Nicotine Response and Nicotinic Receptors in Long-Sleep and Short-Sleep Mice

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DE FIEBRE, C. M., L. J. MEDHURST AND A. C. COLLINS. Nicotine response and nicotinic receptors in long-sleep and short-sleep mice. Alcohol 4(6): 493–501, 1987.—Nicotine response and nicotinic receptor binding were characterized in long-sleep (LS) and short-sleep (SS) mice which have been selectively bred for differential “sleep-time” following ethanol administration. LS mice are more sensitive than SS mice to nicotine as measured by a battery of behavioral and physiological tests and as measured by sensitivity to nicotine-induced seizures. The greater sensitivity of the LS mice is not due to differences in binding of [3H]nicotine. Unlike inbred mouse strains which differ in sensitivity to nicotine-induced seizures, these selected mouse lines do not differ in levels of binding of [3H]alpha-bungarotoxin (BTX) in the hippocampus. Significant differences in BTX binding were found in the cerebellum and striatum. Although these two mouse lines do not differ in blood levels of nicotine following nicotine administration, they differ slightly in brain levels of nicotine indicating differential distribution of the drug. Since this distribution difference is much smaller than the observed behavioral differences, these mice probably differ in CNS sensitivity to nicotine; however, follow-up studies are necessary to test whether the differential response of these mice is due to subtle differences in distribution of nicotine to the brain.

<table>
<thead>
<tr>
<th>Nicotine receptors</th>
<th>Alpha-bungarotoxin</th>
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<tr>
<td>Seizures, nicotine-induced</td>
<td>Selectively bred mouse lines</td>
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ETHANOL and nicotine are widely used by humans and are often used simultaneously. A high correlation exists between smoking and alcohol consumption [6, 10–12, 14, 18, 27, 30, 45, 54]. Alcoholics have a higher tendency to smoke cigarettes than do non-alcoholics and smokers who are alcoholic smoke more cigarettes per day than non-alcoholic smokers [2]. Experimental studies have shown that ethanol increases smoking behavior in alcoholics [19,22]. Furthermore, rats have been found to increase their intake of ethanol during administration of nicotine [46]. A possible explanation of increased smoking behavior following ethanol administration may come from biochemical studies utilizing the nicotinic receptors found within the electric organ of Torpedo. These studies have shown that aliphatic alcohols stabilize nicotinic receptors in a non-functional (desensitized) high affinity state [5, 57–58]. Whether this also occurs within mammalian central nervous system is not known at this time.

In 1889 Langley and Dickinson [26] showed that low doses of nicotine will stimulate autonomic ganglia while higher doses will induce blockade. In addition to interacting with receptors at the autonomic ganglia, nicotine interacts biophysically with specific receptors at the neuromuscular junction and in the brain. This biphasic action is composed of an initial transient stimulation followed by a more long-lasting depression known as desensitization [25]. Not surprisingly, the behavioral and physiological responses to nicotine are multiple and varied. In rodents, among other responses, locomotor activity, heart rate, respiration, startle response and body temperature are affected by nicotine administration [1, 36, 48]. At higher doses nicotine has been shown to cause clonic-tonic convulsions [7,51]. Nicotine acts at receptors in the central nervous system and in the periphery, specifically the nicotinic receptors for the neurotransmitter acetylcholine (ACh). It has been suggested that nicotine may act at two distinct classes of nicotinic receptors in the brain, one characterized by the binding of [3H]alpha-bungarotoxin (BTX) and the other by [3H]nicotine or [3H]ACh binding [8, 32, 34, 38].

