Sensitivity to the Seizure-Inducing Effects of Nicotine is Associated with Strain-Specific Variants of the α5 and α7 Nicotinic Receptor Subunit Genes

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ABSTRACT

Restriction fragment length polymorphisms (rfpls) have been identified for the nicotinic ACh receptor subunit genes α5 and α7 between two mouse strains (C3H/2ibg and DBA/2ibg) that differ in sensitivity to the convulsant effects of nicotine. In this study reported here, F2 animals derived from these two parental strains were tested for their sensitivity to the convulsant effects of nicotine as measured by seizure frequency and overall sensitivity score. Subsequently, the animals were genotyped for the α5 and α7 rfpls. In addition, levels of α-bungarotoxin (α-BTX) binding were measured in four brain regions (colliculi, hippocampus, hypothalamus and striatum) to determine whether there is a correlation among α-BTX binding levels, sensitivity to nicotine and nicotinic ACh receptor subunit genotype. A significant relationship was observed between α5 and α7 genotype and sensitivity to nicotine. In addition, the α7 rfpl significantly correlated with levels of α-BTX binding in hippocampus, colliculi and striatum. The α5 rfpl did not correlate with α-BTX binding levels in any brain region. Levels of α-BTX binding did not correlate with nicotine-induced seizure sensitivity or overall nicotine sensitivity score in any of the four brain regions examined.

Genetic components appear to influence smoking behavior (Carmelli et al., 1992; Heath and Madden, 1994; Heath et al., 1995). Similarly, animal studies have demonstrated that sensitivity to the effects of nicotine on a variety of behavioral and physiological measures is influenced by the genetic constitution of the animal (Marks et al., 1989; Marks et al., 1991; Miner and Collins, 1989; Robinson et al., 1996). Although these studies have demonstrated a clear influence of genetics on nicotine sensitivity, the genes responsible for these differences in sensitivity have not been identified. Because the effects of nicotine are presumably mediated through nAChRs, it is possible that differences in sensitivity to nicotine in both humans and mice are due, in part, to differences in the genes that code for the various nicotinic receptor subunits.

Eleven (10 in mammals) neuronal nAChR subunits have been identified: α2 to α9 (α8 is found only in chickens) and β2 to β4 (Lindstrom, 1995; Elgoyhen et al., 1994). In mice, rfpls have been identified for several of the genes that encode nAChR subunits, including the genes for α5 and α7 (Bessis et al., 1990; Eng et al., 1991; Nagavarapu and Boyd, 1995; Stitzel et al., 1995). The α7 subunit is thought to be the major α-BTX-binding subunit in the mammalian brain (Séguela et al., 1993), although some reports suggest that the α5 subunit can bind α-BTX (McLane et al., 1990; McLane et al., 1991).

Previous studies have shown that sensitivity to nicotine-induced seizures correlates with levels of the nAChR subtype that binds α-BTX with high affinity in the hippocampus (Miner et al., 1986; Miner et al., 1985; Miner et al., 1984; Miner and Collins, 1989), the brain region where nicotine-induced seizures are believed to be initiated (Dunlop et al., 1960; Floris et al., 1964; Brown, 1967; Stumpf and Gogolak, 1967). In general, animals with higher levels of hippocampal α-BTX binding were found to be more sensitive to the convulsant effects of nicotine. It has also been demonstrated that the α7 rfpl is linked to levels of α-BTX binding in hippocampus (Stitzel et al., 1996). In addition, the rfplps for the α5 and α7 nAChR subunit genes have been found between strains that differ in sensitivity to the convulsant effects of nicotine (Nagavarapu and Boyd, 1995; Stitzel et al., 1995). RNAs for both α5 and α7 nAChR subunit genes are found in the hippocampus (Wada et al., 1990; Marks et al., 1992; Marks et al., 1996; Séguela et al., 1993). Overlap in the distribution of these two nAChR subunit RNAs in the hippocampus occurs only in the pyramidal cell layer of the CA1 region, a region

ABBREVIATIONS: α-BTX, α-bungarotoxin; ANOVA, analysis of variance; KRH, Krebs-Ringer’s HEPES; MLA, methyllycaconitine; nAChR, nicotinic ACh receptor; rfpl, restriction fragment length polymorphism.
particularly prone to excitotoxicity due to epileptiform activity (Meldrum, 1991).

