Effects of Nicotine on Hippocampal and Cingulate Activity During Smooth Pursuit Eye Movement in Schizophrenia

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Background: Abnormal smooth pursuit eye movement (SPEM) in schizophrenic patients is a well known phenomenon, but the neurophysiological mechanisms underlying the deficit are unknown. Nicotine temporarily improves SPEM and has been associated with reduced hippocampal hemodynamic activity in schizophrenics. Nicotine’s effect on brain activity in control subjects performing SPEM has not been studied. The purpose of this work was to determine if nicotine differentially affects brain activity in schizophrenic and control subjects during pursuit eye tracking.

Methods: 16 subjects with schizophrenia and 16 control subjects underwent functional MR imaging during SPEM after receiving placebo or nicotine gum. Four brain regions were analyzed for main effects of group, drug, and interactions: hippocampus, cingulate gyrus, frontal eye fields, and area MT.

Results: Nicotine reduced hippocampal activity in both groups, but the effect was greater in control subjects. A group by drug interaction was observed in the anterior cingulate gyrus, where nicotine decreased activity in control subjects and increased activity in schizophrenics.

Conclusions: Nicotine may improve SPEM performance in people with schizophrenia through cholinergic stimulation of the hippocampus and cingulate gyrus. Potential mechanisms include improved inhibitory function and attention.

Key Words: Nicotine, smooth pursuit eye movement, schizophrenia, fMRI, hippocampus, cingulate

The nicotinic cholinergic system is one of several neurotransmitter systems implicated in the pathophysiology of schizophrenia. Evidence for this involvement includes (a) a higher frequency of nicotine dependence in persons with schizophrenia (Hughes et al 1986), (b) the finding of fewer nicotinic receptors in brains of schizophrenic subjects compared to control subjects (Freedman et al 1995; Durany et al 2000), (c) the normalization of two sensory gating deficits, auditory P50 and smooth pursuit eye movements (SPEM), in schizophrenic patients by nicotine (Adler et al 1993; Olincy et al 1998), (d) the modulation of sensory gating by nicotinic agonists in animals (Stevens and Wear 1997), and (e) an association between inhibitory function and nicotinic cholinergic receptor activity in animals (Alkondon et al 1997).

Smooth pursuit eye movements enable the continuous maintenance of foveal vision on a moving target. Performance depends on a complex interaction of sensorimotor transformation, retinal and extraretinal motion processing, attention, and prediction. Parsing out specific components of the pursuit deficit could suggest the underlying neurophysiological mechanisms of the deficit in schizophrenia (Avila et al 2002). For example, increased anticipatory saccades found in people with schizophrenia and their relatives (Clementz et al 1990; Rosenberg et al 1997; Ross et al 1998) may represent a disturbance in attention or internal representation (Rosenberg et al 1997), premature anticipation (Hommer et al 1991), or inhibitory failure (Levin 1984; Rosenberg et al 1997; Ross et al 2002). While the basic neural pathways of pursuit eye movements are relatively well understood, it is not known if specific brain regions underlie this deficit in people with schizophrenia.

Nicotine is the only drug known to improve SPEM deficits in schizophrenic subjects (Avila et al 2003; Olincy et al 2003a; Sherr et al 2002). Nicotinic cholinergic receptors are widely distributed throughout the brain. The α7 nicotinic cholinergic subtype is particularly rich in the hippocampus (Nestler et al 2001). Activation of cholinergic receptors on inhibitory interneurons is one possible mechanism by which nicotine improves pursuit eye movements. Alterations in inhibitory γ-aminobutyric acid (GABA) interneurons, particularly those in the hippocampus and anterior cingulate gyrus, have been implicated in schizophrenia (Benes and Berretta 2001). Several human neuroimaging studies have demonstrated differences in hippocampal activity at rest (Malaspina et al 2004), during smooth pursuit (Tregellas et al 2004), and during memory retrieval in schizophrenic subjects (Heckers et al 1998) compared to control subjects (for review, see Heckers 2001). Hippocampal hyperactivity in schizophrenic subjects was observed during smooth pursuit eye movements using functional magnetic resonance imaging (fMRI) methods (Tregellas et al 2004). Nicotine was associated with a reduction in hippocampal hyperactivity in the patients (Tregellas et al 2005), supporting a hypothesis that nicotine improves smooth pursuit by improving inhibitory function in the hippocampus. It is not known if the reduction in hippocampal activity is specific to schizophrenia or represents a general effect of nicotinic cholinergic stimulation.

