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# Smoking and mental illness

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**Abstract**

Patients with mental illness have a higher incidence of smoking than the general population and are the major consumers of tobacco products. This population includes subjects with schizophrenia, manic depression, depression, posttraumatic stress disorder (PTSD), attention-deficit disorder (ADD), and several other less common diseases. Smoking cessation treatment in this group of patients is difficult, often leading to profound depression. Several recent findings suggest that increased smoking in the mentally ill may have an underlying biological etiology. The mental illness schizophrenia has been most thoroughly studied in this regard. Nicotine administration normalizes several sensory-processing deficits seen in this disease. Animal models of sensory deficits have been used to identify specific nicotinic receptor subunits that are involved in these brain pathways, indicating that the $\alpha 7$ nicotinic receptor subunit may play a role. Genetic linkage in schizophrenic families also supports a role for the $\alpha 7$ subunit with linkage at the $\alpha 7$ locus on chromosome 15. Bipolar disorder has some phenotypes in common with schizophrenia and also exhibits genetic linkage to the $\alpha 7$ locus, suggesting that these two disorders may share a gene defect. The $\alpha 7$ receptor is decreased in expression in schizophrenia. [$^3$H]-Nicotine binding studies in postmortem brain indicate that high-affinity nicotinic receptors may also be affected in schizophrenia. 

*Keywords:* Nicotinic acetylcholine receptors; $\alpha 7$ subunit; Schizophrenia; Bipolar disorder; Smoking; Nicotine addiction; Mental illness; Sensory deficit; Nicotine binding; Neuroleptic; Genetic linkage

## 1. Introduction

A major conundrum in tobacco research today is the neurobiology of tobacco use in the mentally ill. The incidence of smoking in individuals suffering from various forms of mental illness is inordinately high, approximately 60% overall compared to 25% in the general population. It is estimated that of the total number of smokers in the US today, 30% have some form of mental illness. This population purchases the majority of cigarettes sold, since they are also the heaviest smokers, and yet we know very little about any differences in the biology of smoking in this population that might account for their increased rate of tobacco use. This review focuses on what is known about the receptor populations that respond to nicotine in a common mental illness, schizophrenia.

## 2. The mentally ill population

Individuals suffering from a mental illness fall into several major groups. When the phenotypes are parsed, differences can be seen in the extent to which each group smokes and also in the use of alcohol. Although the groupings can be subdivided endlessly by phenotype, four major groups will be considered: schizophrenia, bipolar disorder, depression, and other. The last group, "other" includes posttraumatic stress disorder (PTSD), attention-deficit disorder (ADD), panic disorder, and anxiety. The total number of subjects suffering from some form of mental illness in the US today is estimated to be greater than 12% (American Psychiatric Association, 1994).

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## 3. Smoking incidence

The incidence of smoking is higher in all of the mentally ill groups than in the general population (Glassman, 1993; Dalack et al., 1999; Foulds, 1999; George and Krystal, 2000). It is estimated that 25% of the general population in the US are currently smoking tobacco (Anonymous, 1999). Active smoking cessation programs and the pressure of smoking bans in many buildings assure that the incidence of smoking is likely to continue declining in general. The incidence of smoking in schizophrenia, bipolar disorder, depression, and in control subjects with no history of mental illness is shown in a population of more than 700 subjects from whom tissue samples have been collected in the Denver Schizophrenia Center since 1990 (Fig. 1). Tissues include postmortem brain and blood lymphocytes, both used for measurements of gene expression. The latter have been immortalized by Epstein–Barr virus for use in the laboratory (Anderson and Gusella, 1984). The incidence of smoking is highest in schizophrenics, ~70% compared to ~30% in control subjects. It is agreed that the use of tobacco in schizophrenia is inordinately high and that they are generally heavy smokers (Dalack et al., 1998; Glassman, 1998; George and Krystal, 2000). Indeed, schizophrenics appear to extract more nicotine per cigarette than control smokers, possibly due to deeper inhalation (Olincy et al., 1997). In subjects with other forms of mental illness, the smoking incidence is also high (>50% in bipolar disorder) where it may be associated with depressive symptoms (Glassman, 1998; Glassman et al., 1992). The incidence of smoking was seen to be highest in the schizophrenic population, confirming previous findings. In this group of subjects, the use of alcohol was also queried (Fig. 1). The "use" of alcohol is not intended to mean alcohol abuse, but rather, the intake of alcoholic beverages. Subjects in the depression group smoked less than the controls, but alcohol consumption was inordinately high. Patients with either schizophrenia or bipolar disorder appear to smoke more and drink less than subjects with depression. It is not known why the use of alcohol may be less in schizophrenia and bipolar disorder, although response to medication might be involved.