Despite the correlation between the amount of α-BTX binding in hippocampus and seizure sensitivity, some evidence suggests that α-BTX-sensitive nAChRs may not mediate nicotine-induced seizures. Gasior et al. (1996) demonstrated that methyllycaconitine (MLA), an antagonist selective for α-BTX-sensitive nAChRs, did not block nicotine-induced convulsions. Some of the results of Miner et al. (1985) also indicated that the correlation that has been observed between seizure sensitivity and α-BTX binding levels among populations and inbred mouse strains does not persist in certain populations. When segregating populations from a classic genetic cross (backcross to parentals and F2 animals) were compared for seizure sensitivity and α-BTX binding levels, a significant correlation between the two measures was observed when all three populations were grouped together. However, no significant relationship between the two measures was observed when the most genetically diverse population, the F2 animals, was examined alone. This finding suggests that the relationship between seizure sensitivity and α-BTX binding may be casual rather than causal. The lack of relationship between nicotine-induced seizures and levels of hippocampal α-BTX binding observed in the F2 population also may be due to an insufficient number of animals having been tested—34 F2 animals were tested in the study by Miner et al. (1985)—for this genetically heterogeneous population.

The studies reviewed here attempt to determine whether there is a relationship between nAChR subunit genotype and seizure phenotype in F2 animals derived from a C3H/2ibg by DBA/2ibg cross. This investigation also examines whether a significant relationship exists between nicotine-induced seizure sensitivity and levels of α-BTX binding in four brain regions of F2 animals when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a when a significantly larger number of animals is surveyed—34 F2 animals were a
times. Samples were stored at −20°C as a pellet until use. On the
day of receptor-ligand binding, pellets were resuspended in 10 vol-
umes of ice-cold distilled water.

**Ligand binding.** The binding of α-bungarotoxin to brain mem-
branes was measured as described previously (Marks and Collins,
1982) with modifications for filtration by means of a 96-well cell
harvester. [125I]-α-Bungarotoxin (230–285 Ci/mmol; Amersham, Ar-
lington Heights, IL) binding was carried out at 37°C for 4 h in 96-well
microtiter plates in a final volume of 100 μL. The average concen-
tration of [125I]-α-bungarotoxin used was 2.7 ± 0.28 nM. Nonspecific
binding was determined by the addition of 1 mM L-nicotine during
the incubation. Samples were filtered through two filters, a Gelman
type A/E filter and a MFS GB100R filter, with a cell harvester
(Inotech, Lansing MI). The Gelman A/E filter was presoaked in 1×
KRH/0.5% polyethyleneimine, and the MFS GB100R filter was pre-
soaked in 5% nonfat dry milk/1× KRH before filtration. Individual
filters were placed into 5-ml culture tubes and counted on a Packard
Auto-Gamma 5000 gamma counter.

Protein levels were measured by the method of Lowry (Lowry et
al., 1951).

**Statistics.** One-way ANOVA was used for all statistical analyses.
Duncan’s Multiple Range test was utilized for post-hoc analysis.

**Results**

**Behavioral testing.** The sensitivity of individual F2 ani-
imals to the convulsant effects of nicotine was determined after an i.p.
injection of nicotine at a dose (4 mg/kg) that elicits seizures in 100% of animals of the C3H parental strain but rarely induces seizures in the DBA parental strain. Of 122 F2 animals tested, 24 (19.7%) exhibited clonic or tonic seizure activity. These results are consistent with previous studies that have shown that resistance to the convulsant effects of nicotine is inherited in a dominant fashion (where 25% of the animals would be expected to have seizures) (Miner et al., 1984).