In humans, genetic factors appear to influence alcoholism [17] and smoking behavior [53]. Similarly, genetic factors influence drug response in rodents. Responsiveness to nicotine is regulated by genetic factors [3–4, 15–16, 21, 31, 33, 35–36, 42–44] and acute sensitivity to ethanol [39], ethanol preference [40] and severity of ethanol withdrawal [9,55] are regulated by genetic factors in rodents. Inbred strains of mice have been found to differ in responsiveness to a number of other pharmacologic agents including opiates [49], cocaine [52], and methylxanthines [28]. Because genetic factors are important in human drug taking behaviors, the use of genetically defined stocks of animals may be useful in increasing our understanding of drug taking behaviors. For example, previous studies from our laboratory have attempted to assign a function to nicotinic receptors in regulating behavioral responses to nicotine. We have reported that C3H mice are more sensitive to nicotine-induced seizures than are DBA mice and that C3H mice have a greater
number of hippocampal BTX binding sites than do the less seizure-susceptible DBA mice [42–44]. A classical genetic analysis revealed a reasonable correlation between seizure sensitivity and hippocampal BTX binding in the populations examined (F1, F2, F1 x C57, and F1 x DBA). Recently, we have reported that in 19 inbred strains of mice nicotine-induced seizure sensitivity is correlated with concentration of BTX binding sites [41]. It has been hypothesized that nicotine-induced seizure sensitivity is controlled by the level of hippocampal BTX binding. Interestingly, no differences have been found in levels of nicotine binding.

Studying nicotine response in inbred strains or selected lines of mice that differ in sensitivity to ethanol may allow an assessment of whether sensitivity to these two drugs is regulated by common genes. In the study reported here we have examined nicotine response in the long-term (LS) and short-term (SS) mouse lines which have been selectively bred for differential “sleep time” following an anesthetic dose of ethanol [39]. Furthermore, several biochemical parameters have been measured in an attempt to explain the differential nicotine response that we observed between these two selected mouse lines.

METHOD

Animals

Male and female LS and SS mice were used in this study. Mice were raised at the Institute for Behavioral Genetics, kept on a 12-hr light cycle and allowed free access to food (Wayne Lab Blox) and water. Mice were weaned at 25 days of age and housed with 1-5 like-sex littermates. Animals were 60-90 days old when tested and all testing was conducted between 9:00 a.m. and 4:00 p.m. All animals were drug naive prior to testing and were only used for a single test.

Materials

The radiolabeled compounds used in the receptor binding assays, [3H]BTX (Tyr-1-2H, initial specific activity 136.8 Ci/mmol) and L-[6H]nicotine (N-methyl-3H), initial specific activity 71.9 Ci/mmol), were obtained from New England Nuclear Corp. (Newton, MA). [3H]nicotine was repurified by thin-layer chromatography and stored frozen with a 4-fold excess of mecaprotoacetic acid [47]. Additional L-[3H]nicotine was obtained from Amersham Corp. (Arlington Heights, IL) and was used in the estimation of blood and brain levels of nicotine.

L-Nicotine, mecaprotoacetic acid, bovine serum albumin and polyethyleneimine were purchased from Sigma Chemical Co. (St. Louis, MO). L-Nicotine was periodically redistilled. Glass fiber filters and HEPES, 4-(2-hydroxyethyl)-1-piperazinethaneethanesulfonic acid, were purchased from Boehringer-Mannheim (Indianapolis, IN). Hepane was obtained from Sargent-Welch Scientific Co. (Skokie, IL), polypropylene test tubes from Sarstedt Co. (Princeton, NJ), and scintillation fluid (Safety Solve) and polypropylene scintillation vials from Research Products International (Mount Prospect, IL). Inorganic compounds were reagent grade.

Nicotine Administration

Nicotine was dissolved in physiological saline and was administered by intraperitoneal injection. Injection volume was 0.01 mg/kg body weight.

Nicotine Test Battery

Thirty LS and thirty SS mice of each sex were tested for response to nicotine in a battery of behavioral and physiological tests. Six LS and six SS mice of each sex received injections of 0.0, 0.5, 1.0, 1.5, or 2.0 mg/kg nicotine before being tested for respiration rate, startle response, Y-maze activity, heart rate, and body temperature. We have demonstrated previously that animals that are tested in all five tests do not differ in response to nicotine from animals that are tested in only a single test (i.e., there are no intertest interactions) [36]. Therefore, all animals were tested on all measures following a single injection of nicotine. Testing proceeded in the order listed above. Details of the testing procedure have been presented elsewhere [36].

Data analysis. All test battery data were analyzed using a three-way Analysis of Variance (ANOVA) to determine main effects of line (i.e., mouse line), dose, and sex, as well as interactions among these variables. For those analyses in which significant effects were observed, the results were subjected to Newman-Keuls' post hoc test.