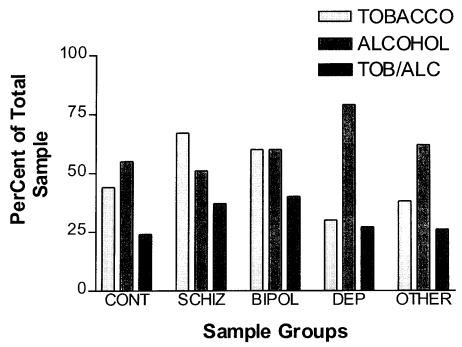


Fig. 1. Smoking incidence and alcohol use in control and mentally ill subjects. Individuals providing consent for molecular genetic studies in the Denver VA Schizophrenia Center were interviewed regarding the use of tobacco products and alcohol. The total number of samples presented in this graph are 733, including tissue from postmortem brain and immortalized lymphoblasts. Collection of postmortem brain carries an exempt status, as approved by the Colorado Multiple Institutional Review Board. Brain tissue was donated, postmortem, by the family. Smoking and alcohol histories were obtained from autopsy reports, hospital and nursing home records and family and physician interviews. Psychiatric evaluation was made from records and interviews by two independent psychiatrists (RF and AO). Subjects from whom blood was obtained were interviewed using the Structural Clinical Interview for Axis I DSM-IV Disorders — patient edition (with psychotic screen) or Structured Clinical Interview for Axis I DSM-IV Disorders — nonpatient edition to determine mental illness status (First et al., 1996a,b). Smoking and alcohol histories were collected using a modified version of the Fagerstrom questionnaire (Fagerstrom and Schneider, 1989). Individuals included control subjects with no history of schizophrenia or bipolar disorder. The patient population consisted of subjects suffering from schizophrenia, bipolar disorder, depression, or another mental illness. The latter group included PTSD, ADD, panic disorder and anxiety. The data are presented as a percentage of the total number of samples collected in each group. CONT, control subjects; SCHIZ, schizophrenia; BIPOL, bipolar disorder; DEP, depression; OTHER, other mental illness; TOB, tobacco; ALC, alcohol.

Smoking cessation has been shown to increase depression in many subjects (Glassman et al., 1990; Borrelli et al., 1996; Covey et al., 1997) and the use of antidepressants in the treatment of smoking cessation is now widespread (Prochazka et al., 1998; Hall et al., 1998; Benowitz and Peng, 2000; Holm and Spencer, 2000). Although antidepressant therapy for smoking cessation does increase the quit rate, it is not clear whether over the long term it will be better than nicotine replacement therapy. The reason may lie in the interaction of these agents with the nicotinic receptors. Several, including bupropion and fluoxetine, have been shown to be noncompetitive inhibitors of nicotinic receptors (Fryer and Lukas, 1999a,b). It is not yet known whether these agents increase receptor numbers as do other nicotinic receptor ligands (Marks et al., 1992; Abdulla et al., 1996; Breese et al., 1997b; Almeida et al., 2000), nor whether continued receptor upregulation may play a role in substitution of these agents for tobacco.

