational attainment, general intelligence, and baseline plasma cotinine. Subjects were studied during *ad libitum* smoking and after 24 h of abstinence from smoking. A subset of subjects from each group also underwent functional magnetic resonance imaging while performing a visuospatial encoding and recognition task. Adolescent tobacco smokers with prenatal exposure experienced greater nicotine withdrawal-related deficits in immediate and delayed visuospatial memory relative to adolescent smokers with no prenatal exposure. Among adolescent smokers with prenatal exposure, nicotine withdrawal was associated with increased activation of left parahippocampal gyrus during early recognition testing of visuospatial stimuli and increased activation of bilateral hippocampus during delayed recognition testing of visuospatial stimuli. These findings extend prior preclinical work and suggest that, in human adolescent tobacco smokers, prenatal exposure to active maternal smoking is associated with alterations in medial temporal lobe function and concomitant deficits in visuospatial memory.

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INTRODUCTION

Epidemiologic data suggest that more than half of tobacco-smoking women continue to smoke through their pregnancies, resulting in at least a half a million births per annum of infants prenatally exposed to tobacco smoke in the United States (Ebrahim *et al*, 2000; Martin *et al*, 2005). Prospective clinical studies have linked prenatal exposure to tobacco smoke to reductions in motor and verbal comprehension scores in children assessed at 13 months of age (Gusella and

Fried, 1984), reductions in measures of language development, and general intellectual function in children assessed at 3, 4, 5, and 6 years of age (Fried and Watkinson, 1990; Fried *et al*, 1992), deficits in verbal and nonverbal learning and memory in preadolescents (Cornelius *et al*, 2001), and reductions in general intelligence in children assessed between 9 and 16 years of age (Fried *et al*, 1998, 2003).

Preclinical studies have provided strong evidence that the disruptive effects of nicotine on brain development contribute significantly to these deleterious effects of gestational exposure to tobacco smoke (Slotkin, 2004). Nicotine, the primary psychoactive component of tobacco smoke, binds to nicotinic acetylcholine receptors (nAChRs), which become detectable in embryonic development shortly before neurulation (Atluri *et al*, 2001; Schneider *et al*, 2002). Acetylcholine plays a critical developmental role in brain maturation (Lauder and Schambra, 1999; Weiss *et al*, 1998; Slotkin, 2004), initially promoting cell division, and

*Correspondence: Dr LK Jacobsen, Psychiatry and Pediatrics, Yale University School of Medicine, 2 Church Street South, Suite 207, New Haven, CT 06519, USA, Tel: +1 203 764 8480, Fax: +1 203 764 8484, E-mail: leslie.jacobsen@yale.edu

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subsequently promoting the switch from replication to differentiation (Slotkin, 1998, 1999). In rodents, inappropriate stimulation of nAChRs by nicotine during prenatal development produces reductions in brain cell number, size, and surface area (Roy *et al*, 1998; Slotkin, 1998, 1999), prolonged reductions in cholinergic neural transmission (Zahalka *et al*, 1992; Navarro *et al*, 1989), and alters the subsequent response to nicotine challenge (Navarro *et al*, 1988; Seidler *et al*, 1992; Levin *et al*, 1993, 1996).

Recent work with a rodent model of adolescent nicotine exposure provided evidence that vulnerability to the developmentally disruptive effects of nicotine extends postnatally into adolescence (Slotkin, 2002). In doses designed to model typical human exposures, nicotine treatment of rats during adolescence altered cell numbers in cortex, midbrain, and hippocampus (Trauth *et al*, 2000), and produced prolonged suppression of cholinergic neural activity and responsiveness (Abreu-Villaca *et al*, 2003; Trauth *et al*, 2001). Consistent with this preclinical work, human adolescent smokers were recently found to have impairments in accuracy of verbal working memory performance relative to nonsmokers who were similar in age, gender, educational attainment, and duration of prenatal exposure to active maternal smoking (Jacobsen *et al*, 2005). Performance decrements were more severe with earlier age at the onset of smoking. In adult humans, cessation of regular tobacco smoking disrupts memory (Pineda *et al*, 1998; West and Hack, 1991) and attention (Shiffman *et al*, 1995; Snyder *et al*, 1989), while administration of nicotine, or resumption of tobacco smoking, reverses these withdrawal-related deficits (Foulds *et al*, 1996; Krebs *et al*, 1994; Pineda *et al*, 1998). Consistent with these observations in adults, memory deficits worsened in adolescent tobacco smokers during nicotine withdrawal (Jacobsen *et al*, 2005).

Rates of tobacco smoking and nicotine dependence among offspring prenatally exposed to active maternal smoking are elevated (Buka *et al*, 2003; Kandel *et al*, 1994; Griesler *et al*, 1998; Cornelius *et al*, 2000). Recent data from the US National Comorbidity Survey indicate that onset of daily smoking is concentrated between the ages of 15 and 20 years, suggesting that adolescence is a critical period for progression from initial to regular tobacco use (Breslau *et al*, 2001). Adolescence is also a period of continued brain development, with ongoing neuroproliferation, apoptosis, synaptic rearrangement, and maturation of central cholinergic systems (Bayer, 1983; Bayer *et al*, 1982; Huttenlocher, 1990; Matthews *et al*, 1974; Nadler *et al*, 1974; Zahalka *et al*, 1993). Rodent models developed to investigate the effects of prenatal nicotine exposure on response to nicotine administration during adolescence have shown that prenatal nicotine exposure reduces the upregulation of nAChRs induced by adolescent nicotine exposure, produces cholinergic hypoactivity during nicotine withdrawal in adolescence (Abreu-Villaca *et al*, 2004b), and magnifies the effects of adolescent nicotine exposure on indices of cell number, size, and surface area (Abreu-Villaca *et al*, 2004a).

To the best of our knowledge, no prior study has examined the potential modifying effects of gestational exposure to active maternal smoking on cognitive or brain functional response to tobacco smoking or nicotine with-

drawal in human adolescents or adults. In the present study, verbal and visuospatial memory were examined in adolescent regular tobacco smokers with and without prenatal exposure to active maternal smoking, during *ad libitum* smoking and during smoking abstinence.