Nicotine-Induced Seizure Sensitivity

Male and female LS and SS mice were tested for nicotine-induced seizure sensitivity following a single injection with this drug. Depending on genotype, animals were injected with 2.0, 3.0, 3.5, 4.0, 5.0, 6.0, or 7.0 mg/kg nicotine and placed in a 10 x 32 x 21.5 cm metal cage, the bottom of which was covered with aspen shavings. Whether a clonic seizure occurred, as well as the latency to that seizure, was recorded for each animal. Animals were observed for 5 min following injection. A minimum of 12 mice of each sex and genotype were tested at each dose.

Data analysis. Due to the nonparametric nature of the seizure data, sex differences were assessed using Wilcoxon tests [50]. ED50 values for each selected mouse line were computed using a log-dose probit regression analysis and slopes from the dose-response curves of each mouse line were computed by regression analysis. Testing for differences in the dose-response curves of the two mouse lines was conducted via sequential testing for: (1) differences in homogeneity of variance (an F-test), (2) differences in slope (a t-test) and (3) superimposability of the curves (a t-test) [13]. Non-superimposable dose-response curves that did not differ in homogeneity of variance or slope are an indication that the test groups differ in nicotine-induced seizure sensitivity. This method of analysis takes into account all points on the dose-response curves and is therefore more powerful than using a t-test to test for differences in ED50.

Estimation of Blood and Brain Nicotine Levels

Differential behavioral response to a drug by animals of different genetic stocks may be due to differential intrinsic sensitivity (i.e., CNS sensitivity) or may be due to differential absorption, distribution or elimination of the drug. For this reason, we estimated the levels of nicotine in blood and brain of LS and SS mice following nicotine administration. Brain and blood levels of nicotine were estimated 1, 2, 5, 10, 15 and 30 min following a 4.0 mg/kg injection of nicotine. Five animals of each sex and each selected line were decapitated at the appropriate time after receiving an injection of nicotine to which 2 μCi of radioactive nicotine had been added as a tracer ([3H]nicotine). A sample of blood (50-450 μl) was collected from the trunks of the animals and the brain
was removed, blotted and weighed. Brain tissue was homogenized in 2 ml saline and centrifuged for 10 min at 14,000 × g in a Sorvall RC2-B centrifuge refrigerated at 4°C. Blood was added to 1.5 ml saline and centrifuged similarly. Prior to centrifugation, 10 μg of cold nicotine was added to the tubes to inhibit the binding of radiolabeled nicotine to glassware. A 1.6 ml aliquot of the brain supernatant was added to 1 ml of 0.1 N NaOH and 7.5 ml of purified heptane containing 1.5% isooamy alcohol. Similarly, an aliquot of the blood supernatant (1.4 ml) was added to NaOH and heptane. The heptane had been purified by successive washings with 1 N NaOH, 1 N HCL and three washings with distilled water. From this point, the extraction of nicotine proceeded in an identical fashion for both blood and brain. The nicotine was extracted by shaking for 20 min. After centrifugation for 5 min at 1500 × g in an International Universal Model centrifuge, 5 ml of the supernatant (organic phase) was pipetted into a tube containing 2.5 ml of 0.1 N HCL. The contents of the tube were mixed by shaking for 5 min and centrifuged as above for 5 min at 1500 × g. The organic phase was removed by aspiration and a 1 ml aliquot of the acid phase was then transferred into polypropylene scintillation vials (7 ml). Scintillation fluid (2.5 ml) was added and the vials were shaken manually until a homogeneous gel was produced. Radioactivity was determined on an LS 1800 liquid scintillation spectrometer (Beckman Instruments, Fullerton, CA) with an efficiency of 39.5%. Previous studies in our laboratory have demonstrated that this method separates nicotine from its more polar metabolites [20].

Data analysis. Data were analyzed for differences in nicotine levels via three-way Analysis of Variance to determine the main effects of line (i.e., mouse line), sampling time and sex, as well as interactions among these variables. For those analyses in which significant effects were observed, the results were subjected to Newman-Keuls' post hoc test. Where differences were found between the two selected mouse lines, individual t-tests were performed to determine at what times the mouse lines differed.