## 4. What are the underlying biological etiologies of smoking in the mentally ill?

The reasons for the high incidence of smoking in the mentally ill are likely to be as complex as the illnesses themselves. Although there are some common phenotypic symptoms in schizophrenia and the affective disorders, there are also major differences. Genetic evidence, discussed below, suggests commonality at some loci and not at others. Smoking cessation in all the disorders leads to a worsening of symptoms (Glassman et al., 1990; Greeman and McClellan, 1991; Dalack et al., 1999) and it has been suggested that use of tobacco may be a form of self-medication (Adler et al., 1998; Leonard et al., 1998a).

The role of nicotine in the mesolimbic dopamine pathways has been well characterized (Nisell et al., 1997; Balfour et al., 1998; Di Chiara, 2000). Dopaminergic pathways most certainly play a role in the psychopathology of schizophrenia, where blockade of D2 receptors with typical neuroleptics leads to improvements in symptomatology (Weinberger, 1997; Carlsson et al., 1997). Cognitive deficits, also seen in the mentally ill (Leger et al., 2000; Low et al., 2000), may be responsive to nicotine treatment (Mirza and Stolerman, 1998; Levin and Simon, 1998; Levin et al., 1998). Since nicotine is known to stimulate release of a large number of neurotransmitters (Wonnacott, 1997; Guo et al., 1998), it is not yet known which systems are involved in these effects of nicotine. However, additional information has been acquired by measuring the genetics and pharmacology of endophenotypes present in mental illness.

## 5. Direct assay of behavioral phenotypes in schizophrenia and bipolar disorder

The use of behavioral phenotypes in the study of schizophrenia was first proposed by Peter Venables in 1964. Venables suggested that sensory overload, which he referred to as ''flooding,'' pointed to a defect in important brain mechanisms regulating perception of sensory stimuli. The failure of such an inhibitory filter for sensory input could possibly lead to the paranoia and delusions frequently seen in schizophrenia. This has stimulated the investigation of several behavioral paradigms in schizophrenia and others are suggested. The two best characterized of the endophenotypes are auditory sensory gating (Freedman et al., 1983, 1991; Clementz et al., 1998) and smooth pursuit eye movement (Holzman, 1985; Clementz et al., 1997). Prepulse inhibition of startle responses has also been used for measurement of sensory deficits in mental illness (Cadenhead et al., 1993), and impaired attention has been proposed as a phenotype for molecular studies in schizophrenia (Cornblatt and Malhotra, 2001). Use of a single endophenotype for genetic and molecular studies is thought to limit the number of genes that might be involved, thereby simplifying the approach. It does not assume, however, that a single gene is involved, even in these simpler models.

The endophenotypes most thoroughly studied, particularly with regard to tobacco use are auditory sensory gating (Freedman et al., 1987, 1991; Baker et al., 1987) and smooth pursuit eye movement (Holzman, 1983; Clementz and Sweeney, 1990; Radant and Hommer, 1992). Auditory sensory gating is measured using an auditory-evoked potential combined with a conditioning/testing paradigm. Electrodes on the skull surface record a wave with a 50-ms latency (P50) following paired auditory stimuli that are delivered 0.5 s apart. In a normal response, the subject decrements the amplitude of the second of the two paired-click stimuli through the action of an inhibitory pathway activated by the first stimulus (Adler et al., 1991). Eye tracking is assessed using an infrared photoelectrode limbus detection device in a smooth pursuit task (Grove et al., 1992).

