CHAPTER TEN

The Nicotine Hypothesis

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1. INTRODUCTION

1.1. Nicotine

The effects of acetylcholine (ACh), the neurotransmitter, are separated into two broad categories, muscarinic and nicotinic. Both muscarinic and nicotinic acetylcholine receptors (AChRs) are widely distributed throughout the human body including the nervous system. The manifold manifestations of the effects of ACh on the central nervous system (CNS) are mediated through stimulation of both muscarinic and nicotinic AChRs. This review focuses on the role of dysfunction of central neuronal nicotinic acetylcholine receptors (nAChRs) in the pathogenesis and the pathophysiology of schizophrenia and other neuropsychiatric disorders.

Nicotine, an exogenous substance available in a vast spectrum of forms for human consumption, exerts profound effects throughout the human body. There are several subtypes of neuronal nAChRs in the human body. Ethnic, familial, cultural, environmental, and social influences alter the propensity of a particular person to utilize nicotine in its many forms. Nicotine is extensively metabolized, primarily in the liver (Schroeder, 2005), but also in the lung (Turner et al., 1975), resulting in a variety of metabolites. Cytochrome P450 2A6 (CYP 2A6), a member of the cytochrome P450 mixed-function oxidase system, is the primary enzyme responsible for the oxidation of nicotine and cotinine. The rate of metabolism of nicotine is influenced by genetic, environmental, and other influences. The likelihood that a person will develop nicotine dependence is directly proportional to the persons's rate of metabolism of nicotine (Benowitz, 2008).

Tobacco consumption by smoking cigarettes is a popular form of obtaining nicotine. Cigarette smoking and secondhand exposure to cigarette smoke constitute major public health problems with significant morbidity and mortality (Brašić et al., 2009; Eisner et al., 2007). Facilitating the smoking cessation of smokers may be the most beneficial intervention of a physician (Schroeder, 2005). Cigarette smoking

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constitutes a lethal preventable health liability. Cigarette smoking results in approximate annual death rates of 440, 000 in the United States and 5,000,000 in the world (Schroeder, 2004). Cigarette smoking has resulted in disabilities, including chronic obstructive pulmonary disease and pulmonary carcinoma (Anonymous, 2003). Total fatal outcomes of cigarette smoking exceed the total fatal outcomes due to human immunodeficiency virus, alcohol and other substances, motor vehicle accidents, and suicide (Centers for Disease Control and Prevention, 2011). Overall nonsmokers live 10 years longer than smokers (Doll et al., 2004). Fatalities due to cigarette smoking are particularly high in people with mental disorders including the abuse of other substances; a third of the deaths in this population result from cigarette smoking (Williams, et al., 2005; Lasser et al., 2000). Even among nonsmokers nine percent of deaths are attributable to inhalation of secondhand cigarette smoke (Anoymous, 2002). Infertility, unfavorable outcomes of pregnancy, carcinomal of the breast, and poor vision due to cataracts and macular degeneration are several of the disorders resulting at least in part from cigarette smoking (Office on Smoking and Health, National Center for Chronic Disease Prevention and Health Promotion, 2004). Many of the adverse health effects of cigarette smoking can be avoided by the consumption of nicotine in a variety of alternative forms including gum, patch, lozenge, nasal spray, and inhaler (Schroeder, 2005). The inhaled particles of smoke pass through the pulmonary circulation into the arterial blood supply to the brain. In the brain the nicotine passes the blood–brain barrier to bind to neuronal nAChRs, sites on postsynaptic neurons (Benowitz, 2008).

1.2. Acetylcholine Receptors

ACh acts widely throughout the organs of the body including the nervous system. Neurons, nerve cells, communicate with each other by secreting a chemical compound across a synapse, the gap between neurons. The receiving postsynaptic neuron contains receptors, structures that bind to the substance emitted by the initiating neuron. Neuronal AChRs exist in two broad groups, muscarinic and nicotinic.

1.3. Muscarinic Acetylcholine Receptors

There exist at least five neuronal muscarinic acetylcholine receptors (mAChRs) subtypes categorized as (A) the group that couples through $G_{q/11}$ proteins to utilize phopholipase-C to mobilize calcium, M_1, M_3, and M_5, and (B) the group that utilizes $G_{i/0}$ proteins to diminish cyclic adenosine monophosphate (cAMP) in cells via reductions of adenylate cyclase, M_2 and M_4 (Langmead et al., 2008). In the CNS, the M_1 subtype predominates primarily in the cortex, the hippocampus, the striatum, and the thalamus (Langmead et al., 2008). The M_1 mAChR is a target of pharmacotherapeutic interventions to improve the cognitive deficits of Alzheimer's disease (AD) and other dementias (Langmead et al., 2008).