Prior work has shown that reducing cholinergic neurotransmission disrupts memory (Rosier *et al*, 1998, 1999; Mewaldt and Ghoneim, 1979). Given that nicotine withdrawal induces cholinergic hypoactivity in adolescent rodents with gestational exposure to nicotine (Abreu-Villaca *et al*, 2004b), we anticipated that nicotine withdrawal-related disruption of memory would be greater in adolescents with prenatal exposure to active maternal smoking than in adolescents without such exposure. Medial temporal lobe structures, including hippocampus and parahippocampal gyrus, provide critical support for both encoding and retrieval of explicit memory (Cabeza *et al*, 2001; Eichenbaum *et al*, 1996; Squire, 1992), and evidence suggests that hippocampal cholinergic neurotransmission modulates the efficacy of encoding (Sperling *et al*, 2002). A subset of subjects were studied using functional magnetic resonance imaging during performance of a task designed to permit separate examination of neural circuits mediating encoding and recognition memory. Prior work has shown that enhanced cholinergic neurotransmission increases the selectivity of perceptual processing during encoding, thereby reducing demand on, and activation of, regions involved in higher-order processes (Furey *et al*, 2000). Given evidence that nicotine withdrawal induces cholinergic hypoactivity in adolescent rodents with gestational exposure to nicotine (Abreu-Villaca *et al*, 2004b), we hypothesized that efficacy of encoding would be impaired during nicotine withdrawal in adolescents with prenatal exposure to active maternal smoking, and that this would increase the demand on and activation of hippocampus during recognition memory testing.

METHODS

Participants

In total, 35 adolescent daily tobacco smokers with prenatal exposure to active maternal smoking were compared with 26 adolescent daily tobacco smokers with no prenatal exposure to active maternal smoking. All participants were recruited from the community by newspaper advertisement and flyer distribution in the context of an ongoing study of adolescent tobacco use. All subjects were free of medical and psychiatric illness and substance abuse or dependence disorders, other than nicotine dependence, as determined by physical exam and structured clinical interview using the Schedule for Affective Disorders and Schizophrenia for School Aged Children (Kaufman *et al*, 1996) performed at initial screening. Detailed drug use history was obtained using the Comprehensive Addiction Severity Index for Adolescents (Meyers *et al*, 1995). Subjects were required to abstain from all drug use for 1 month prior to assessment and from alcohol for 48 h prior to assessment. Abstinence from substance use prior to assessment was confirmed by urine toxicology screen.

At initial screening, general intelligence was estimated using the Kaufman Brief Intelligence Test (Bowers and

Pantle, 1998), reading achievement was estimated using the Word Attack subtest of the Woodcock–Johnson Revised Test of Achievement, and self-reported symptoms of depression, anxiety, stressful life events, and inattention were assessed using the Beck Depression Scale (Beck *et al*, 1961), the Multidimensional Anxiety Scale for Children (March *et al*, 1997), the Life Events Questionnaire (Masten *et al*, 1994), and the Conners Adolescent Self-Report Scale (Conners, 1998), respectively. Prenatal exposure was assessed at the time of initial screening using a semi-structured interview of the parents regarding the average number of cigarettes smoked per day by the mother during each trimester of her pregnancy with the subject. The mother's consumption of other substances and alcohol during the pregnancy, and the rate of smoking by other persons living in the home during the pregnancy (prenatal environmental exposure), were also assessed during this interview.

Parental consent was obtained for subjects 17 years of age and younger. This study was approved by the Yale University School of Medicine Human Investigation Committee. Subjects provided written assent or, for 18 year-olds, consent for study participation.

Procedure

All subjects were tested twice, during *ad libitum* smoking and after 24 h of abstinence from tobacco. Test sessions were conducted in the late afternoon for all subjects. Prior to the abstinence test session, subjects were instructed to refrain from using any tobacco products for 24 h. Subjects were told what time to stop smoking and were provided reminders and encouragement to comply with the tobacco restriction by telephone contact. To avoid the emergence of nicotine withdrawal during the smoking test session, subjects were permitted to smoke their own brand of cigarettes during a break midway through the test session. Order of smoking and abstinence test sessions was randomized. At the beginning of each test session, nicotine withdrawal was assessed using the Minnesota Nicotine Withdrawal Scale (Hughes and Hatsukami, 1998), and tobacco craving was assessed using the Tiffany Scale for Smoking Urges (Tiffany and Drobes, 1991) and the Shiffman Jarvik Scale (Shiffman and Jarvik, 1976).

During each test session, blood or, in 21 subjects who declined phlebotomy, saliva was obtained to measure nicotine and cotinine, the primary metabolite of nicotine, using gas chromatography (Jacob *et al*, 1981). The proportion of subjects declining phlebotomy did not differ across groups ($\chi^2 = 1.2$, $p = 0.3$). To reduce food contamination, saliva was collected after subjects rinsed their mouths with water. Saliva and plasma were frozen at -30°C immediately following collection. All samples were shipped on dry ice to the Clinical Pharmacology Laboratory of the University of California, San Francisco, where they were assayed for nicotine and cotinine. Expired air carbon monoxide (CO), a product of combustion, was also measured at each test session (Micro CO meter: Micro Direct, Auburn, ME). While examiners were not blind to prenatal exposure status of the subjects, they were blind to the hypotheses under study.