Both gating of the P50 response and smooth pursuit eye movement are abnormal in schizophrenic subjects. Although abnormal responses for these paradigms are also seen in subjects with no history of major psychiatric disorders, they are inherited in families of schizophrenics in an apparent autosomal dominant manner (Holzman et al., 1984; Freedman et al., 1997; Ross et al., 1998; Waldo et al., 2000). Patients with bipolar disorder also have abnormal auditory gating, but only during their manic phase; P50 responses normalize as the subject becomes euthymic (Baker et al., 1987). Additionally, a related inhibitory deficit in startle response has been found to be abnormal in schizophrenics, their first-degree relatives and patients with schizotypal personality disorder (Cadenhead et al., 2000). These sensory deficits and eye-tracking phenotypes, therefore, represent inherited predisposing factors for mental illness that can be measured in patients and families, and that can also be studied at the molecular level in the laboratory. As more than 10% of normal subjects have high P50 ratios, it is likely that functional polymorphisms in the genes that regulate this pathway are segregating in the general population.

## 6. Normalization of sensory deficits by nicotine

Several of these sensory deficits, seen in schizophrenics and their first-degree relatives, are normalized by nicotine, suggesting that the high incidence of tobacco use in the mentally ill is an attempt at self-medication (Adler et al., 1998; Leonard et al., 1998a, 2000). Nicotine, administered either as gum or in cigarettes, normalizes the P50 deficit in both schizophrenics and their nongating relatives (Adler et al., 1992, 1993). An effect on desensitization of nicotinic receptors was suggested by a study where schizophrenic smokers were found to normalize auditory gating after a short period of sleep, consistent with resensitization of nicotine desensitized receptors. This effect was decreased when the patients wore a transdermal nicotine patch during the sleep period (Griffith et al., 1998).

Smoking also normalizes the abnormal leading saccadic eye-tracking patterns in schizophrenia (Olincy et al., 1998). Nicotine acting through nicotinic receptors may, therefore, be affecting a common inhibitory pathway in both behavioral paradigms. A mechanism involving the neurotransmitter GABA is likely to be involved, as nicotinic receptors are present on GABAergic interneurons in human brain (Freedman et al., 1993), and nicotine-stimulated GABA release has been demonstrated in both rodent and human tissues (Alkondon et al., 1997; Hilmas et al., 1999; MacDermott et al., 1999).

## 7. Effects of neuroleptic treatment on auditory sensory gating and eye tracking

Typical (haloperidol) and atypical (clozapine) neuroleptic medication have differing effects on sensory-processing measures. Haloperidol, thought to act principally as a dopamine D2 receptor antagonist (Farde et al., 1992), has no effect on the auditory-evoked potential deficits seen in schizophrenics and their first-degree relatives (Freedman et al., 1983; Adler et al., 1990). The atypical neuroleptic clozapine, however, normalizes the P50 deficit (Nagamoto et al., 1996). Clozapine has also been shown, in several studies, to decrease the incidence of smoking in schizophrenia (McEvoy et al., 1994, 1995, George et al., 1995). Clozapine is an antagonist at the serotonin 5HT3 receptor, where blockade induces the release of several neurotransmitter types, including acetylcholine (Imperato et al., 1993; Parada et al., 1998; Shirazi et al., 2000). It is postulated that release of acetylcholine and action at nicotinic receptors is the mechanism for normalization of the P50 deficit by clozapine (Nagamoto et al.,1996; Adler et al., 1998), and may explain the effects on smoking levels seen in schizophrenics treated with this atypical neuroleptic.

Nicotinic cholinergic systems have also been implicated in cognition, in both animal and human studies (Levin and Rezvani, 2000; Rezvani and Levin, 2001). Although haloperidol does not appear to affect cognitive measures (Levin, 1997), improvements in cognitive function in schizophrenics have been noted with clozapine (Meltzer and McGurk, 1999; Galletly et al., 2000). Whether the effect of clozapine on cognition in schizophrenia is mediated by increased acetylcholine release is not known. Another principal action of clozapine is at the 5HT2a receptors; the effects on sensory-processing pathways are likely, therefore, to be complex (Damask et al., 1996; D'Souza et al., 1997; Seeman et al., 1997).