Moderate levels of nicotinic cholinergic receptors, with a significant portion of the α4β2 subtype (Chattopadhyay et al 2005), are found in the cingulate gyrus, a region also implicated in the smooth pursuit pathway (Berman et al 1999; Schmid et al 2001; Tanabe et al 2002). Several studies have shown that nicotine modulates cingulate gyrus activity with concomitant improvements in attentional performance (Jacobsen et al 2004; Lawrence et al 2002). Given these findings, it is possible that attentional facilitation may partly explain nicotine’s beneficial effect on pursuit eye movements (Depatie et al 2002).

The purpose of this study was to extend previous work to a placebo-controlled, case-controlled, crossover design to com-
pare the effects of nicotine on brain activity in schizophrenic subjects to that of control subjects during smooth pursuit eye movements. We postulated that nicotine improves the pursuit deficit in people with schizophrenia by reducing hippocampal hyperactivity (e.g., improving inhibitory function) and increasing frontal and cingulate gyrus activity (e.g., improving attention). We tested for the effects of drug and group using anatomically defined regions-of-interest (ROIs): hippocampus, cingulate gyrus, frontal eye fields, and area MT.

Methods and Materials

Subjects

16 subjects with DSM-IV schizophrenia (n = 15) or schizoaffective disorder, depressive type (n = 1) and 16 control subjects participated in this study. Subjects were recruited from the Denver Veterans Affairs Medical Center and the Denver Metro Area Mental Health Clinics. Control subjects were recruited from the Denver metro area via an email advertisement and word of mouth. Capacity to participate was informally assessed through the consent process. Participants were required to describe the procedures associated with the study as well as the most common and most serious side effects of nicotine. They were also assessed for their ability to follow directions during the gum chewing procedure. Diagnoses were confirmed using the Structured Diagnostic Interview for DSM-IV or the Diagnostic Interview for Genetic Studies (Nurnberger et al 1994) and severity of symptoms was assessed using the Positive and Negative Syndrome Scale (Kay et al 1987). One subject was neuroleptic-naïve, one subject was taking a typical, 13 subjects were taking atypical, and 1 subject was taking both typical and atypical neuroleptics. There were no differences in age, education, gender, or smoking or nicotine dependence. A medical history was gathered from and physical exam performed on all subjects to ensure that no subjects suffered from known cardiac atherosclerotic disease, uncontrolled hypertension, diabetes, or neurologic illness. Three subjects with schizophrenia were taking cholesterol or lipid lowering agents. One subject with schizophrenia was taking an antihypertensive medication.

Smokers were asked to abstain from smoking for 6 hours prior to scanning. They were monitored for two hours prior to magnetic resonance (MR) scanning to ensure non-smoking status. Carbon monoxide levels were measured to verify subjects had not smoked recently. If carbon monoxide levels exceeded 14 ppm, the subject was asked to return on another day after abstaining for 6 hours prior to the scan. Only one individual had an elevated carbon monoxide level and he returned 7 days later. All volunteers provided written, informed consent approved by the Colorado Multiple Institutional Review Board.

Task Design

Subjects performed a visual smooth pursuit task adapted from Radant and Hommer (1992) in the MR scanner. The task consisted of tracking a white dot that moved horizontally across a black background over a visual angle of 28° at a constant velocity of 16.7°/sec followed by a 700 msec fixation period at the edges. The paradigm was a block design with 4 cycles of task and rest per run. During “rest” the subject was told to “look straight ahead” at a black screen.