Assessment of Cognitive Performance

Visuospatial memory was assessed using the Brief Visuospatial Memory Test-Revised (BVMT-R) (Benedict *et al*, 1996), a figural learning task. For this task, subjects view a stimulus page with six geometric figures on it. After 10 s, the stimulus page is removed and subjects must draw as many of the figures as possible in the correct location. This procedure is repeated twice for a total of three administrations (immediate recall trials), and is followed by a 25-min delay interval during which the subject engages in verbal tasks. After the 25-min delay, the subject is asked once again to draw from memory as many of the figures as possible in the correct location (delayed recall trial). Following the delayed recall trial, a recognition test is administered during which the subject views 12 geometric figures in sequence, including the six figures learned during the immediate recall trials. The subject indicates whether each figure is old or new. Scores derived included total immediate recall (the sum of scores obtained during the three immediate recall trials), delayed recall, and recognition hits (the number of true positives on the recognition task). Verbal memory was assessed using the Hopkins Verbal Learning Test-Revised (HVLTR) (Shapiro *et al*, 1999), an auditory word list learning task whose structure is identical to that of the BVMT-R. During the immediate recall trials of the HVLTR, the subject is read a list of 12 words and is asked to repeat as many of them as possible after each of three readings. Delayed recall is tested after a 25-min interval, during which the subject is engaged in nonverbal tasks. Recognition memory is tested using a randomized list of 24 words that includes the 12 words learned during the immediate recall trials. After each word is read, the subject indicates whether each word is old or new. As with the BVMT-R, the HVLTR includes measures of total immediate recall, delayed recall, and recognition hits. To minimize item-related practice effects (Benedict and Zgaljardic, 1998), different forms of the HVLTR and BVMT-R were administered at each of the two testing sessions.

Assessment of Brain Function

To explore the effects of prenatal exposure to active maternal smoking on the functioning of neural-circuitry-mediating components of mnemonic processing, seven subjects with prenatal exposure and six subjects with no prenatal exposure also underwent fMRI scanning while performing a modified version of a previously described task (Shaywitz *et al*, 1999; Mencl *et al*, 2000) that incorporated a mixed design, including block and event-related features (Donaldson *et al*, 2001; Otten *et al*, 2002; Visscher *et al*, 2003), to permit separate assessment of neural-circuitry-mediating encoding, rehearsal, and early and delayed recognition of verbal and nonverbal stimuli. Imaging and assessment of verbal and visuospatial memory were performed during the same test session.

The subgroup of adolescent tobacco smokers with and without prenatal exposure to tobacco smoke, who underwent fMRI scanning and were selected randomly from the larger group and did not differ in age (mean \pm standard deviation (SD): prenatally exposed = 16.6 ± 1.2 years, no

prenatal exposure = 17.0 ± 0.9 years), gender (all prenatally exposed subjects were females, four subjects with no prenatal exposure were female), years of education (prenatally exposed = 10.0 ± 1.5 years, no prenatal exposure = 10.5 ± 0.8 years), years of parent education (prenatally exposed = 13.8 ± 1.7 years, no prenatal exposure = 13.8 ± 1.7 years), consumption of tobacco (number of cigarettes smoked per day: prenatally exposed = 11.2 ± 6.4 , no prenatal exposure = 9.1 ± 4.2), alcohol (number of drinks consumed per week: prenatally exposed = 2.0 ± 4.4 , no prenatal exposure = 0.2 ± 0.2), and cannabis (lifetime episodes of cannabis use: prenatally exposed = 130 ± 169 , no prenatal exposure = 201 ± 286), symptoms of nicotine dependence (FTND score: prenatally exposed = 3.1 ± 2.6 , no prenatal exposure = 3.5 ± 2.5), general intelligence (KBIT composite score: prenatally exposed = 94.0 ± 7.1 , no prenatal exposure = 91.8 ± 6.8), reading achievement (WJR Word Attack subtest standard score: prenatally exposed = 102.6 ± 16.0 , no prenatal exposure = 105.7 ± 15.0), or symptoms of anxiety (MASC score: prenatally exposed = 39.7 ± 15.1 , no prenatal exposure = 32.3 ± 6.0), depression (Beck Depression score: prenatally exposed = 7.1 ± 6.1 , no prenatal exposure = 8.0 ± 6.3), or inattention (Conners Adolescent Self-Report Scale score: prenatally exposed = 17.4 ± 10.0 , no prenatal exposure = 14.7 ± 12.2) at the initial screening visit. Prenatally exposed subjects included three subjects with exposure restricted to the first trimester and four subjects with exposure throughout gestation.

Each imaging run consisted of four cycles, the components of which included rest, encode, rehearse, and forced choice recognition, each of which were 20 s long. Verbal and nonverbal cycles alternated during each run. The structure of the nonverbal cycles is schematically depicted in Figure 1. During the nonverbal cycle encode phase, subjects saw six characters from South Asian languages (Tamil, Marathi, Bengali, Telugu, Hindi, Gujarati, Sanskrit, Kannada, and Gurmukhi) sequentially for 3.333 s each, followed by a 20-s rehearsal period during which subjects were instructed to remember the words just presented. During forced choice recognition, subjects saw three familiar characters and three new characters, with the sequence of old and new

randomized across recognition blocks and runs. Subjects indicated with a button press whether each character was old (seen during encoding) or new. Verbal cycles were identical in design to the nonverbal cycles, except that stimuli were monosyllabic English words. Each block type began with a visual prompt, reminding subjects to rest, study (encode), rehearse, or test (forced choice recognition). Each run was 5 min 40 s long. After six runs, a sagittal anatomic MP-RAGE three-dimensional image was obtained. This was followed by two runs in which delayed recognition memory was assessed. Each of these last two runs consisted of five cycles that included a 20-s rest block followed by a 20-s verbal recognition and a 20-s nonverbal recognition block. The order of verbal and nonverbal recognition blocks was randomized across cycles. Each delayed recognition block consisted of three stimuli presented during the encoding blocks, but not the recognition blocks during the first six runs, and three new stimuli. Subjects again indicated whether each stimulus was old or new with a button press. Due to time constraints, only stimuli presented during encoding blocks within the first five runs were included as targets in the final two (delayed recognition) runs. The time duration between the end of the fifth run and the beginning of the seventh run was 16.1 ± 1.9 min.

Subjects were scanned twice, during *ad libitum* smoking and after 24 h of abstinence from tobacco products, and the order of smoking and abstinence scans was randomized. Subjects were scanned using a 3.0-T Siemens Trio MRI system. Axial oblique T1-weighted anatomic images were acquired parallel to the AC/PC line using a FLASH sequence (25 contiguous slices, slice thickness = 5 mm, TE = 2.47 ms, TR = 300 ms, FA = 60° , matrix = 256×256 pixels, FOV = 20 cm^2). Epibold functional images were acquired in the same relative slice locations using a single-shot, gradient echo pulse sequence (TE = 30 ms, TR = 2000 ms, FA = 80° , matrix = 64×64 pixels, FOV = 20 cm^2). In all, 170 images were acquired per slice per run. The high-resolution anatomic scan was acquired using a sagittal MP-RAGE sequence (TE = 3.66 ms, TR = 2530 ms, FA = 7° , FOV = 256×256 pixels, slice thickness = 1 mm, 176 slices, NEX = 1).