## 8. Nicotinic receptors in animal models of sensory processing

Processing of sensory information also occurs in animals, where invasive pharmacological techniques can be used. The work described above suggested that the nicotinic acetylcholine receptors might be involved in the brain pathways active in sensory processing. Using a paradigm of paired auditory stimuli, similar to that used in the human experiments, gating of the second or test response was observed in anesthetized rats (Adler et al., 1986; Bickford-Wimer et al., 1990). Although an antagonist of the high-affinity nicotinic receptors, mecamylamine, did not affect the gated response, an antagonist of the low-affinity receptor, $\alpha$-bungarotoxin, resulted in a loss in gating observed as an increased test to conditioning response (Luntz-Leybman et al., 1992). Nicotinic receptors containing $\alpha 7$ subunits are thought to be the principal, low-affinity nicotinic receptors in mammalian brain (Séguéla et al., 1993). Another specific antagonist of the $\alpha 7$ nicotinic receptor, methyllycaconitine (Alkondon et al., 1992; Davies et al., 1999), also produced a loss of auditory gating in an awake, behaving rat model (Rollins et al., 1993). Antisense oligonucleotides to the translation start site of rat $\alpha 7$ mRNA, delivered intracerebroventricularly, resulted in a decrease in $\alpha$-bungarotoxin binding of 40% and in a loss of the gated response to paired auditory stimuli (Rollins et al., 1993).

A genetic model for the auditory-evoked potential deficit exists in inbred mouse strains. The DBA/2Ibg strain has lower levels of $\alpha$-bungarotoxin binding than the C3H strain (Miner et al., 1986; Marks et al., 1989), and has a similar inhibitory deficit in auditory gating seen in schizophrenia (Stevens et al., 1996). Gating was correlated with the level of $\alpha$-bungarotoxin in the mouse strains examined. Nicotine normalizes the abnormal auditory gating seen in the DBA strains and, further, a specific agonist of $\alpha 7$ nicotinic receptors, GTS-21, also normalizes the deficit (Stevens et al., 1998), suggesting that the $\alpha 7$ nicotinic receptor plays an important role in this phenotype. A murine knock-out of the $\alpha 7$ receptor has been generated (Orr-Urtreger et al., 1997), where acoustic startle and prepulse inhibition were found to be normal (Paylor et al., 1998). These animals have auditory-gating responses that are less than those found in the C57Bl/6 parent, but the difference was not significant (unpublished results, KES). The embryonic stem cells used for the construction of the $\alpha 7$ knock-out were from a 129 mouse strain, which has been found to have abnormal sensorimotor gating (Lijam et al., 1997). Compensatory processes, operative as a result of gene deletion, have been found in other knock-outs (Fauchey et al., 2000), suggesting that the sensory-processing measures in the $\alpha 7$ knock-out mouse must be considered with caution.

## 9. Human genetics of auditory gating

The studies in inbred mouse strains suggested that inhibition of auditory responses is inherited in these animal models. Auditory-gating deficits are also inherited in human subjects. While gating deficits are present in more than 80% of schizophrenic subjects, they are also present in about 50% of the first-degree relatives of schizophrenics and at a lower level in the general population (Waldo et al., 1991). Not all of the first-degree relatives have schizophrenia, suggesting that the auditory–gating deficit is one of several predisposition factors in the disorder (Waldo et al., 2000). Because the P50 deficit is present in 50% of members of schizophrenic families, it appears to be inherited as an autosomal dominant trait and thus represents a more penetrant phenotype for genetic linkage than schizophrenia itself. Genetic linkage studies done by this group showed that the P50 deficit was genetically linked in

schizophrenic families to a chromosomal locus at 15q14 with a lod score of 5.3, $\Theta = 0.00$ (Freedman et al., 1997). This locus contains the human $\alpha 7$ nicotinic receptor gene. Linkage at this locus was also found to schizophrenia and has been replicated by our laboratory and by many other laboratories in diverse populations (Kaufmann et al., 1998; Leonard et al., 1998b; Riley et al., 2000; Stöber et al., 2000; Stassen et al., 2000; Liu et al., in press; Tsuang et al., in press; Xu et al., in press; Freedman et al., 2001). Although linkage was not found in all cohorts examined (Neves-Pereira et al., 1998; Curtis et al., 1999), in many populations around the world the locus at 15q14 may contain a gene for schizophrenia.