MR Parameters

The study was performed on a 1.5T Siemens Vision MR system (Siemens AG, Iselin, New Jersey) using a standard quadrature head coil. A high resolution 3D T1-weighted anatomical scan (repetition time (TR) = 45, echo time (TE) = 20, flip angle (FA) = 45, 2562 matrix, 240 mm2 field of view (FOV), 1.5 mm thick coronal slices) was acquired, followed by the functional images using gradient-echo echoplanar imaging (TR = 2500, TE = 50, 642 matrix, 240 mm2 FOV, 20 axial slices angled parallel to the planum sphenoidale, 6 mm thick, 1 mm gap). Each run consisted of a 10 sec equilibration period, followed by 4 cycles of 25 sec task/25 sec rest. Two runs were acquired prior to drug administration (“pre-drug” scans) to control for any placebo effect. Subjects were then removed from the scanner and given either nicotine or placebo gum. Following drug administration subjects returned to the scanner to perform two additional runs of the smooth pursuit task (“post-drug” scans). Subjects returned the following week to repeat the procedure with either placebo or nicotine, whichever they did not receive previously.

Drug Administration

After the “pre-drug” scans, subjects were given either nicotine or placebo gum, in a counterbalanced, single-blinded design. Nicotine was administered as nicotine polacrilex (Nicorette) gum. Smokers received 6 mg (three 2 mg pieces) and nonsmokers received 4 mg (two 2 mg pieces). The pieces were pressed together with one piece of similar tasting placebo gum to maximize taste similarity between the nicotine and placebo condition. The placebo gum consisted of 3 (for the non-smokers) or 5 (for the smokers) pieces of Orbit brand peppermint gum adhered to one another to form one large piece of gum. The nicotine gum consisted of 2 (or 3) pieces of 2 mg Nicorette and 1 (or 2) pieces of Orbit brand peppermint gum adhered to one another to form one large piece of gum. Subjects were not allowed to look at the gum prior to placing it in their mouth to reduce any visual cues to the gum’s identity. The adhering of the gum pieces was performed to reduce any tactile clues to the gum’s identity and the use of a combination of Orbit and Nicorette in the nicotine condition was utilized to reduce any taste or olfactory clues to the gum’s identity. Subjects chewed the gum for 10 minutes and were asked not to swallow their saliva but instead spit every 2 minutes into a cup. During both nicotine and placebo administration, blood pressure and heart rate were monitored. After the 10 minute gum chewing session, subjects returned to the magnet for the “post-drug” scans. Following scanning, subjects were monitored for 1 hour to ensure that any changes in blood pressure or heart rate returned to normal. During the week prior to MR imaging, all subjects were given 4 mg nicotine gum and monitored to ensure they were able to tolerate the drug safely.

fMRI Data Analysis

Preprocessing. Spatial pre-processing, model specification and estimation, and statistical inference were performed with Statistical Parametric Mapping (SPM2). The first four image volumes from each run were excluded for saturation effects. Images were motion-corrected and normalized to the Montreal Neurological Institute (MNI) template. One subject was excluded because motion exceeded 2 mm. A 4 mm full width half maximum Gaussian smoothing kernel was applied to the normalized images. The effective smoothing was approximately 7 × 7 × 9 mm3.