Several radiotracers have been developed to delineate the muscarinic system. *RS 3-quinuclidinyl-4-[^{123}I]iodobenzilate (RS [^{123}I]IQNB)* is a radiotracer used for clinical studies in humans to quantify changes in the concentrations of mAChRs (Eckelman, 2006). Since decrements in the uptake of regions of interest identified through imaging with *RS [^{123}I]IQNB* can be due to either (A) a reduced number of mAChRs per cell or (B) a reduced concentration of cells with mAChRs, this radiotracer has not gained widespread clinical use (Eckelman, 2006). *3-[[4-(3-[^{18}F]fluoropropylsulfanyl)-1,2,5-thiadiazol-3-yl]]-1-methyl-1,2,5,6-tetrahydropyridine. ([^{18}F]FP-TZTP),* a radiotracer to quantitatively estimate the function of the muscarinic system in humans, is a promising agent to monitor this system (Eckelman, 2006). The development of novel techniques to characterize the muscarinic system in health and disease promises to enhance the diagnosis and treatment of neurologic and psychiatric disorders, as well as other diseases.

1.4. Nicotinic Acetylcholine Receptors

nAChRs are channels operated by contact with the ligand. When opened, the channels allow the passage of sodium and calcium ions. Each nAChR is made up of five components. Twelve subunits are categorized as alpha (α) isoforms, α_2 to α_{10}, and beta (β) isoforms, β_2 to β_4. The $\alpha_4\beta_2$ constitute the predominant high-affinity nAChR subtype in the rat and human brain. The α_4 subunit participates in the sensitivity to nicotine. The β_2 subunit plays a role in dopamine release. The β_2 subunit also mediates the behavioral effects of nicotine. An asterisk ($\star$) indicates the preceding subunit may occur in multiples in the site on the postsynaptic receptor membrane. Thus, $\alpha_4\beta_2\star$ indicates that multiple β_2 isoforms are combined with a single α_4 isoform. The α_7 subunit likely mediates synaptic transmission and sensory gating (Benowitz, 2008).

High-affinity nAChRs are those nAChRs that bind *[^{3}H]nicotine* and *[^{3}H]cytisine.* These high-affinity sites are thought to be composed of α_4 and β_2 subunits. In the human brain the $\alpha_4\beta_2\star$ nAChR subtype is distributed with high density in the thalamus, the basal ganglia, other central structures, and the dentate gyrus, and with moderate density in the cortex, the hippocampal pyramidal layer, the limbic system, and other structures (Gotti et al., 1997).

nAChRs differentiate cigarette smokers from nonsmokers. While increased stimulation by agonists typically cause downregulation, a reduction in the number of cell surface receptors, chronic exposure to high levels of tobacco through cigarette smoking paradoxically causes upregulation, an increment in the number (B_{max}) of high-affinity $\alpha_4\beta_2\star$ nAChRs in the cortex, the hippocampus, the cerebellum, the striatum (Court et al., 1998; Gotti et al., 1997; Perry et al., 1999), the nucleus accumbens, and the ventral tegmental areas of the midbrain (Schroeder, 2005). The principal pharmacological site of action of nicotine appears to be the high-affinity $\alpha_4\beta_2\star$ nAChRs. Furthermore, human cigarette smokers exhibit a dose-dependent increase in high-affinity $\alpha_4/\beta_2\star$ nAChR *[^{3}H]nicotine* binding in the hippocampus and the thalamus (Breese et al., 1997).

High-affinity nAChR binding appears to differentiate people with schizophrenia from people without schizophrenia (Leonard et al., 1998). Both decreased inhibition of the P50 auditory evoked potential to repeated stimulation (Leonard et al., 1998) and the phenotype of schizophrenia have been associated with 15q13-q14 in the region of the α_7 nAChR (Craddock et al., 1999). The absence of a radiotracer for the α_7 nAChR hinders its investigation. However, dysfunction of the nAChR system in schizophrenia is not exclusively of the low-affinity α_7 subtype. For example, an increase in in vitro high-affinity nAChR ([^{3}H]nicotine) binding in the striatum, the hippocampus, and some thalamic nuclei in people with schizophrenia who smoke cigarettes has been reported by some researchers (Court et al., 1998). On the other hand, others have reported reductions in in vitro nAChR *([^{3}H]nicotine)* binding in the hippocampus, the cortex, and the caudate of people with schizophrenia who smoke cigarettes in contrast to people without schizophrenia who smoke cigarettes (Leonard et al., 1999). Additionally, in vitro $\alpha_4\beta_2\star$ nAChR *[^{3}H]cytisine* density is decreased in the postmortem striata of people with both schizophrenia and Parkinson's disease in contrast to normal control subjects (Durany et al., 2000). Furthermore α7 nAChR are reduced in the postmortem brains of people with schizophrenia (Kucinski et al., 2010).

Preparations of nicotine may be salutatory for some individuals with schizophrenia just as for some people with Parkingson's disease (Durany et al., 2000). The neuroprotective effects of nicotine observed in vitro in laboratory animals (Costa et al., 2001) likely hold in some humans.

Future research is needed to compare and contrast the number of high-affinity $\alpha_4/\beta_2\star$ neuronal nAChRs in the hippocampus, amygdala, cingulate, and other limbic structures, as well as the hypothalamus, striatum, and cortex of people with and without schizophrenia, using with positron emission tomography (PET).