These mice were also scored for their overall sensitivity to
the high-dose effects of nicotine on a scale of 1 (least sensi-
tive) to 5 (tonic seizure) as described in “Materials and Meth-
ods” (fig. 1). Of the mice that did not show nicotine-induced
seizure activity, 35.4% (34/96) were scored as least sensitive
(a score of 1); 26.0% (25/96) were scored as moderately sensi-
tive (a score of 2) and 38.5% (37/96) were scored as sensitive
but without the appearance of convulsions (a score of 3). Of
the 24 mice that seized, 21 had clonic seizures (a score of 4),
and the remaining 3 had clonic/tonic seizures (a score of 5).
Two of the animals were not assigned sensitivity scores be-
cause they died after testing without showing signs of sei-
zure.

**Comparison of genotype and nicotine sensitivity.** It is
possible that the differences in sensitivity to the high-dose
effects of nicotine are due to a strain-specific variant of a gene
or genes that code(s) for subunits of the nAChR family. Pre-
viously, a rflp has been described for the nAChR α7 subunit
gene between two mouse strains, C3H/2ibg and DBA/2ibg
(Nagavarapu and Boyd, 1995; Stitzel et al., 1995). We have
also identified a rflp for the nAChR α5 subunit gene be-
tween these two strains (fig. 2). To assess whether there is any
relationship between nicotine-induced seizure sensitivity and
the α5 or α7 genotypes of the tested F2 mice, genomic
DNA was isolated from the animals and the α5 and α7
genotypes of each were determined by rflp analysis. A com-
parison of phenotype with genotype revealed that animals homozygous for the C3H variant of the α5 rflp seized with a
higher frequency (40%) than animals homozygous for the
DBA variant of α5 (7.7%) (table 1). Heterozygotes for the α5
rflp had a seizure frequency (10.2%) essentially the same as
the α5 DBA variant homozygotes. Animals homozygous for
the C3H variant of α7 also had an increased seizure fre-
cency (32.4%) compared with the α7 heterozygotes (18.2%) and
the α7 DBA variant homozygotes (4%). However, unlike

![Fig. 1. Distribution of nicotine sensitivity scores across the F2 population. Animals were injected i.p. with 4 mg/kg nicotine and scored for their sensitivity to nicotine as described in "Materials and Methods." The number of animals rated for each sensitivity score is shown.](image)

![Fig. 2. Restriction fragment length polymorphisms for the nicotinic ACh receptor subunit genes α5 and α7. Representative autoradiograms of F2 animals homozygous for the DBA variant (DD), heterozygous (HD) or homozygous for the C3H variant (HH) for either the α5 or the α7 subunit gene as determined by rflp analysis. The α5 and α7 polymorphisms were detected with the restriction endonucleases EcoRV and PvuII, respectively, as described in "Materials and Methods." Size standards (HindIII digested Lambda DNA) are indicated by lines adjacent to the autoradiograms. For the α5 polymorphism, the standards shown are, from top to bottom, 23,130 bp, 9416 bp, 6557 bp and 4361 bp. The size standards shown for the α7 polymorphism are, from top to bottom, 4361 bp, 2322 bp, 2027 bp and 564 bp.](image)
the α5 heterozygotes, the α7 heterozygotes had a seizure frequency midway between the two homozygous populations.

A comparison of overall sensitivity score with genotype gave similar results (fig. 3). C3H-like homozygotes had significantly (α5: F(2,108) = 9.88; P < .0005; α7: F(2,114) = 4.34; P < .05) higher sensitivity scores (α5: 3.16 ± 0.17; α7: 2.88 ± 0.17) than either their heterozygous (α5: 2.22 ± 0.14; α7: 2.38 ± 0.16) or their DBA-like homozygous counterparts (α5: 2.02 ± 0.2; α7: 2.04 ± 0.2) for both α5 and α7. Once again, heterozygotes for the α5 rflp had an average sensitivity score very similar to that of the α5 DBA-like homozygotes, and α7 heterozygotes had an average score midway between those of the two α7 homozygous populations.