Model. Data were analyzed using two-stage mixed effects model (Friston et al 1999). The time series model consisted of a boxcar convolved with a hemodynamic response function. Parameter estimates of for each individual (e.g., SPM contrast images comparing “post-drug” scan to “pre-drug” scan) were then entered into the second level and analyzed using a one-way ANOVA.
analysis of variance (ANOVA) with constant. The 4 “columns” of the design matrix represented nicotine-control, placebo-control, nicotine-schizophrenia, and placebo-schizophrenia. Four brain regions were analyzed: hippocampus, cingulate gyrus, frontal eye fields, and area MT. We focused on the hippocampus based on previous findings of hyperactivity in schizophrenic subjects and reduced activity with nicotine (Tregellas et al 2004, 2005). The cingulate gyrus was selected because of its moderate cholinergic receptor density and involvement in smooth pursuit. Frontal eye fields and area MT were selected as they are known to be involved in smooth pursuit eye movements. Hippocampal and anterior cingulate ROIs were anatomically, not functionally, defined. Right and left hippocampal formations were manually outlined on the “single-subject T1 MNI template” using MRIcro (Rorden and Brett 2000), converted to Analyze format (Mayo Clinic, Rochester, Minnesota), and imported into the MarsBaR tool (Brett et al 2002). Anterior was arbitrarily separated from posterior hippocampus at the posterior margin of the thalamus. Right and left sides were combined as there were no a priori hypotheses about sidedness and separate analyses revealed similar findings on each side. The anterior and posterior cingulate gyrus was defined using the Anatomical Automatic Labeling (AAL) for SPM2 (Tzourio-Mazoyer et al 2002). Examples of the hippocampal and anterior cingulate gyrus ROIs used for this study are shown in Figure 1. Frontal eye fields and area MT were defined as 2 centimeter diameter spheres centered on an average of Talairach coordinates reported in the literature (frontal eye field: ± 38, −8, 52; area MT: ± 45, −80, −7; Berman et al 1999; Cullham et al 1998; Tanabe et al 2002). Data were extracted from the ROI using the MarsBaR toolbox (Brett et al 2002) at the second level of analysis. All voxels in the ROI were averaged, entered into the model, and estimated. The response variable was the parameter estimate for main effects of group (schizophrenic vs. control), drug (nicotine vs. placebo), and group by drug interaction. One-tailed t-tests were used for the hippocampus because our a priori hypothesis predicted reduced activation associated with nicotine (Tregellas et al 2005). Two-tailed t-tests were used for all other regions for which there were no a priori hypotheses. Extracted data were also analyzed using cross-over analysis of variance to test for covariate effects of smoking, gender, age, order of drug, and scan session (Statistical Analysis Software, Cary, North Carolina).

A secondary, exploratory, whole brain analysis of drug and group conditions was performed. Statistical parametric maps were set at an arbitrary threshold of $p < .005$, cluster size $= 20$ voxels, degrees of freedom $= 48$. We tested for pure effects of group by comparing only pre-drug scans across group.

**Results**

**Subjects**

The groups were not significantly different in age, gender, smoking, nicotine dependence, or education. There were expected differences in Positive and Negative Syndrome Scale for Schizophrenia (PANSS) scores (Table 1).

### Hippocampus, Cingulate Gyrus, Frontal Eye Fields, Area MT

#### Main Effect of Drug

There was a significant main effect of drug in the anterior hippocampus. Nicotine was associated with less anterior hippocampal activation across groups ($t = 1.71$, $p < .05$) (Figure 2). Nicotine was associated with less posterior hippocampal activation across groups, but this did not reach statistical significance ($t = 1.6$, $p < .06$). There was a non-significant main

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<th>Table 1. Group Demographics</th>
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<td>Age (years)</td>
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<tr>
<td>38.1 (6.4)</td>
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<td>Gender (% male)</td>
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<td>PANSS (Total)**</td>
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Data are presented as Mean(SD). For categorical variables, used Fisher’s exact 2-tailed.

*Fagerstrom Test for Nicotine Dependence (FTND; Heatherton et al 1991).

**Positive and negative syndrome scale for schizophrenia (PANSS;Kay et al 1987).
effect of drug on area MT (t = 1.5, p < .14). There was no main effect of drug on the frontal eye fields.

Main Effect of Group. There was no main effect of group in any of the regions.

Group by Drug Interaction. There was a significant group by drug interaction in the anterior cingulate gyrus. Compared to placebo, nicotine was associated with less anterior cingulate activity in control subjects, but more activity in schizophrenic subjects (t = 2.3, p < .04)(Figure 3).

Smoking, Gender, Age, Order of Drug, Scan Session. There were no carry-over effects. Covariates smoking, gender, age, and order of drug did not contribute to the model. There was a small but significant effect of scan session on anterior cingulate response (F = 13.4, p < .001). The mean response in anterior cingulate was lower in the second session (.14 vs. -.09) across group and drug conditions, and this effect was more significant in control subjects.