A goal of future research is to demonstrate an objective measure to quantify high-affinity neuronal nAChR binding in the living human brain. If there exist marked reductions in high-affinity neuronal nAChR binding in the living brains of people with schizophrenia, then we shall have a tool to identify the deficits in people with schizophrenia and other neuropsychiatric disorders as well as a means to quantify the effects of pharmacological and other interventions to treat the deficits. Thus, there will be a means to assess people with schizophrenia and to monitor their progress during novel therapies. The needed technique will provide the means to quantitatively measure the occupancy by novel therapeutic agents of nicotinic receptors in the brains to facilitate the determination of optimal therapeutic doses. This procedure will thus facilitate the development of innovative pharmacological agents for schizophrenia, nicotine dependence, and other neuropsychiatric disorders.

The needed procedure likely will contribute to the knowledge about schizophrenia and other neuropsychiatric disorders, as well as nicotine dependence and other addictions.

2.1. SCHIZOPHRENIA

The high prevalence of cigarette smoking in individuals with schizophrenia (Gotti et al., 1997; Leonard et al., 1998) suggests that dysfunction of the nAChRs may (1) play a greater role in the relentless progressive deterioration common in people with schizophrenia who smoke cigarettes, and (2) provide clues to the development of novel effective treatments. The results of this review are crucial to determine biological markers for subtypes of people with schizophrenia, nicotine dependence, and other neuropsychiatric disorders.

The dopamine hypothesis of schizophrenia attributes positive symptoms to increased dopaminergic subcortical neurotransmission and negative symptoms to decreased dopaminergic cortical neurotransmission (Kucinski et al., 2010).

If a brain imaging procedure can determine alterations in the density and distributions of neuronal nAChRs in the living human brain of people with schizophrenia who do and do not smoke cigarettes, then those who likely will benefit from the administration of nicotine and nicotinic agonists can possibly be identified. Additionally, a subclass of people with schizophrenia who will not benefit from nicotinic agonists can likely be identified. They can then be spared the adverse effects of fruitless administrations of nicotine and nicotinic agonists.

The demonstration that a positive allosteric modulator of the α7 neuronal nAChR enhanced the current flow through α7 neuronal nAChR in the presence of ACh in cell cultures suggests that this agent exhibits the potential to alleviate the cognitive impairments associated with auditory gating inhibition in people with schizophrenia (Timmermann et al., 2007). This research suggests that positive allosteric modulators of neuronal nAChR may be fruitful agents to ameliorate the auditory gating inhibitions characteristic of people with schizophrenia. Additionally, other novel therapeutic interventions for schizophrenia and related neuropsychiatric disorders, as well as nicotine dependence and other addictions will likely be based on the results of this protocol.

Schizophrenia is a devastating disorder without effective treatments presenting in adolescence or young adulthood and afflicting approximately one percent of the population (American Psychiatric Association, 2000). Both genetic and environmental items likely play a role in the pathogenesis of schizophrenia (Tandon et al., 2008), a disorder likely resulting from alterations in the brain development during the prenatal period (Lafargue and Brasic, 2000).

The observation that many people with schizophrenia smoke cigarettes (Herrán et al., 2000; Williams and Ziedonis, 2004) suggests that the brains of these individuals may respond abnormally to nicotine. In contrast to control smokers without schizophrenia, smokers with schizophrenia have greater levels of nicotine and cotinine due to an increased nicotine intake per cigarette. Thus, people with schizophrenia are able to obtain large amounts

of nicotine from cigarettes (Williams et al., 2005). Greater positive symptoms and fewer negative symptoms are seen in people with schizophrenia who smoke cigarettes heavily (Williams and Ziedonis, 2004). Although smoking may reduce negative symptoms in people with schizophrenia (Gotti and Clementi, 2004), people with schizophrenia with nicotine dependence have poor outcomes (Aguilar et al., 2005).

Anecdotal studies suggest that nicotine and nicotine agonists are effective to treat some people with neurobiological disorders, e.g. Tourette syndrome (Dursun and Reveley, 1997; Sanberg et al., 1997). This review may (1) identify biological subgroups of people with schizophrenia who may be helped by nicotine preparations and related compounds and (2) provide the means to document the effects of therapies on the underlying deficits in high-affinity $\alpha4\beta2^{\star}$ neuronal nAChRs of the affected portions of the brains of individuals with schizophrenia and related conditions (Brašić et al., 2009, 2010; Wong et al., 2002; Zhou et al., 2002, 2004).

Research on autopsy material supports the hypothesis that nAChRs in the brain are dysfunctional in people with schizophrenia and nicotine dependence. In postmortem studies the neuronal nAChRs normally stimulated by nicotine are reduced in density in the brains of people with schizophrenia (Breese et al., 2000; Leonard et al., 2000). While postmortem studies suggest that binding to high-affinity neuronal nAChRs in the brains of people with schizophrenia who smoke cigarettes is half that of people without schizophrenia who smoke cigarettes (Breese et al., 2000; De Luca et al., 2006; Leonard et al., 1998), the difference is likely reduced by the postmortem changes.