Receptor Binding Assays

Tissue preparation. Six male and six female LS and SS mice were sacrificed by cervical dislocation; brains were removed and placed on an ice-cold platform. The tissue was rinsed and dissected into eight regions: cerebral cortex, hindbrain, pons-medulla, cerebellum, hypothalamus, striatum, hippocampus, superior and inferior colliculi and midbrain (primarily thalamus). Brain regions were placed in 10 volumes of ice-cold buffer (Krebs-Ringer's HEPES: NaCl, 118 mM; KCl, 4.8 mM; MgSO4, 1.2 mM; CaCl2, 2.5 mM; and HEPES, 20 mM; pH adjusted to 7.5 with NaOH). The tissue was homogenized with a glass-Teflon homogenizer. The tissue preparation used was essentially that described by Romano and Goldstein [47]. Briefly, the homogenate was centrifuged for 20 min at 18,000 × g in a Sorvall RC2-B centrifuge refrigerated at 4°C. After centrifugation, the resulting pellet was resuspended in 20 volumes of ice-cold water, incubated at 4°C for 1 hr and centrifuged as described above. The resulting pellet was resuspended in 10 volumes Krebs-Ringer's HEPES and centrifuged as described. The buffer was then discarded and 10 volumes of fresh buffer were added. The sample was then frozen at -70°C until assay. Prior to each centrifugation, the sample was incubated at 37°C for 10 min to promote hydrolysis of endogenous ACh.

On the day of the assay, the pellet was thawed and resuspended in the overlying buffer and centrifuged as described above. Supernatant was discarded and the pellet was resuspended in approximately 10 volumes of fresh Krebs-Ringer's HEPES for use in the assays. For very small brain regions (e.g., hypothalamus) slightly more than 10 volumes of buffer were added to assure that there was enough homogenate for use in the binding assays.

L-[3H]nicotine binding. The binding of [3H]nicotine was measured using a modification of the method of Romano and Goldstein [47] as described previously [32]. Binding assays were conducted at 4°C in a final volume containing 250 μl containing 70 to 700 μg of protein. Protein concentrations varied according to size of brain region assayed. A single concentration of radiolabeled nicotine (4.2 ±0.2 nM) was used for these assays. In half of the assays, the affinity of nicotine for the binding sites in cortex was estimated by displacement of the radiolabeled nicotine by unlabeled nicotine. The displacement experiment was used to calculate the Kd and the Bmax for ligand binding after conversion of the results to a form suitable for use in Scatchard plots. Blanks were determined by including 10-4 M nicotine in the assays.

[3H]IBTX binding. The binding of [3H]IBTX was measured as described previously [32]. Binding assays were conducted at 37°C in a final volume containing 35 to 350 μg of protein in an incubation volume of 500 μl. Protein concentrations varied according to size of brain region assayed. For all brain regions, a single concentration of [3H]IBTX (1.78±0.12 nM) was used. In half of the assays of cortical binding, five additional concentrations of [3H]IBTX were used and Kd and Bmax values were calculated from Scatchard plots. Blanks were determined by including 10-3 M nicotine in the assays.

Protein assay. Protein was measured using the method of Lowry et al. [29] with bovine serum albumin as the standard.

Scintillation counting. After the samples were washed, the glass fiber filters were placed in polypropylene scintillation vials (7 ml) and 2.5 ml of scintillation fluid was added. The samples were mechanically shaken for 30 min and radioactivity was determined on an LS 1800 liquid scintillation spectrometer (Beckman Instruments, Fullerton, CA). Both tritium and [3H] were counted at 45% efficiency.

Data analysis. All kinetic analyses were conducted by linear regression of Scatchard plots of data. Testing for differences between the selected mouse lines in Kd and Bmax was done by t-tests. Binding data were also analyzed using t-tests to determine differences between the mouse lines as well as sex differences in each brain region.

Nicotine Test Battery

The effects of nicotine on test battery response of the LS and SS mouse lines are presented in Fig. 1. Data from all six tests (respiration, startle response, Y-maze crosses, Y-maze rear, heart rate and body temperature) are included in the figure. The responses for each test were analyzed separately using three-way ANOVAs (line × dose × sex). No significant sex or dose differences were found for any of the tests indicating that for these two mouse lines the sexes do not differ in response to nicotine on these measures. Figure 1 therefore shows results with the two sexes combined. There is a significant effect of dose on each of the tests, except for the startle response test, indicating that nicotine does affect response
FIG. 1. Dose-response curves for nicotine test battery response in LS and SS mice. Data represent the mean±SEM of 12 animals of each genotype tested at each dose.

on five of these measures. There are significant differences between the two mouse lines on all tests, except startle response and respiration, indicating that these selected mouse lines differ in response to nicotine. Visual analysis of Fig. 1 reveals that the LS mice are more sensitive than the SS to the effects of nicotine on these tests. The results of the statistical analyses are presented in Table 1.