Data Analysis

Accuracy of task performance during scanning was assessed by dividing the number of correct responses by the total number of trials (proportion correct). Data were analyzed using linear mixed-effects regression analysis implemented in SPlus (Insightful Cooperation, Seattle, WA) with subject modeled as a random effect. Models that included fixed effects for group (prenatally exposed vs no prenatal exposure), smoking condition, and the interaction between group and smoking condition were estimated. The potential confounding effects of group differences in gender, years of parental education, environmental tobacco smoke exposure during gestation, rate of smoking, and severity of nicotine dependence (FTND score) were controlled by entering these variables in the regression models. Effects of trimesters of exposure to active maternal smoking were examined by comparing subjects exposed for one trimester to those exposed for three trimesters, using regression models that

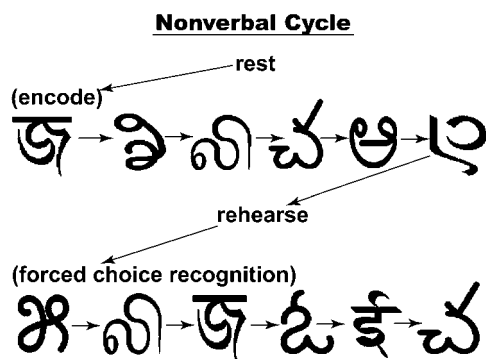


Figure 1 Schematic representation of the structure of the nonverbal cycles during fMRI scanning. During the nonverbal cycle encode phase, subjects saw six characters from South Asian languages sequentially for 3.333 s each, followed by a 20-s rehearsal period. During forced choice recognition, subjects saw three familiar characters and three new characters, with the sequence of old and new randomized across recognition blocks and runs.

included fixed effects for trimesters of exposure (one or three), smoking condition, and the interaction between trimesters of exposure and smoking condition.

Previous work has shown that salivary concentrations of cotinine in youth are, on average, 20% higher than plasma concentrations of cotinine (Jarvis *et al*, 2003). Therefore, salivary cotinine concentrations were multiplied by a factor of 0.8 before combining them with measures of cotinine from plasma. Plasma nicotine concentrations were estimated from salivary nicotine concentrations using a previously described power model shown to produce good correlations between plasma and salivary nicotine concentrations (Teneggi *et al*, 2002). The estimated plasma nicotine concentration less than 2 ng/ml prior to the abstinence test session was accepted as evidence of abstinence from tobacco use.

Image analysis was performed using software written in Matlab (MathWorks, Natick, MA). Prior to statistical analysis, images were corrected for slice acquisition time, motion corrected for three translational directions, and for the three possible rotations using SPM 99 (Friston *et al*, 1995), and then spatially filtered using a Gaussian filter of full-width at half-maximum (FWHM) of 3.125 mm. Individual subject maps of activation associated with transient processes (time-locked responses evoked by the encoding and recognition trials) and sustained processes (average activation levels during the self-guided rehearsal blocks) were generated using regression-based methods implemen-

ted within the general linear model (GLM). Transient effects were coded in the GLM with a reference hemodynamic response function modeled as a gamma function ($\tau = 0.9$, $n = 4$, delay = 2.3; peaking at 5 s post-stimulus) (Boynton *et al*, 1996). The sustained effects of rehearsal were similarly coded by convolving a block-length boxcar function with this gamma function (Boynton *et al*, 1996; Visscher *et al*, 2003). Prior to across-subjects comparisons, fMRI data were spatially normalized to standard stereotactic space using an affine transformation (Duncan *et al*, 2004). Group by smoking condition interaction effects on task-related activation were assessed using voxelwise mixed-model repeated-measures analysis of variance (ANOVA) (Woods, 1996; Kirk, 1982; Holmes and Friston, 1998), with group assignment as a between-subjects variable, smoking condition as a within-subjects variable, and subject treated as random effect within each group. A voxel threshold of $\alpha = 0.001$ and a cluster threshold of eight contiguous significant voxels were applied.

RESULTS

Demographic, clinical, and cognitive characteristics of both groups are presented in Table 1.

The groups did not differ in age, years of education, consumption of alcoholic beverages or cannabis, general intelligence, reading achievement, symptoms of depression

Table 1 Demographic, Clinical and Cognitive Characteristics of Adolescent Tobacco Smokers: 35 Subjects With and 26 Subjects Without Prenatal Exposure to Active Maternal Smoking^a

Characteristic	Prenatally exposed	No prenatal exposure	t/χ^2	<i>p</i>
Age (years)	16.9 (1.3)	16.9 (0.8)	0.1	0.9
Girls (no., %)	29 (83)	15 (58)	4.7	<0.05
Education (years)	10.0 (1.3)	10.2 (0.9)	0.6	0.6
Parent education (years)	13.4 (2.1)	14.4 (2.5)	1.8	0.08
Rate of tobacco smoking (no. of cigarettes per day)	13.6 (7.1)	9.2 (4.9)	2.7	<0.01
Range	2–30	1–20		
Estimated plasma cotinine, 1930 h <i>ad libitum</i> smoking (ng/ml)	123.3 (90.4)	122.7 (85.8)	0.03	1.0
FTND ^b score	3.9 (2.2)	2.2 (1.9)	3.2	<0.01
Rate of alcohol consumption (drinks per week)	3.8 (8.0)	1.3 (2.4)	1.5	0.1
Lifetime episodes of cannabis use	411 (720)	231 (506)	1.1	0.3
Weeks prenatal exposure to active maternal smoking	24.7 (17.1)	0	7.3	<0.0001
Weeks prenatal exposure to environmental tobacco smoke	28.8 (17.7)	8.6 (16.1)	4.6	<0.0001
KBIT ^c composite score	95.4 (8.3)	96.0 (7.1)	0.3	0.7
WJR word attack SS ^d	99.4 (14.9)	103.6 (13.4)	1.2	0.2
Beck Depression score (Beck <i>et al</i> , 1961)	5.2 (5.1)	5.4 (4.5)	0.2	0.8
MASC ^e	33.4 (13.6)	33.7 (10.0)	0.1	0.9
Life Events ^f	9.8 (4.6)	8.1 (4.2)	1.5	0.1
Conners ^g	16.5 (9.6)	16.6 (9.4)	0.04	1.0

^aData are presented as mean (SD) unless otherwise specified.