Likely, the apparent alterations in neuronal nAChRs of people with schizophrenia represent an interaction between an inherent genetic effect and a drug effect of nicotine (De Luca et al., 2006). Since the cholinergic genes, CHRNA4 and CHRNB2, confer susceptibility to schizophrenia, some individuals with polymorphisms in CHRNA4 and CHRNB2 may be vulnerable to develop both nicotine dependence and schizophrenia (De Luca et al., 2006). Furthermore, among people with schizophrenia, heavy cigarette smoking is associated with the CHRNA4 rs3746372 allele 1 and CHRNA4 rs3787116 and rs3746372 suggesting an interaction between schizophrenia and the number of cigarettes smoked (Voineskos et al., 2007). At postmortem the density of neuronal nAChRs in the cortex exists in a continuum from highest density to lowest density as follows: (1) people without schizophrenia who smoke cigarettes; (2) people with schizophrenia who smoke cigarettes; (3) healthy people without schizophrenia who do not smoke cigarettes, i.e. normal control subjects; and (4) people with schizophrenia who do not smoke cigarettes (Breese et al., 2000).

In schizophrenia dysfunction of neuronal nAChRs may play an important role in the cognitive and attention deficits and relentless progressive deterioration (Sacco et al., 2005). Abnormalities in the transmission of auditory sensory information through the amygdala (Cromwell et al., 2005) likely plays a role in the symptoms of schizophrenia. People with schizophrenia exhibit alterations in P50 (Fresnán et al., 2007), N100 (Turetsky et al., 2008), N200 (Groom et al., 2008), and P300 (Begré et al., 2008; Groom

et al., 2008) auditory evoked potentials. The α7 neuronal nAChR subtype is likely associated with the P50 auditory inhibition marker on human chromosome 15 (Leonard et al., 1998; Leonard and Freedman, 2006; Li et al., 2004; Williams and Ziedonis, 2004). In contrast to healthy controls increased memory load led to increases in event-related desynchronization and synchronization in people with schizophrenia and, to a lesser extent, their co-twins discordant for schizophrenia (Bachman et al., 2008). Nicotine may reduce the disturbed information sensory gating of people with schizophrenia.

Exposure to smoking and related scenes produces activation in the hippocampus of smokers. Smoking-related cues result in activation of associative learning portions of the brain including the hippocampus. After rats addicted to nicotine experience withdrawal, the activation of high-affinity nicotinic receptors returns to baseline levels within 24 h. However, CA1 pyramidal neurons may exhibit an increased sensitivity to nicotine resulting in persistent susceptibility to utilize nicotine long after the last use of nicotine (Penton et al., 2011).

Utilizing kinetic modeling (Fujita et al., 1999, 2000; Yokoi et al., 1999), we demonstrated that *(S)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine (5-[^{123}I]IA),* a novel potent radioligand prepared by radioiododestannylation (Musachio et al., 2001) for high-affinity $\alpha_4\beta_2$* neuronal nAChRs (Musachio et al., 1999), provides a means to evaluate the density and the distribution of neuronal nAChRs in the living human brain of healthy adults (Brašić et al., 2009; Wong et al., 2001). Dynamic single photon emission computed tomography (SPECT) on a Trionix TriadXLT collected images over 6 h with 20 acquisitions (Brašić et al., 2009; Wong et al., 2001). A 2-compartmental (plasma and brain tissue) model was used for the kinetic analysis and parametric imaging of volumes of interest (VOIs) drawn on co-registered magnetic resonance imaging (MRI) scans (Brašić et al., 2009; Wong et al., 2001). The plasma radioactivity of centrifuged whole arterial blood obtained during the scan was measured with a gamma counter (Brašić et al., 2009). Plasma radiometabolites were also assayed with high performance lipid chromatography (HPLC) (Brašić et al., 2009; Hilton et al., 2000). The metabolite-corrected plasma input function was used for kinetic modeling (Brašić et al., 2009; Fujita et al., 1999, 2000). Binding potentials were calculated by the mathematical modeling utilizing the procedures for other radioligands of this series (Brašić et al., 2009; Yokoi et al., 1999). High plasma nicotine level was significantly associated with low *5-[^{123}I]IA* binding in the caudate head, the cerebellum, the cortex, the fusiform gyrus, the hippocampus, the parahippocampus, the pons, the putamen, and the thalamus. These findings confirm that *5-[^{123}I]IA* competes with nicotine to occupy nAChRs. We conclude that *5-[^{123}I]IA* is a safe, well-tolerated, and effective pharmacologic agent for human subjects to estimate high-affinity α_4/β_2 neuronal in the living human brain (Brašić et al., 2001, 2004, 2009; Wong et al., 2001; Zhou et al., 2001).

Since the high-resolution research tomography (HRRT), a brain dedicated PET, has superior resolution of 2 mm, 3–5 times smaller than SPECT, we now seek to perform HRRT, in place of SPECT, using PET [^{18}F] radiotracers, with greater binding

to nAChRs developed by (Horti and Wong, 2009) in place of *5-[^{123}I]IA*, an SPECT radiotracer.