When the α5 and α7 genotypes of the animals were combined and compared with seizure frequency and overall nicotine sensitivity, the two nAChR loci appeared to act in combination relative to these measures. For example, animals homozygous for the C3H variant of both α5 and α7 rflps exhibit a higher seizure frequency (54.5%) and average sensitivity score (3.54 ± 0.16) (fig. 4) than did those animals homozygous for the C3H variant of either α5 (40%; 3.16 ± 0.17) or α7 (32.4%; 2.88 ± 0.17) alone (compare table 1 with table 2 and fig. 3 with fig. 4). In addition, animals homozygous for the DBA variant of both nAChR loci had a lower seizure frequency (no animals seized) than animals homozygous for the DBA variant of α5 (7.7%) or α7 (4%) alone. Although the overall sensitivity score for the combined DBA-like homozygotes (1.83 ± 0.4) was lower than that of animals homozygous for the DBA variant of α5 (2.02 ± 0.2) or α7 (2.04 ± 0.2) alone, the difference was not statistically significant.

### Comparison of α-BTX binding levels in brain with nAChR subunit genotype and nicotine sensitivity

Previous findings have indicated that sensitivity to the convulsant effects of nicotine may not correlate with levels of α-BTX binding in the hippocampus in F2 animals even though these two measures do significantly correlate across populations and between inbred mouse strains (Miner et al., 1985). In addition, α7 genotype has been shown to exhibit a significant effect on levels of α-BTX binding in several brain regions, including hippocampus (Stitzel et al., 1996). Therefore, levels of α-BTX binding were measured in four brain regions (colliculi, hippocampus, hypothalamus and striatum) and compared with α5 genotype, α7 genotype, seizure frequency and sensitivity score for those animals tested for their high-dose sensitivity to nicotine. As previously demonstrated, α7 genotype was significantly associated with levels of α-BTX binding in hippocampus (F(2,118) = 10.96, P < .0001), colliculi (F(2,119) = 6.36, P < .005) and striatum (F(2,118) = 6.29, P < .005) (fig. 5A). No significant relationship between α5 genotype and levels of α-BTX binding was observed (fig. 5B).

In accordance with an earlier study by Miner et al. (1985), levels of α-BTX binding were not significantly different in hippocampus or any other brain region between the F2 animals that had seizures and those that did not after a challenge dose of nicotine (fig. 6). Moreover, there was no significant relationship between α-BTX binding levels in any brain region and nicotine sensitivity scores (fig. 7).

### Discussion

This study demonstrated an association between sensitivity to the high-dose effects of nicotine, as measured by seizure frequency and overall sensitivity score, and polymorphisms for the nAChR subunit genes, α5 and α7, in F2 animals.
derived from a cross between C3H/2ibg and DBA/2ibg mice. The \( a^5 \) genotype appears to have a dominant effect relative to the sensitivity measures; \( a^5 \) heterozygotes exhibit seizure frequencies and overall sensitivity scores that are indistinguishable from the DBA variant homozygotes. On the other hand, \( a^7 \) heterozygotes had seizure frequencies and average sensitivity scores intermediate between those of animals of the homozygous \( a^7 \) genotypes. This result indicates that the inheritance of high-dose sensitivity with respect to \( a^7 \) genotype is additive. Together, the \( a^5 \) and \( a^7 \) genotypes act in combination with respect to nicotine sensitivity. The observation that dominance and additive effects are seen with the \( a^5 \) and \( a^7 \) genotypes, respectively, is consistent with previous classic genetic cross studies that established that the inheritance of sensitivity to nicotine-induced seizures has both additive and dominance components (Miner et al., 1984). To the best of our knowledge, this is the first study to find linkage between specific genetic loci and sensitivity to nicotine.