Bipolar disorder has also been genetically linked to the 15q14 locus, suggesting that schizophrenia and the affective disorders may share a common predisposition gene defect at the $\alpha 7$ nicotinic receptor gene locus (Edenberg et al., 1997; Craddock et al., 1999; Turecki et al., 2000). That smoking incidence is also higher in patients suffering from bipolar disorder than in normal controls is of note. Bipolar disorder shares some phenotype similarities to schizophrenia. Subjects with bipolar disorder also have a deficit in gating of the P50, but only during the manic phase of the disease. When the patient becomes euthymic, gating improves (Baker et al., 1987; Adler et al., 1990). It is possible that the P50 deficit is regulated by more than one gene in both schizophrenics and bipolar disorder, but that defects in one of these genes, perhaps the $\alpha 7$ nicotinic receptor, are shared in the two diseases.

## 10. Expression of the low-affinity nicotinic acetylcholine receptor in schizophrenia

The correlation of low levels of $\alpha 7$ receptor expression with loss of auditory gating in the mouse models suggested that low levels of $\alpha 7$ expression might also be found in schizophrenia. Using receptor autoradiography, we measured binding of [$^{125}$I]-$\alpha$-bungarotoxin in postmortem hippocampus isolated from both schizophrenic and control subjects, matched for smoking history. Binding was found on both the cell somata and on cellular processes of large nonprincipal cells in both the dentate hilar area, in CA3 and in CA1. A decrease of 50% in binding was found overall in the schizophrenic subjects (Freedman et al., 1995). Decreased expression of $\alpha 7$, also measured by receptor autoradiography, has been replicated by our laboratory and other investigators in the reticular thalamic nucleus (RTN) (Leonard et al., 1998b,c; Court et al., 1999) and in the cortex (Guan et al., 1999). Court et al. also found a large reduction of $\alpha 7$ expression in dementia with Lewy bodies. The decrease found in the RTN is of particular interest as this narrow, discrete nucleus provides the principal inhibitory input into the thalamus (deBiasi et al., 1986; Mitrofanis and Guillery, 1993). The RTN is a major localization of expression for the $\alpha 7$ nicotinic receptor subunit in human brain (Breese et al., 1997a). We are presently measuring nicotinic receptor subunit expression in multiple regions of postmortem brain, using a coupled immunoprecipitation/western blot assay (Benhammou et al., 2000). Preliminary results in hippocampal tissue suggest that $\alpha 7$ protein is decreased in this region in schizophrenics (Fig. 2), consistent with the earlier autoradiographic studies.

## 11. High-affinity nicotinic receptor expression is also decreased in schizophrenia

In human brain, [$^3$H]-nicotine binding, which measures high-affinity nicotinic acetylcholine receptors, is increased in smokers compared to nonsmokers (Benwell et al., 1988; Breese et al., 1997b; Perry et al., 1999). In subjects who had quit smoking for some time before death, receptor levels had returned to control levels. Receptor number is correlated with the packs of cigarettes used per day, but not with the length of time subjects have smoked in their lifetime (Breese et al.,1997b), suggesting that receptor numbers are regulated by nicotine dose in human brain as was found in rodent models (Marks et al., 1986; Flores et al., 1997).