We have demonstrated that *2-[^{18}F]fluoro-3-(2(S)-azetidinylmethoxy)pyridine (2-[^{18}F]FA)*, (Schildan et al., 2007; Sorger et al., 2007) a compound with the same chemical moiety to bind to the high-affinity $\alpha_4\beta_2$ neuronal nAChRs as *5-[^{123}I]IA,* has approximately twice the uptake in the thalami of healthy nonsmoking control adult volunteers than smoking adults with schizophrenia who smoked cigarettes the day of the scan (Brašić et al., 2010). Utilizing *2-[^{18}F]FA,* Brody et al. (2006) demonstrated that in human smokers inhalation of one or two mouthfuls of cigarette smoke led to uptake in half the $\alpha_4\beta_2\star$ neuronal nAChRs and that uptake of half the $\alpha_4\beta_2\star$ neuronal nAChRs occurred with plasma nicotine levels of 0.87 ng/mL (Brody et al., 2006). Additionally, PET with *2-[^{18}F]FA* demonstrated greater regional uptake in the brainstem and the cerebellum in smokers than in nonsmokers (Wüllner et al., 2008).

2.2. NACHR RADIOTRACERS

Several agents have been developed to image nAChRs (Brašić et al., 2009). *5-[^{123}I]IA, 2-[^{18}F]FA,* and *6-[^{18}F]fluoro-3-(2(S)-azetidinylmethoxy)pyridine (6-[^{18}F]FA)* are agents available for nuclear neuroimaging in humans with relatively slow uptake requiring several hours of administration of radiotracer to attain equilibrium (Horti et al., 2010). While some healthy adults can tolerate the hours required for the administration and the uptake of *5-[^{123}I]IA, 2-[^{18}F]FA,* and *6-[^{18}F]FA,* many patients with schizophrenia and other neuropsychiatric disorders cannot tolerate the discomforts and the inconveniences of these scans. Another drawback to the utilization of *5-[^{123}I]IA* (Brašić et al., 2009), *2-[^{18}F]FA* (Brašić et al., 2010), and *6-[^{18}F]FA* is the relatively limited uptake of these compounds outside the thalamus. This is a particular drawback for the evaluation of schizophrenia and other neuropsychiatric disorders with likely deficits in the dorsolateral prefrontal cortex and other extrathalamic regions of the brain.

2.3. ALZHEIMER'S DISEASE

2.3.1. Overview

Decrements in ACh transmitting neurons are hypothesized to play a role in the pathogenesis and pathophysiology of mild cognitive impairment (MCI) and AD (Kendziorra et al., 2010). Quantitative measurements of the density and the distribution of the high-affinity $\alpha_4\beta_2\star$ neuronal nAChRs provides a tool to guage the possible deficits present in MCI and progressive in AD. Additionally estimation of the extent of dysfunction of high-affinity $\alpha_4\beta_2\star$ neuronal nAChRs in MCI, AD, and related disorders may provide a tool to monitor the beneficial and adverse effects of therapeutic interventions for these disorders.

2-[^{18}F]FA (370 MBq) was administered by continuous infusion in a vein in an antecubital fossa over 90 s to 24 nonsmoking participants including 8 with MCI and 9

with AD as well as 7 age-matched healthy control subjects (Kendziorra et al., 2010). PET (ECAT EXACT HR+, CTI/Siemens, Knoxville, TN, USA) was performed in the 2-D acquisition mode to obtain 63 slices with a resolution of 4.7 mm full-width half maximum (Kendziorra et al., 2010), in the first 120 min (4 × 15 s, 4 × 1 min, 5 × 2 min, 5 × 5 min, and 8 × 6 min) as well as 6 h after the radiotracer administration (4 × 15 min) (Kendziorra et al., 2010). Input function arterial blood sampling was obtained to determine the counts of radioactivity with a Cobra gamma counter (Packard Instrument Company, Meriden, CT, USA) (16 in 3 min, then samples at 3, 4, 5, 6, 8, 10, 12, 14, 18, 25, 35, 50, 60 min, and then every 30 min until 7 h). Samples of arterial blood were obtained to measure the nonmetabolized fraction at 14, 60, 120, 240, 330, and 420 min (Kendziorra et al., 2010). Utilizing coregistered PET and MRI visualizations, the nondisplaceable binding potential (BP_{ND}) (Innis et al., 2007) was calculated with the corpus callosum or the cerebellum as the reference region (Kendziorra et al., 2010). Since both patients with MCI and AD demonstrated reduced BP_{ND}s in the regions affected in AD, the corpus callosum was utilized as the reference region. Progression to AD was observed solely in those people with MCI who had reductions BP_{ND}s. The cognitive impairment was inversely proportional to the BP_{ND}.