^bFagerstrom Test for Nicotine Dependence (Heatherton *et al*, 1991).

^cKaufman Brief Intelligence Test (Bowers and Pantle, 1998).

^dWoodcock-Johnson Revised Test of Achievement Word Attack subtest standard scores.

^eMultidimensional Anxiety Scale for Children (March *et al*, 1997).

^fLife Events Questionnaire (Masten *et al*, 1994).

^gConners Adolescent Self-Report Scale (Conners, 1998).

or level of anxiety at the initial screening visit, number of stressful life events, or symptoms of inattention. The prenatally exposed group included more girls and there was a trend for parents of subjects with no prenatal exposure to have received, on average, 1 more year of education than parents of subjects with prenatal exposure. Consistent with previous evidence that maternal smoking during pregnancy increases the risk of smoking in offspring (Buka *et al*, 2003; Cornelius *et al*, 2000; Griesler *et al*, 1998; Kandel *et al*, 1994), the rate of tobacco smoking and symptoms of nicotine dependence were higher among prenatally exposed adolescent smokers than among adolescents with no prenatal exposure. However, estimated plasma cotinine measured at 7:30 pm during a day of *ad libitum* smoking did not differ across groups.

Adolescents with prenatal exposure also had more environmental tobacco smoke exposure during gestation (persons other than the mother smoking in the home during gestation of the subject). Among the 35 adolescents with prenatal exposure, exposure was restricted to the first trimester in 16, while 19 were exposed throughout gestation. Reported maternal use of other drugs during pregnancy was as follows: among mothers who smoked tobacco during gestation of the subject, five consumed alcohol during three trimesters, one consumed alcohol during two trimesters, and one consumed alcohol during one trimester. Two of the mothers who consumed alcohol during gestation of the subject also consumed cannabis, one during three trimesters, and one during two trimesters. One mother who consumed alcohol during gestation of the subject also consumed cocaine during three trimesters. Among mothers who did not smoke tobacco during gestation of the subject, two consumed alcohol during three trimesters and none consumed cannabis or cocaine. No subject in either group was exposed to opiates, amphetamine, hallucinogens, sedative-hypnotics, or inhalants during gestation. The proportion of subjects exposed to alcohol during gestation did not significantly differ across groups ($\chi^2 = 1.8$, $p = 0.2$), nor did the proportion of subjects exposed to cannabis ($\chi^2 = 1.5$, $p = 0.2$; Fisher exact $p = 0.5$), or cocaine ($\chi^2 = 0.8$, $p = 0.4$; Fisher exact $p = 1.0$) during gestation.

Five subjects with prenatal exposure had experimented with illicit substances other than cannabis; three had tried cocaine, one had tried oral opiates, one had tried sedative-hypnotics, and one had tried hallucinogens. Similarly, five subjects with no prenatal exposure had experimented with illicit substances other than cannabis; three had tried cocaine, one had tried amphetamine, one had tried oral opiates, and one had tried hallucinogens. All subjects denied previous use of methylenedioxymethamphetamine (ecstasy), inhalants, anabolic steroids, and injected drugs.

Nicotine Plasma Levels and Effects of Prenatal Exposure and Smoking Condition on Performance

The average duration between test sessions was 13.5 ± 12.3 days and did not differ between groups. Abstinence significantly reduced nicotine, cotinine, and expired air CO (a product of combustion: effect of smoking condition: $\beta = 17.3$, $t = 11.5$, $p < 0.0001$ for nicotine, $\beta = 58.8$, $t = 4.2$, $p = 0.0001$ for cotinine, $\beta = 8.4$, $t = 7.5$, $p < 0.0001$ for expired air CO), and this effect did not differ between

groups. Estimated plasma nicotine concentrations were consistent with compliance with the tobacco restriction prior to the abstinence test session (smoking condition: nicotine = 16.2 ± 7.6 ng/ml, abstinence: nicotine = 0.5 ± 0.8 ng/ml, range = 0–2.0 ng/ml). Consistent with previous reports (Jacobsen *et al*, 2005), smoking abstinence increased tobacco craving (Tiffany: $\beta = -28.6$, $t = -4.2$, $p = 0.0001$; Shifman Jarvik Scale: $\beta = -1.6$, $t = -4.1$, $p = 0.0001$). The effect of smoking abstinence on symptoms of nicotine withdrawal was marginally significant (Minnesota Nicotine Withdrawal Scale: $\beta = -4.2$, $t = -1.9$, $p = 0.056$). Group by smoking condition interaction effects on symptoms of craving and nicotine withdrawal were not significant.

Assessment of visuospatial memory revealed a significant effect of group on BVMT-R total immediate recall ($\beta = -5.5$, $t = -3.6$, $p = 0.0008$), which was modified by smoking condition (group by smoking condition $\beta = 3.1$, $t = 2.3$, $p = 0.02$; Figure 2a). During *ad libitum* smoking, prenatally exposed subjects had poorer immediate visuospatial recall than subjects with no prenatal exposure. During the abstinence condition, immediate recall improved in subjects with no prenatal exposure, but worsened in prenatally exposed subjects. Significant group and group by smoking condition interaction effects were also observed for BVMT-R delayed recall. During *ad libitum* smoking, delayed recall of figures was similar among subjects with and without prenatal exposure. However, during smoking abstinence, visuospatial delayed recall declined in prenatally exposed subjects and improved in subjects with no prenatal exposure (effect of group: $\beta = -1.6$, $t = 2.9$, $p = 0.006$; group by smoking condition interaction effect: $\beta = 1.1$, $t = 2.2$, $p = 0.03$; Figure 2b). Group and group by smoking condition interaction effects on HVLT-R performance were