This study also confirmed the linkage of nAChR \( a^7 \) subunit genotype with levels of \( \alpha \)-BTX binding in several brain regions, which suggests that the strain-specific variants of the \( a^7 \) gene have a significant influence on determining levels of \( \alpha \)-BTX binding in a brain region-specific fashion. No correlation between levels of hippocampal \( \alpha \)-BTX binding and nicotine-induced seizure sensitivity was observed in this study. This result is in agreement with a previous study by Miner et al. (1985) that indicated that such a relationship does not exist in F2 animals even though a relationship is seen across populations and between inbred mouse strains.

An explanation for the apparent correlation between nicotine-induced seizure sensitivity and levels of hippocampal \( \alpha \)-BTX binding across populations but not among individuals is that the two measures are controlled by different genes that are linked. However, it is possible for seizure susceptibility and levels of \( \alpha \)-BTX binding to be linked to the same gene if one of the measures is influenced by a second gene that does not affect the other measure. This is, in fact, what we observe for high-dose sensitivity and binding levels of \( \alpha \)-BTX. Both measures are linked to the \( a^7 \) locus, whereas only the high-dose sensitivity measures are linked to the \( a^5 \) locus. Moreover, there is a significant effect of \( a^5 \) genotype on seizure frequency and sensitivity score among animals of the same \( a^7 \) genotype; the seizure frequency and average sensitiv-
are linked to seizure susceptibility raises two questions: Does a native neuronal nAChR exist that is made up of these two nAChR subunits? And if so, does this subtype of nAChR mediate nicotine-induced seizures? RNAs for both subunits are found in the hippocampus (Wada et al., 1990; Marks et al., 1992; Marks et al., 1996), the brain region where nicotine-induced seizures are believed to be initiated (Dunlop et al., 1960; Floris et al., 1964; Brown, 1967; Stumpf and Gogolak, 1967). α7 RNA is localized in the pyramidal cell layers CA1, CA2 and CA3 and in the polymorphic areas of the dentate gyrus, whereas α5 RNA is restricted to the pyramidal cell layer of CA1. Thus overlap in the localization of α5 and α7 RNAs occurs in the CA1 region of the hippocampus, a region that is especially prone to excitotoxicity due to epileptiform activity (Meldrum, 1991). In addition, a preliminary quantitative autoradiographic study (L. Caton, unpublished data) indicates that nicotine-induced seizure sensitivity may, in fact, be correlated with levels of α-BTX binding in F2 animals, but only in one subregion of the hippocampus (out of 13 subregions examined), the ventral portion of the stratum lacunosum-moleculare of the CA1. Therefore, the association between nicotine-induced seizure sensitivity and levels of α-BTX binding may exist in F2 animals, but only in the hippocampal subregion where α5 and α7 are co-localized. These observations suggest that nicotine-induced seizures may be mediated by a novel α5/α7 heteromeric nAChR or by one of two distinct nAChRs: a heteromeric nAChR containing α5 and a homomeric/heteromeric nAChR containing α7. If an α5/α7 hetero-oligomeric nAChR does exist and does mediate nicotine-induced convulsions, it may have a pharmacology distinct from that of classical neuronal α-BTX-sensitive nAChRs because nicotine-induced seizures do not appear to be blocked by methyllycaconitine (Giasor et al., 1996). The possibility that co-expression of an α5 subunit with the α7 subunit may lead to the formation of a hetero-oligomeric nAChR with unique pharmacology and function is not implausible, given the recent finding that the α5 nAChR subunit can alter the pharmacology and electrophysiological properties of an α4/β2 nAChR subtype when co-expressed with these nAChR subunits in vitro (Ramirez-Latorre et al., 1996).