In schizophrenic subjects, we found a lack of nicotine-induced increases in both [$^3$H]-nicotine and [$^3$H]-epibatidine binding in multiple brain regions, compared to postmortem brain from normal smokers (Breese et al., 2000). At every smoking level, schizophrenics had fewer high-affinity nicotinic receptors. High-affinity nicotinic receptors were also found to be decreased in postmortem

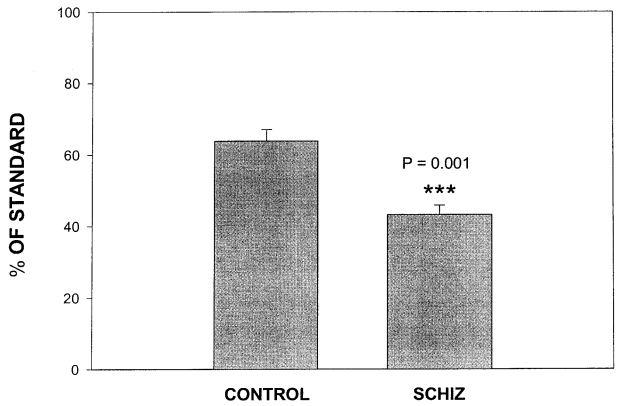


Fig. 2. Expression of $\alpha 7$ nicotinic receptor subunit protein in human hippocampus. A coupled immunoprecipitation/Western blot assay was used to measure $\alpha 7$ protein expression in human postmortem hippocampus. We have previously described the assay and antibodies used (Benhammou et al., 2000). Briefly, an antibody to the human $\alpha 7$ subunit, raised in goat, was used for immunoprecipitation of sample homogenates. The resultant protein was separated on polyacrylamide gels, blotted to membrane, and a second human $\alpha 7$ antibody, raised in rabbit, was used for detection of the $\alpha 7$ band. Quantification was done using an Alpha-Inotec Imaging System. The results were normalized to a single sample of human cortex that is run on each gel. Data are reported as a percentage of this standard. A Student's *t* test was used to determine the significance of the difference in expression.

striatum from schizophrenic subjects, using the ligand [$^3$H]-cytisine (Durany et al., 2000). These findings do not appear to be related to neuroleptic treatment (Lee et al., 2001). Rats were treated for 6 weeks with saline, nicotine, haloperidol or halperidol and nicotine together. We found no generalized effect of haldol on either binding levels or nicotinic receptor up-regulation by nicotine, suggesting that the decreases in high-affinity nicotinic receptors seen in schizophrenic postmortem brain are not likely to be related to typical neuroleptic medication. Effects of an antypical neuroleptic such as clozapine on nicotinic receptor regulation have not yet been investigated.

We have recently developed an assay for high-affinity nicotinic receptor expression in blood cells (Benhammou et al., 2000). Nicotinic receptors are expressed in many types of peripheral tissues (Drebing et al., 1998), including lymphocytes and polymorphonuclear cells (PMN). [$^3$H]-Nicotine binding is also regulated by tobacco use in leukocytes. The receptor levels are correlated, as in human brain, with the number of cigarettes smoked per day. Coupled immunoprecipitation/western blot assays were used to show that lymphocytes express both $\alpha4\beta2$ and $\alpha3\beta4$ receptors, but PMN express principally $\alpha3\beta4$ (Benhammou et al., 2000). Neither cell type expresses $\alpha7$ receptors, although lymphocytes immortalized with Epstein–Barr virus (Anderson and Gusella, 1984), do express $\alpha7$ (unpublished results). As receptor numbers are responsive to nicotine dose, this assay should be useful for study of high-affinity receptor levels in schizophrenic pedigrees where brain tissue is not available.