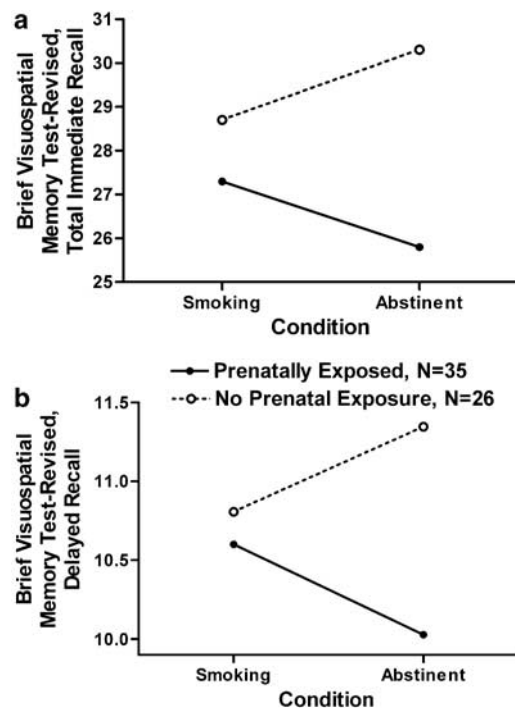


Figure 2 Group by smoking condition interaction effects on BVMT-R total immediate recall (a; $\beta = 3.0$, $t = 2.3$, $p = 0.02$) and delayed recall (b; $\beta = 1.1$, $t = 2.2$, $p = 0.03$).

not significant. Adding the number of lifetime episodes of cannabis use and rate of alcohol consumption by the subjects into these regression models did not change the significance of any of these results.

Examination of the effects of the number of trimesters of exposure revealed no significant differences between subjects exposed to active maternal smoking for one or three trimesters on BVMT-R performance (p -values > 0.4).

Within the subgroup of subjects who underwent fMRI scanning, smoking abstinence produced significant declines in plasma nicotine and expired air CO concentrations ($\beta = 13.4$, $t = 4.8$, $p = 0.009$ for plasma nicotine; $\beta = 8.5$, $t = 3.9$, $p = 0.002$ for expired air CO), and these effects did not differ across groups. Group and group by smoking condition interaction effects on accuracy and speed of task performance during scanning were not significant (p -values > 0.2).

Effects of Prenatal Exposure and Smoking Condition on Task-Related Medial Temporal Lobe Activation

Given the evidence from BVMT-R performance suggesting that neural circuits subserving visuospatial memory may be rendered vulnerable to the destabilizing effects of nicotine withdrawal by prenatal exposure to active maternal smoking, we focused the analysis of imaging data on identification of medial temporal lobe regions showing significant group by smoking condition interaction effects during mnemonic processing of visuospatial stimuli. Group by smoking condition interaction effects on medial temporal lobe activation during encoding and rehearsal were not

significant. A significant group by smoking condition interaction effect was observed in a region of the left parahippocampal gyrus (Talairach coordinates: $X = -17$, $Y = -40$, $Z = -4$, volume = 72 mm^3) during testing of early recognition memory for familiar stimuli. Smoking abstinence was associated with increased activation of the left parahippocampal gyrus in subjects with prenatal exposure and decreased activation of this structure in subjects with no prenatal exposure.

Significant group by smoking condition interaction effects were observed at the left hippocampus ($X = -29$, $Y = -29$, $Z = -7$), the lateral aspect of the right hippocampus ($X = 38$, $Y = -23$, $Z = -7$), and the left insular cortex ($X = -33$, $Y = 4$, $Z = -7$) during testing of delayed recognition memory for familiar stimuli. These results are presented in Figure 3. Linear contrasts were applied to identify regions that increase in activity during nicotine withdrawal to a greater degree in subjects with prenatal exposure relative to subjects with no prenatal exposure as red/yellow. Regions demonstrating the opposite pattern (decreasing in activity during nicotine withdrawal to a greater degree in subjects with prenatal exposure relative to subjects with no prenatal exposure) are shown in blue/purple. Data are displayed per radiological convention, with the right side of the brain on the left side of the figure. Average percent signal change of the left and right hippocampal voxels demonstrating significant group by smoking condition interaction effects are plotted for each group and smoking condition. During testing of delayed recognition memory, smoking abstinence was associated with increased activation of the left and right hippocampus