Whole Brain Analysis
Whole brain analysis did not result in statistically significant main effects or interactions, after correction for multiple comparisons using either family wise or false discovery rate methods. At a less conservative statistical threshold (p < .005, t < 2.6), whole brain SPMs were consistent with the ROI results (e.g. a main effect of nicotine in the hippocampus [Figure 4] and a drug by group interaction in the cingulate gyrus [Figure 5]). There was a non-significant main effect of group (schizophrenic > control) in the right basal ganglia (Talairach coordinates −26, 8, −5 mm). A comparison of pre-drug scans alone showed bilateral fusiform gyrus hyperactivity in schizophrenic subjects compared to control subjects (Figure 6), partially replicating previous results (Hong et al 2005; Tregellas et al 2004).

Discussion
The present study is an extension of an earlier paper reporting nicotine-associated decreased fMRI blood-oxygen-level-depen-
been found at rest and during cognitive tasks (Heckers 2001; Malaspina et al 2004). Tregellas et al (2004) found hippocampal hyperactivity in schizophrenic subjects during smooth pursuit eye movements, compatible with reduced inhibitory function in hippocampus.

Although nicotine reduced hippocampal activity in both groups, the magnitude of nicotine's effect was “dampened” in schizophrenic subjects compared to control subjects. This may reflect abnormal expression or function of hippocampal nicotinic cholinergic receptors. Post-mortem studies comparing schizophrenic to control subjects, across several levels of smoking, have shown reduced expression of high- and low-affinity neuronal nicotinic receptors in the hippocampus and other brain regions (Breese et al 2000; Freedman et al 1995). A dampened response to the drug is compatible with impaired hippocampal recruitment which has been observed using PET during memory retrieval in schizophrenic subjects (Heckers et al 1998).

Among several components of smooth pursuit, reduced gain and increased leading saccades have been proposed as potential markers for schizophrenia. Some researchers have hypothesized that leading saccades represent a failure of the frontal lobe to inhibit the generation of inappropriate saccades (Levin 1984). The association of abnormal SPEM with impairment on the Wisconsin card sorting test (Litman et al 1991) and an oculomotor delayed memory task (Park and Holzman 1993) implicate inhibitory failure of the pre-frontal cortex including frontal eye fields (Hommer et al 1991). Hong et al (2005) found reduced activity in frontal and supplemental eye fields in schizophrenic subjects compared to control subjects after matching for performance. Our findings did not support a significant effect of nicotine on frontal eye fields.

Figure 4. Main effect of drug. Compared to placebo, nicotine reduced blood-oxygen-level-dependent signal in the right hippocampus. (Second level analysis of variance, threshold t ≥ 2.66, cluster size 20 voxels, degrees of freedom = 48. Color bar corresponds to t-value).

Figure 5. Group by drug interaction in the anterior cingulate gyrus. Nicotine was associated with less activity in control subjects and greater activity in schizophrenic subjects during smooth pursuit eye movements. (Second level analysis of variance, threshold t ≥ 2.66, cluster size 20 voxels, degrees of freedom = 48. Color bar corresponds to t-value).
The second, somewhat unexpected, finding was a significant drug by group interaction in the anterior cingulate gyrus. Compared to placebo, nicotine increased anterior cingulate activity in schizophrenic subjects and decreased it in control subjects during the task (Figures 3 and 5). The anterior cingulate gyrus plays a crucial role in the regulation of attention, particularly when responses are complex or conflict (Botvinick et al 2004). Since the smooth pursuit task is a simple one not requiring a complex response (only to “follow the dot”), nicotine’s effect on cingulate activity is unlikely to involve response conflict. Anterior cingulate activity may be important in the predictive nature of smooth pursuit eye tracking (Berman et al 1999; Schmid et al 2001). Nicotine has been associated with decreased regional cerebral blood flow (Ernst et al 2001; Ghatan et al 1998) and BOLD fMRI “deactivation” (Lawrence et al 2002) in the anterior cingulate gyrus, independent of smoking status. These effects have been interpreted as representing either a reallocation of resources in order to focus on task or an overall euphoriant effect. In our study, the hemodynamic response to nicotine in the cingulate was increased in patients and decreased in control subjects. Decreased activity in control subjects may reflect reallocation of resources whereas hyperactivity in schizophrenic subjects may reflect an improvement in baseline dysfunctional attention, leading to better eye movement performance. This is consistent with prior work suggesting that nicotine improves pursuit in schizophrenic subjects and their relatives by improving attention (Depatie et al 2002; Rosenberg et al 1997). Modulating attention during the task (Rosenberg et al 1997) would allow future fMRI studies to more directly address if nicotine alters cingulate activity during smooth pursuit through attentional mechanisms. Our finding of a significant differential response in the cingulate gyrus is consistent with evidence for differences in structure (Goldstein et al 1999; Kubicki et al 2003), cytoarchitecture (Benes et al 1992), and metabolism in the cingulate gyrus of schizophrenic subjects compared to control subjects.