2.3.2. Sample Size Determination

Pilot studies of schizophrenia are often hindered by limited knowledge of the study variables. While the sample sizes of this study are so small that detailed statistical analysis is inappropriate, a strategy can be developed to assess small numbers of subjects in research studies. Utilizing a hypothesized proportional difference in the variable of interest between the experimental group and healthy volunteers, the minimal sample size to detect the hypothesized variations in the study variable in the experimental (P_1) and control (P_2) groups can be estimated. Proportions are estimated to range between 0 and 1 ($0 < P_1 < 1$, and $0 < P_2 < 1$). For example, suppose that based on prior research with 20 subjects in each group, we hypothesize that typical values of the study variable are exhibited by (1) a low proportion of subjects with schizophrenia, say, $P_1 = 0.05$, and (2) a high proportion of healthy adults, say, $P_2 = 0.75$. We estimate the power for a two-sample comparison of proportions to test the null hypothesis, H_0: $P_1 = P_2$, where the proportion of typical values of the variable of interest is P_1 in subjects with schizophrenia and P_2 in healthy volunteers. We assume that alpha (α) = 0.0500 (two-tailed), that there are 20 participants with schizophrenia, and that there are 20 control participants. The power resulting from the expected and slightly different proportions are listed in Table 1 (StataCorp, 1999). Even if the proportions obtained in the study deviate slightly from the hypothesized proportions, reasonable power is obtained with a sample size of 20 in each of the two groups as demonstrated by Table 1. For a pilot study of participants with schizophrenia or another rare disorder, these are reasonable powers with a sample size of 20 in each group (Brašić et al., 2003, StataCorp, 1999). The power resulting from the expected and slightly different proportions are listed in Table 1 (StataCorp, 1999).

Table 1 Power for Variable Proportions in 20 Participants with Study Condition (P_1) and 20 Healthy Control Participants (P_2) (StataCorp, 1999)

P_1	P_2	Power
0.05	0.65	0.6894
0.05	0.70	0.7832
0.05	0.75	0.8671
0.05	0.80	0.9338
0.10	0.65	0.5572
0.10	0.70	0.6582
0.10	0.75	0.7582
0.10	0.80	0.8501

Even if the proportions obtained in the study deviate slightly from the hypothesized proportions, reasonable power is obtained with the sample size of 10 in each of the two groups as demonstrated by Table 1. For a pilot study these are reasonable powers with a sample size of 10 in each of two groups (StataCorp, 1999).

3. CONCLUSIONS

The pathways for ACh, dopamine, serotonin, and other neurotransmitters are intimately related. Exposure to nicotine, nicotinic agonists, cocaine and other dopaminergic agents, and other substances affects the other neurotransmitter systems. For this reason, quantitative records of exposure to nicotine and other substances need to be obtained at baseline as well as during and after scans and other measurements of nicotinic receptors and other neurotransmitters. For this reason, we include tools to capture the key information for exposure to cigarettes and other forms of nicotine, various forms of cocaine (Please refer to Table A.1 in the appendix).

Characterization of people with schizophrenia remains a challenge for clinicians and researchers. Several procedures have been developed to standardize the criteria for schizophrenia (American Psychiatric Association, 2000; Brašić et al., 2009, 2010). Tools to measure the nuances of both positive (Andreasen, 1984b) and negative (Andreasen, 1984a) symptoms of schizophrenia will be invaluable to the identification of subtypes of people with schizophrenia. The effort required to accurately structured tools to identify the subtle differences between groups of individuals with schizophrenia (Andreasen, 1984a,b) will be rewarded by the precise characterization of subclasses of the disorder.

The Cocaine Administration Preference Questionnaire (Table A.1 in the Appendix) is a twelve-item form to be completed by the patient repeatedly before, during, and after treatments. Scores of individual items are tabulated for each section—A. Nasal inhalation, B. Smoking a joint, and C. Intravenous injection. For each section, a positive

score indicates preference for the indicated route of administration, while a negative score indicates a dislike of the indicated route of administration. A score of zero for a section indicates indifference to the suggested route of administration (Table A.1 in the Appendix).

The density and the distribution of nicotinic receptors likely are influenced by the phase of the menstrual cycle. By analogy the binding potential for dopamine $D_{2/3}$ receptors ($D_{2/3}$ Rs) in increased in the periovulatory and luteal phases and is decreased for the follicular phase (Wong et al., 1988). Like nicotinic receptors and other types of receptors throughout the brain exhibit similar waxing and waning with the menstrual cycle. For this reason, capturing the salient data to determine the phase of the menstrual cycle when the patient enters studies is a key foundation for meaningful data analysis. For this reason, all women are asked to complete both the Menstrual Cycle Questionnaire (Table A.2 in the Appendix) to obtain clinical relevant clinical data and the Menstrual Cycle Diary (Table A.3 in the Appendix) to obtain retrospective data to determine the specific phase of the menstrual cycle. The Menstrual Cycle Questionnaire (Table A.2 in the Appendix) is completed retrospectively by each woman one the date of screening for each study. The Menstrual Cycle Diary (Table A.3 in the Appendix) is given to each woman on the date of screening for each study to be completed prospectively to document the start and stop dates for all menstrual cycles from the start of the study until the completion of the study. If the woman records the start and the stop dates for at least three menstrual cycles, then a reasonable estimation of the phase of the menstrual cycle on the study date. Additionally, plasma is obtained from each woman on the study date for measurement of prolactin and progesterone levels to confirm the data recorded by the patient.