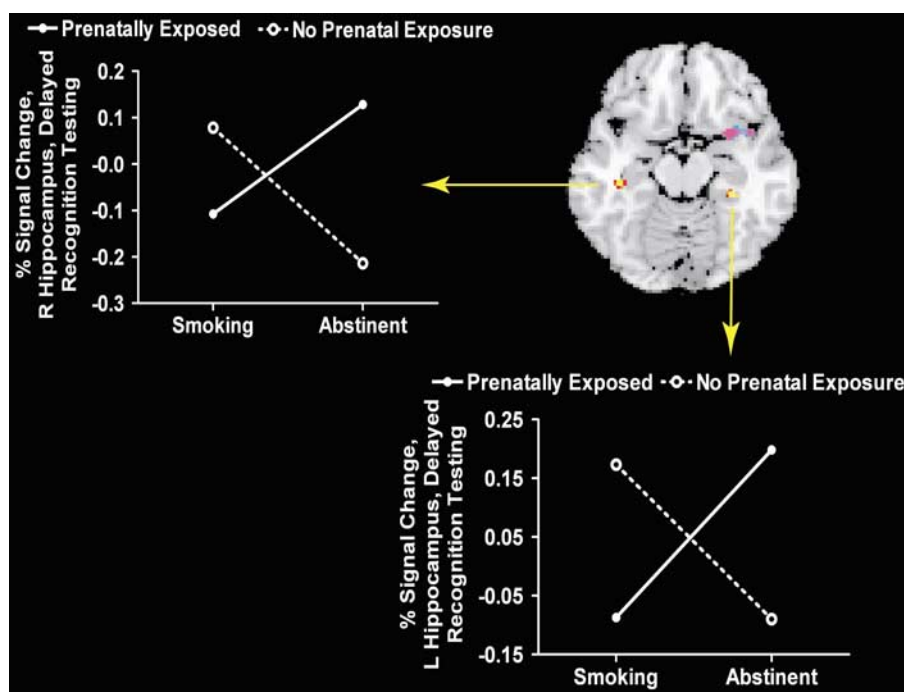


Figure 3 Group by smoking condition interaction effects on activation of the left and right hippocampus during delayed recognition testing of visuospatial stimuli. Images are displayed per radiological convention, with the right side of the brain on the left side of the figure. Linear contrasts were applied to identify regions where prenatally exposed subjects show significant increases in activity relative to controls during smoking abstinence as red/yellow. Regions demonstrating the opposite pattern (significantly decreased activation during smoking abstinence in prenatally exposed subjects relative to controls) are shown in blue/purple. Voxel threshold: $p < 0.001$, cluster threshold: eight contiguous significant voxels. Plots are shown of the average percent signal change for the left and right hippocampal voxels demonstrating significant group by smoking condition interaction effects.

in subjects with prenatal exposure and decreased activation of these structures in subjects with no prenatal exposure.

DISCUSSION

In the present study, adolescent tobacco smokers with prenatal exposure to active maternal smoking experienced greater nicotine withdrawal-related deficits in immediate and delayed visuospatial memory relative to adolescent smokers with no prenatal exposure to maternal smoking. Among adolescent smokers with prenatal exposure, nicotine withdrawal was associated with increased activation of the left parahippocampal gyrus during early recognition testing of visuospatial stimuli and increased activation of bilateral hippocampus during delayed recognition testing of visuospatial stimuli. These findings are consistent with prior work in animals and in humans, indicating that gestational exposure to nicotine absorbed during active maternal smoking exerts long-lasting effects on brain development that alter the subsequent brain response to nicotine during adolescence (Kirkland *et al*, 2000; Cornelius *et al*, 2000; Griesler *et al*, 1998; Kandel *et al*, 1994; Abreu-Villaca *et al*, 2004a, b).

Work in rodents has shown that combining prenatal with adolescent exposure to nicotine produces additive effects on neurons and synaptic function (Abreu-Villaca *et al*, 2004a, b). Prenatal nicotine exposure increases hippocampal size, DNA content, and neuronal packing density (Roy *et al*, 2002; Abreu-Villaca *et al*, 2004a). The hippocampus is unique among brain regions in retaining the capacity to generate new neurons into adolescence (McEwen, 1999, 2001), and has been shown to exhibit synaptic sprouting and reinnervation in response to lesioning throughout the juvenile period (McWilliams and Lynch, 1983, 1984). Hippocampal changes pursuant to gestational exposure to nicotine thus likely reflect adaptive rearrangement of neuronal populations and synapses (Abreu-Villaca *et al*, 2004a), and are magnified when followed by adolescent nicotine administration (Abreu-Villaca *et al*, 2004a). The present findings suggest that, in human adolescents, changes in hippocampal architecture associated with combined prenatal and adolescent exposure to active tobacco smoking produce alterations in hippocampal function during nicotine withdrawal and concomitant deficits in visuospatial memory.

Animal studies have also demonstrated evidence that prenatal exposure to nicotine impairs cholinergic responsiveness in adolescence, blunting the ability of nicotine to upregulate nAChRs and desensitizing neuronal responses to nicotine challenge (Abreu-Villaca *et al*, 2004b; Seidler *et al*, 1992). In addition, nicotine withdrawal has been found to induce cholinergic hypoactivity in adolescent rodents with prenatal exposure to nicotine (Abreu-Villaca *et al*, 2004b). Given that reductions in cholinergic neurotransmission disrupt memory in humans (Rosier *et al*, 1998, 1999; Mewaldt and Ghoneim, 1979), the present observations of significant decrements in visuospatial memory that emerge during nicotine withdrawal in adolescent smokers with exposure to active maternal smoking are consistent with these preclinical findings. In the present study, self-administration of nicotine via tobacco smoking was

associated with a reversal of withdrawal-related deficits in memory function in prenatally exposed adolescents. Cholinergic hypoactivity induced by nicotine withdrawal in prenatally exposed adolescent rodents is prolonged (Abreu-Villaca *et al*, 2004a). Together, these observations lend strength to the proposal that cholinergic hypoactivity may be a mechanism by which prenatal exposure elevates the risk of regular tobacco smoking, nicotine dependence, and relapse to smoking during quit attempts in offspring, as nicotine may be taken to self-medicate brain functional deficits resulting from cholinergic hypoactivity (Abreu-Villaca *et al*, 2004a). Reduced cholinergic reactivity and nicotine response in adolescents with prenatal exposure would also drive higher tobacco consumption in order to achieve the requisite restoration of 'normal' cholinergic function, and thus may have contributed to the higher rate of cigarette consumption observed in the present sample of prenatally exposed adolescents and in other samples of prenatally exposed adults (Buka *et al*, 2003). Conversely, cognitive deficits experienced by adolescent smokers with prenatal exposure to maternal smoking in school, where smoking is not permitted, may place them at greater risk of academic failure.

In the present study, BVMT-R total immediate and delayed recall tended to improve during smoking abstinence in adolescents with no prenatal exposure to active maternal smoking. Nicotinic receptors desensitize following exposure to nicotine, with desensitization occurring over seconds and resensitization occurring over minutes (Grady *et al*, 1994). Work in rats has shown that nicotine administration induces upregulation of nAChRs (Nguyen *et al*, 2003), while postmortem work in humans has shown that tobacco smoking increases nAChR-binding sites in adult smokers in a dose-dependent manner (Perry *et al*, 1999). During smoking abstinence, these subjects have an increased number of sensitized nAChR receptors available to endogenous acetylcholine (Leonard and Giordano, 2002). Enhancement of cholinergic neurotransmission has been shown to improve memory performance by improving the selectivity of perceptual processing during encoding (Furey *et al*, 2000). Two prior studies have observed improved memory performance in healthy adult smokers during acute smoking abstinence (George *et al*, 2002; Jacobsen *et al*, 2004). The present findings suggest that this effect occurs in adolescent smokers and is modified by prenatal exposure to active maternal smoking.