One reason for the significant main effect of nicotine was a concomitant placebo effect. In control subjects, placebo was associated with an increased BOLD response during the eye movement task in cingulate gyrus. In all cases, gum was administered in the latter half of the MR session when we would expect to see decreased activity with task repetition (Tanabe et al 2002) implying there was a true placebo effect. The reason for the placebo effect is uncertain but may have to do with differences in expectation between the groups. The cingulate gyrus has been implicated in expectation especially when paradigms involve nociceptive stimuli (Pariente et al 2005). Our results underscore the importance of placebo-controlled experimental designs. It is unlikely that group or drug effects were confounded by differences in systemic hemodynamic response (e.g. due to atherosclerotic disease or poor cardiac function) as the groups were of similar age and medical health, having been screened for coronary artery disease, cerebrovascular disease, and hypertension. Although we cannot exclude unknown atherosclerotic disease, the post-drug design would help control for differences in vasculature. There were no differences in blood pressure or heart rate after nicotine or placebo gum.

The finding of a significant reduction in cingulate gyrus activity during the second compared to first week of scanning was unexpected, but consistent with a habituation to the eye movement stimulus. That control subjects habituated more than schizophrenic subjects suggests a difference in the perception of novelty across groups.

The exploratory whole brain analysis of pre-drug scans revealed moderate hyperactivity in bilateral fusiform gyri of schizophrenic subjects compared to control subjects, consistent with previous work (Tregellas et al 2004) and partially overlapping the medial temporal occipital hyperactivity reported by Hong et al (2005). The finding of hyperactivity may be related to abnormalities in retinal versus extraretinal or impaired gaze processing.

While nicotinic effects observed in this study may involve direct cholinergic receptor projections to cortex and hippocampus, it is equally possible that these effects reflect nicotinic...
modulation of dopaminergic, serotonergic, and/or GABA transmission, as has been amply shown in animal studies.

A limitation of this study is the lack of eye tracking measurements in the magnet. Another limitation is that individual differences in smoking status could influence the BOLD response to nicotine. This is unlikely as smoking status did not contribute to the model when analyzed as a covariate. Previous studies suggest that the eye tracking deficit appears to be a function of underlying disease rather than smoking status (Domino et al 1997; Olincy et al 2003a; Ross et al 1999; Thaker et al 1991). Although it is possible that differences in neuroleptics could confound the BOLD response in the patients, this is unlikely since 13 of 16 patients were on atypical antipsychotic medications. Atypicals have been previously shown not to significantly affect the BOLD response during finger tapping (Braus et al 1999). As in all fMRI studies, an ROI approach increases statistical power at the expense of excluding potentially important brain structures. Although it is possible that nicotine mediates its effects on other regions in the pursuit pathways, our whole brain exploratory analysis did not support this. It is noted that the reduction in hippocampal activity secondary to nicotine, while significant, was small. Additional studies with larger sample sizes will be needed to confirm a role of the hippocampus in SPEM dysfunction in schizophrenia.

In summary, this study demonstrated significant effects of nicotine on the hippocampus and anterior cingulate gyrus during smooth pursuit eye movements. Nicotine was associated with reduced hippocampal activity across groups and it differentially affected anterior cingulate gyrus activity depending on diagnosis. The present findings provide evidence that some, but not all, of nicotine’s ability to improve smooth pursuit eye movement in schizophrenic subjects may occur through mechanisms that involve improved inhibition and attention.

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