While identification of the phase of the menstrual cycle is crucial to the interpretation of scans and other quantitative measurements of nicotinic receptors and other neurotransmitters, caution is required to conduct research on pregnant women. The effects of exposures to radiation, chemicals, toxins, and other experimental agents on fetuses may be deleterious. For this reason participants who are pregnant and who may be pregnant are prudently excluded from research studies with experimental interventions. Women who are likely to become pregnant during the course of a research study are wisely excluded from imaging studies of nAChRs in the adult brain. Women may be told that they are welcome to return to participate in studies once they are not pregnant.

Characterization of the smoking preferences of all participants in research studies of nAChRs in the brain is needed to identify the subgroups for analyses. For this reason the Smoking Preferences Questionnaire (Table A.4 in Appendix 1) is administered to all potential participants as part of the screening procedure at baseline, during, and after protocols to visualize nAChRs.

Additionally researchers who administer nicotine patches will benefit from the administration of the Nicotine Patch Adverse Events Scale (Table A.5 in Appendix 2) to each patient before, during, and after each administration an a nicotine patch.

GLOSSARY OF ABBREVIATIONS

2-[^{18}F]FA, 2-[^{18}F]fluoro-3-(2(*S*)-azetidinylmethoxy)pyridine a radiotracer to visualize the high-affinity α4β2* neuronal nicotinic acetylcholine receptors (nAChRs) by means of positron emission tomography (PET).

5-[^{123}I]IA, (*S*)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine a radiotracer to visualize the high-affinity α4β2* neuronal nicotinic acetylcholine receptors (nAChRs) by means of single-photon computed tomography (SPECT).

6-[^{18}F]FA, 6-[^{18}F]fluoro-3-(2(*S*)-azetidinylmethoxy)pyridine a radiotracer to visualize the high-affinity α4β2* neuronal nicotinic acetylcholine receptors (nAChRs) by means of positron emission tomography (PET).

[^{18}F]AZAN, (−)-2-(6-[^{18}F]fluoro-2,3′-bipyridin-5′-yl)-7-methyl-7-aza-bicyclo[2.2.1]heptane ([^{18}F]JHU87522) a radiotracer to visualize the high-affinity α4β2* neuronal nicotinic acetylcholine receptors (nAChRs) by means of positron emission tomography (PET).

[^{18}F]FP-TZTP, 3-[[4-(3-[^{18}F]fluoropropylsulfanyl)-1,2,5-thiadiazol-3-yl]]-1-methyl-1,2,5,6-tetrahydropyridine a radiotracer to quantitatively estimate the function of the muscarinic system in humans.

ACh acetylcholine, an excitatory neurotransmitter

AChRs acetylcholine receptors, structures on postsynaptic neurons that send excitatory impulses on the neurons when activated by binding to acetylcholine (ACh) transmitted from the presynaptic neuron across the synapse.

AD Alzheimer's disease, a disorder typically presenting in people aged more than 65 years characterized by the progressive decline of cognitive functions.

BP_{ND} binding potential

CHRNA4 a cholinergic gene conferring susceptibility to schizophrenia.

CHRNB2 a cholinergic gene conferring susceptibility to schizophrenia.

CNS central nervous system, the brain and the spinal cord.

cAMP cyclic adenosine monophosphate, a compound to provide energy for cellular activities.

HPLC high performance lipid chromatography, a technique to separate proteins present in fluids

HRRT high resolution research tomography, a positron emission tomography (PET) tool with resolution approaching 2 mm.

mAChRs mucarinic acetylcholine receptors, acetylcholine receptors (AChRs) activated by muscarine.

nAChRs nicotinic acetylcholine receptors, acetylcholine receptors (AChRs) activated by nicotine.

MCI mild cognitive impairment, slight decline in intellectual function.

PET positron emission tomography, an imaging technique to visualize the physiology of organs through the detection of positrons released through the decay of radiotracers administered to the patient.

RS [^{123}I]IQNB, RS 3-quinuclidinyl-4-[^{123}I]iodobenzilate a radiotracer for clinical studies in humans to quantify changes in the concentrations of mAChRs.

SPECT single-photon computed tomography, an imaging technique to visualize the physiology of organs through the detection of photons released through the decay of radiotracers administered to the patient.

VOIs volumes of interest, three-dimensional structures to be represented by means of imaging techniques.

APPENDIX

Table A.1 Cocaine Administration Preference Questionnaire. This form is completed repeatedly by the patient before, during, and after each protocol. For each section a positive score indicates a preference, a negative score indicates a dislike, and a score of zero indicates indifference.

Name_____

Date_____

Instructions: Please answer all items based on how you feel right now. Please encircle your response.