Prior work in rodents using tone-shock fear conditioning and the Hebb-Williams maze has provided evidence that reduction of cholinergic neurotransmission is disruptive to encoding, but not to retrieval (Rogers and Kesner, 2003, 2004). In the present study, significant group differences in modulation of hippocampal and parahippocampal activation by smoking condition were evident only during early and delayed recognition testing and not during encoding or rehearsal. Increased hippocampal and parahippocampal activation observed during smoking abstinence in prenatally exposed adolescents during recognition testing may reflect compensatory activation in response to deficient encoding. Supporting this interpretation, prior work has shown that enhanced cholinergic neurotransmission increases the activation of primary sensory cortices during encoding and reduces the activation of brain regions

involved in higher-order processing during mnemonic tasks (Furey *et al*, 2000). This finding suggests that increased cholinergic neurotransmission enhances sensory processing during encoding, thereby reducing the demand on brain regions that mediate higher-order mnemonic processes (Furey *et al*, 2000). If, as suggested by preclinical and postmortem studies reviewed above, smoking abstinence leads to an increase in sensitized nAChR receptors and cholinergic neurotransmission in adolescent smokers without prenatal exposure to active maternal smoking, then reduced medial temporal lobe activation observed in these subjects during delayed recognition testing during smoking abstinence may stem from cholinergically mediated enhancements in encoding, leading to reduced hippocampal and parahippocampal load during recognition testing. Conversely, if prenatally exposed adolescent smokers do not upregulate nAChRs during smoking and thus experience decreased cholinergic neurotransmission during smoking abstinence, then increased medial temporal lobe activation observed in these subjects during delayed recognition testing during smoking abstinence may stem from less effective encoding, leading to increased hippocampal and parahippocampal load. Our failure to observe significant group differences in the effect of smoking cessation on medial temporal lobe activation during encoding may stem from the limited size of the sample undergoing imaging in the present study. Accordingly, findings regarding the effect of prenatal exposure to active maternal smoking on changes in hippocampal and parahippocampal function induced by smoking cessation in adolescents during mnemonic processing must be viewed as preliminary until replicated.

The possibility that the group differences in cognitive performance and brain function observed in this study stem from factors unrelated to prenatal exposure to active maternal smoking cannot be excluded by the present data. However, the fact that group by smoking condition interaction effects remained significant after group matching and statistical controls for potentially confounding variables, including cannabis and alcohol use, argues against this possibility, as does the resemblance of our findings to the effects predicted from animal models of prenatal and adolescent nicotine exposure and withdrawal. More females than males participated in this preliminary study. Preclinical work has suggested that the combined effects of prenatal and adolescent exposure to nicotine on protein biomarkers may be more marked in female rodents (Abreu-Villaca *et al*, 2004a), while the combined effects of prenatal and adolescent exposure on some markers of cholinergic tone may be more pronounced in male rodents (Abreu-Villaca *et al*, 2004b). Recent clinical work has suggested that the effects of adolescent tobacco smoking on cognitive performance may be more pronounced in males (Jacobsen *et al*, 2005). Thus, the effect of gender on the vulnerability of the brain to the combined effects of prenatal and adolescent nicotine exposure is likely complicated. Although the findings observed in the present study remained significant after statistically controlling for the differences in gender distribution across groups, further work is needed in larger samples balanced for gender in order to determine how gender may modify the effects of combined prenatal and adolescent exposure to tobacco

smoking on brain function. An effect of prenatal exposure on verbal memory was not detected in this study. However, the possibility that the divergent findings regarding effects of prenatal exposure on verbal and visuospatial memory stem from subtle differences in the difficulty of the HVLT-R and BVMT-R tasks, as opposed to differences in the vulnerability of neural systems supporting verbal and visuospatial memory to damage resulting from prenatal exposure to nicotine, cannot be excluded by the present data. Other limitations include the measurement of prenatal exposure by retrospective self-report. Work comparing prospectively and retrospectively collected data about pregnancy has supported the accuracy of pregnancy data collected by retrospective self-report (Buka *et al*, 2004). Similarly, comparison of serum cotinine concentrations and self-reported smoking behavior during pregnancy has yielded evidence of significant agreement between these measures (Buka *et al*, 2003). Nevertheless, rates of smoking during pregnancy may have been underreported in the present study due to the social stigma attached to this behavior. Underreporting of maternal smoking during pregnancy in the unexposed group would tend to reduce the observed group differences. Thus, to the extent that this occurred, the actual effects of gestational exposure to active maternal smoking on brain response to smoking and nicotine withdrawal during adolescence may be larger than those reported herein. Finally, the possibility that genetic factors shared by mothers and their offspring may mediate both increased risk for smoking and increased vulnerability to the development of memory deficits during nicotine withdrawal cannot be excluded by these data.

In summary, the present results indicate that adolescent smokers exposed to active maternal smoking during gestation experience greater abstinence-induced deficits in visuospatial memory and concomitant changes in hippocampal and parahippocampal function relative to adolescent smokers with no gestational exposure to active maternal smoking. These findings are consistent with preclinical data showing that combined prenatal and adolescent exposure to nicotine produces reductions in cholinergic neurotransmission and more pronounced changes in hippocampal function than does either prenatal or adolescent exposure alone (Abreu-Villaca *et al*, 2004a,b). At present, approximately 11% of women worldwide who give birth are unable to stop smoking during their pregnancies (Martin *et al*, 2005; Windsor, 2001). The present findings underscore the importance of developing more effective treatments for smoking cessation that do not involve nicotine replacement for these women. Equally critical, the fact that exposure limited to the first trimester and exposure throughout gestation produced similar effects emphasizes the need to focus treatment efforts on women of childbearing age, in addition to pregnant women. Further, these data extend previous work linking prenatal exposure to active maternal smoking with elevated risk of subsequent tobacco smoking and nicotine dependence, and suggest that prenatal exposure may define a subgroup of adolescent smokers who may particularly benefit from smoking cessation treatment that addresses cessation-related changes in brain function. Further work is needed to develop therapies designed to reduce the functional deficits associated with smoking abstinence in this population of adolescents.

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