The lack of inhibition of nicotine-induced seizures by MLA alone may also indicate that the convulsions induced by nicotine are the result of inactivation/desensitization, rather than activation, of the affected nAChR by nicotine. In fact, MLA alone will induce seizures very similar in appearance to nicotine-induced seizures (P. Dobelis, unpublished data). In an effect consistent with the idea that nicotine-induced seizures may be due to inactivation/desensitization of the α-BTX-sensitive nAChR, α-BTX, the classic antagonist of this subtype of nAChR, induces seizures when administered intracerebroventricularly (Cohen et al., 1981).

Even though the nAChR subunit genes for both α5 and α7 are linked to sensitivity to the high-dose effects of nicotine, we cannot rule out the possibility that genes linked to these nAChR genes, and not the α5 and α7 nAChR genes themselves, are responsible for the associations between nAChR genotype and nicotine sensitivity. Judging on the basis of physical maps from chicken, rat and human, α5 is part of a gene cluster that includes the nAChR α3 and β4 genes (Couturier et al., 1990; Boulter et al., 1990; Raimondi et al., 1992). Consequently, if such a cluster were to exist in mice also, it would be impossible to rule out the involvement of either or
Both of these nAChR genes in regulating sensitivity to the high-dose effects of nicotine. This is especially true in light of the fact that a small subpopulation of hippocampal neurons appear to express a receptor that has a pharmacology quite similar to that of α3β4-containing nAChRs (Alkondon and Albuquerque, 1993).

Recently, it has been shown that a mutation in the nAChR α4 subunit gene is linked to nocturnal frontal lobe epilepsy in humans (Steinlein et al., 1995). This finding suggests that nicotinic cholinergic systems may play a role in at least some forms of epilepsy. Wherein the data described in the present study demonstrate that the nAChR α5 and α7 subunit genes are associated with nicotine-induced convulsions, other studies that sought to identify loci that are linked to epilepsy in mouse models detected loci proximal to both the nAChR α5 and α7 genes. Frankel et al. (1995) found a single locus, termed sfz1, linked to handling-induced convulsions. This locus is mapped to within 2 centiMorgans of the nAChR α7 subunit gene on chromosome 7. An investigation by Rise et al., (1991) described a locus, E1/1, that is linked to seizure susceptibility. This locus maps to within 4 centiMorgans of the nAChR α3 locus on chromosome 9, which implicates both α5 and β4 as a result of linkage. Thus, in addition to being linked to nicotine-induced seizure sensitivity, the nAChR α5 and α7 subunit genes may be considered as candidates for genes that regulate general epileptic activity. The α7 nAChR subunit is particularly intriguing in light of the observation that mice engineered to lack a functional α7 subunit have epileptiform-like wave patterns in hippocampus (Orr-Urtreger et al., 1996).

Although polymorphisms have been identified for the nAChR α5 and α7 subunit genes and these polymorphisms were found to be linked to sensitivity to the high-dose effects of nicotine, the exact nature of the polymorphism is not yet known for either gene. A point mutation in α7 between C3H/21bg and DBA/21bg mice has been identified in the protein-coding portion of the gene, but this mutation does not alter the amino acid sequence (Stitzel et al., 1996). However, mutations need not alter the open reading frame of a gene to have a profound effect on behavior. For example, the mouse mutant spastic, which is a prototype of inherited myoclonus, is due to the insertion of a LINE-1 element into intron 6 of the β subunit of the glycine receptor (Kingsmore et al., 1994). Efforts are under way to identify the region or regions of the α7 gene that are polymorphic between C3H/21bg and DBA/21bg mice. A murine nAChR α5 cDNA has recently been cloned (J. Stitzel, unpublished data), and RNAs from C3H/21bg and DBA/21bg mice are being investigated for differences in α5 sequence. Identification and characterization of the polymorphic region of each gene are essential to determine whether variations in these genes are, in fact, involved in determining strain-specific differences in sensitivity to nicotine.

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