A. Nasal administration preference items

1. I like to snort cocaine (through the nose).	No = 0	Yes = 1
2. I like to see others snort cocaine (through the nose).	No = 0	Yes = 1
3. Seeing others snort cocaine (through the nose) turns me on.	No = 0	Yes = 1
4. Seeing other people snort cocaine (through the nose) turns me off.	No = 0	Yes = –1

Nasal administration preference score (Total of scores 1–4) _____

B. Smoking administration preference items

5. I like to smoke cocaine.	No = 0	Yes = 1
6. I like to see others smoke cocaine.	No = 0	Yes = 1
7. Seeing other people smoke cocaine turns me on.	No = 0	Yes = 1
8. Seeing other people smoke cocaine turns me off.	No = 0	Yes = –1

Smoking administration preference score (Total of scores 5–8) _____

C. Intravenous administration preference items

9. I like to shoot up (inject intravenously) cocaine.	No = 0	Yes = 1
10. I like to see others shoot up (inject intravenously) cocaine.	No = 0	Yes = 1
11. Seeing other people shoot up (inject intravenously) cocaine turns me on.	No = 0	Yes = 1
12. Seeing other people shoot up (inject intravenously) cocaine turns me off.	No = 0	Yes = –1

Intravenous administration preference score (Total of scores 9–12) _____

Table A.2 Menstrual Cycle Questionnaire. To obtain the relevant data to determine the phase of the menstrual cycle on the date of the study, please ask the patient to complete this form for every assessment.

Instructions: Please complete this form as accurately as possible. You may write any additional information on the reverse page.

Subject Name: _____

History Number: _____

Date: _____/ _____/ _____

1. Age of menarche (when you started your first period): _____

2. Do you use birth control? (circle one) Yes No

If "Yes," please describe._____

If "Yes," how long have you used this form of birth control?:
_____

3. Average number of days between periods of menstruation: _____

4. Have you ever had problems associated with menstruation? Please describe.
_____

5. What was the first day of last month's menstruation? _____

6. What was the first day of your last menstruation? _____

7. Have you ever been pregnant? (circle one) Yes No

If "Yes", please describe.
_____
_____
_____

Table A.3 Menstrual Cycle. To obtain the relevant data to determine the phase of the menstrual cycle on the date of the study, please ask the patient to complete this form on screening for every study. Please ask the patient to return to the study physician on completion of the study.

Instructions: Please record the start date and the stop date for every menstrual cycle until the completion of the study. Please give this diary to the study physician on the date you complete the study. Please record additional information on the back of this page.

Subject Name: _____

Date: _____/ _____/ _____

Menstrual cycle 1

Start Date: _____/ _____/ _____

Stop Date: _____/ _____/ _____

Menstrual cycle 2

Start Date: _____/ _____/ _____

Stop Date: _____/ _____/ _____

Menstrual cycle 3

Start Date: _____/ _____/ _____

Stop Date: _____/ _____/ _____

Menstrual cycle 4

Start Date: _____/ _____/ _____

Stop Date: _____/ _____/ _____

Menstrual cycle 4

Start Date: _____/ _____/ _____

Stop Date: _____/ _____/ _____

Table A.4 Smoking Preferences Questionnaire. To obtain the relevant data to determine the smoking preferences of all subjects on the date of the study, please ask the patient to complete this form on screening, during, and after every study. Please ask the patient to return to the study physician on completion of the study.

Name_____

Date_____

Time_____

Please answer all items based on your personal lifetime experience. You may record additional information on the back of this page. Thank you.

1. How many cigarettes do you smoke daily?_____

2.How many years in your entire lifetime have you smoked cigarettes?_____

3. What brand of cigarettes do you smoke today?_____

4. What brand of cigarettes have you smoked most in your lifetime?_____

5. How many times in your lifetime have you quit smoking cigarettes?_____

6. What is the longest period of time you quit smoking cigarettes?_____

7. What is the length of the most recent period you quit smoking cigarettes?_____

Table A.5 Nicotine Patch Advere Events Checkelist. To obtain the relevant data to determine the adverse events experienced by each subject resulting from exposure to nicotine patches on the date of the study, please ask the patient to complete this form on screening, during, and after every administration of nicotine patches during the course of the study. Please ask the patient to return to the study physician on completion of the study.

Nicotine Patch Adverse Events Checklist

Name_____
Rater_____
Date_____

Please rate your experience with each item in the week after the most recent nicotine patch application.

	A	Q	M	MO	MS	VS
1. Skin redness	0	1	2	3	4	5
2. Itching	0	1	2	3	4	5
3. Burning	0	1	2	3	4	5
4. Insomnia	0	1	2	3	4	5
5. Abnormal dreams	0	1	2	3	4	5
6. Nervousness	0	1	2	3	4	5
7. Muscle cramps	0	1	2	3	4	5
8. Upset stomach	0	1	2	3	4	5
9. Diarrhea	0	1	2	3	4	5
10. Incoordination	0	1	2	3	4	5
11. Fever	0	1	2	3	4	5

A = absent; Q = questionable; M = mild; MO = moderate; MS = moderately severe; VS = very severe

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