## 12. Polymorphism in the human α7 gene

We isolated both cDNA (GenBank accession: U40583) and genomic clones for the human $\alpha7$ nicotinic receptor subunit gene (Gault et al., 1998). The gene is coded for by 10 exons with a gene size of approximately 75 kb. A putative promoter region of 2.6 kb was also isolated. Generation of a yeast artificial chromosome (YAC) map across the linkage region on chromosome 15q14 and mapping of noncoding polymorphisms in the gene revealed that the human $\alpha7$ gene is partially duplicated. Exons 5–10 are duplicated and inserted, with a large cassette of DNA containing other genes, into a locus approximately 1 Mb proximal to the full-length $\alpha7$ gene and next to novel exons designated A,B,C,D. The duplicated exons 5–10 are expressed as mRNA with these novel exons (GenBank accession number AF029838) (Gault et al., 1998) in both the brain and in the periphery (Drebing et al., 1998). The function of this novel gene containing $\alpha7$ coding sequences is under investigation in the laboratory. A translation product in frame with $\alpha7$ amino acid sequence would contain all membrane-spanning regions and part of the binding site, including the vicinal cysteines; it would be missing the signal peptide.

The coding regions of the full-length $\alpha7$ gene and duplicated $\alpha7$ gene have been screened for polymorphism in schizophrenia. Although complete results will be reported elsewhere, preliminary data suggest that no major coding mutations are found in the coding region in schizophrenics (Gault et al., 1999). However, multiple polymorphisms in the core promoter region of the full-length $\alpha7$ gene were found that are associated with both schizophrenia and with the P50 gating deficit (Leonard et al., 2001). Functional testing of these polymorphisms is in progress.

## 13. Discussion

Progress has been made in understanding the biological basis of smoking in the mentally ill, at least for schizophrenia in which the incidence of smoking is the most prevalent. Schizophrenics appear to have decreased expression of both high- and low-affinity nicotinic receptors. Further, nicotinic receptor up-regulation, seen in human tobacco use, may not be present in these patients. Expression of the $\alpha7$ nicotinic receptor subunit has been studied more thoroughly than other subunits, since it was implicated in sensory-processing deficits in schizophrenia and first-degree relatives. Although the actual mechanisms for the decreases in nicotinic receptors in schizophrenia remain under investigation, some speculation is possible. There are data to suggest that the decrease in $\alpha7$ expression is a genetically inherited trait. Both linkage analyses in families and sib-pair analysis support this hypothesis. Indeed, polymorphisms in the gene are under investigation. The positive and replicated linkage results include the map location of both the duplicated and full-length $\alpha7$ genes. Although a function for the duplicated $\alpha7$ gene has not been established, it is completely missing in some schizophrenic subjects (Gault et al., 1998). No controls missing both copies have yet been found. It is possible that the duplicated transcript is regulatory in some way.

The decreases in high-affinity receptors in schizophrenia are more puzzling. No replicated sites of genetic linkage are found at the map locations for these subunits. It is, therefore, possible that the low levels of high-affinity receptors are an epigenetic effect of a genetically determined decrease in $\alpha7$ expression. In that regard, an early study in cultured chick ganglia suggested some sort of interdependence between $\alpha3$- and $\alpha7$-containing receptors (Listerud et al., 1991).

The elucidation of the gene mutations involved in complex disorders has been and is an arduous endeavor. There are now more than 10 sites of replicated genetic linkage (Baron, 2001), suggesting that 10 genes may play a role in disease development. Because schizophrenia is a heterogeneous disease, only a subset of these gene variants may be necessary for development of the disorder and the subset may not be the same in all patients. The measure of endophenotypes such as the P50 gating deficit and abnormal eye tracking provides assayable inborn traits, perhaps of only small contribution, in which to test our hypotheses concerning the nicotinic receptor gene family. It is also of interest that smoking incidence in bipolar disease, an affective

disorder, may be genetically related in some way to schizophrenia since they share linkage at the locus of the α7 gene cluster. As we take the molecular biology of nicotinic receptors into the realm of clinical measurements, it will become clear whether decreased expression of the nicotinic acetylcholine receptor gene family plays a role, even though small, in the development of the schizophrenia spectrum disorders. The challenges will then lie in relating defects in these genes to other susceptibility genes and factors.

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