**Nicotine-Induced Seizure Sensitivity**

The dose-response curves for nicotine-induced seizures in LS and SS mice are presented in Fig. 2. Since no sex differences were observed, this figure presents data with the two sexes combined. ED$_{50}$ values, the dose at which 50% of the animals seize, were calculated for these two mouse lines using a log-dose probit regression analysis. The ED$_{50}$ for SS mice (4.91 mg/kg) is nearly 50% higher than is the ED$_{50}$ for the LS mice (3.34 mg/kg). Analysis of the dose-response curves revealed that the two selected mouse lines differ in nicotine-induced seizure sensitivity with the LS line being more sensitive, t(4)=4.05, p<0.05. The LS and SS dose-response curves did not differ due to differences in homogeneity of variance or slope.

FIG. 2. Dose-response curves for nicotine-induced seizures in LS and SS mice. Each point represents the percent of animals tested that exhibited a clonic seizure at the indicated dose of nicotine; a minimum of 12 animals of each sex and genotype were tested at each dose.
Estimation of whole brain levels of nicotine following injection with 4 mg/kg. In the top panel, the sexes are combined and each point represents the mean±SEM of 10 animals tested. In the lower panels, the sexes are separated and each point represents the mean±SEM of 5 animals tested. *p<0.02; **p<0.01.

**TABLE 1**

<table>
<thead>
<tr>
<th>Test</th>
<th>Source</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration</td>
<td>Dose</td>
<td>F(4,102)=19.705</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Y-maze Crosses</td>
<td>Dose</td>
<td>F(4,102)=12.497</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Line</td>
<td>F(1,102)=27.046</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Y-maze Retars</td>
<td>Dose</td>
<td>F(4,102)=34.424</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Line</td>
<td>F(1,102)=27.141</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Line × Dose</td>
<td>F(4,102)=3.896</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>Dose</td>
<td>F(4,102)=8.298</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Line</td>
<td>F(1,102)=43.202</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Line × Dose</td>
<td>F(4,102)=2.495</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body</td>
<td>Dose</td>
<td>F(4,102)=19.394</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Temperature</td>
<td>Line</td>
<td>F(1,102)=21.031</td>
<td>&lt;0.0001</td>
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</table>

Three-way ANOVA (line × dose × sex).

Figure 3 shows the levels of nicotine in brain 1, 2, 5, 10, 15, and 30 min following a 4.0 mg/kg injection in LS and SS mice. In this figure, brain nicotine levels are shown for these two selected mouse lines with the sexes combined (top panel) and with the sexes separated (lower panels). Three-way analysis of variance showed a significant effect of sampling time, F(3,96)=56.887, p<0.0001. LS mice have overall greater brain nicotine levels than do SS mice, F(1,96)=24.212, p<0.0001, but this overall difference is primarily due to a significant difference in brain nicotine levels at the 2 minute time point following nicotine injection, t(18)=2.79, p<0.01. Although smaller than the difference found between the two mouse lines, a sex difference was found with males having higher brain nicotine levels than females, F(1,96)=9.881, p<0.01.

Since sex differences were found, analyses were conducted on the two sexes separately. For both sexes there is a significant effect of sampling time; males, F(5,48)=31.143, p<0.0001; females, F(5,48)=26.148, p<0.0001. In both sexes, the LS and SS mouse lines differ in brain nicotine levels when these differences are assessed across all time points.
points via analysis of variance; however, these differences are more pronounced in females than in males; males, F(1,48)=6.889, p<0.02; females, F(1,48)=21.231, p<0.0001. At no individual sampling time were significant differences found between LS and SS males. In females, however, significant differences were found at 2 min, r(8)=2.92, p=0.02; and at 5 min, r(7)=3.11, p=0.02.

Figure 4 shows the levels of nicotine in blood 1, 2, 5, 10, and 30 min following a 4.0 mg/kg injection in LS and SS mice. In this figure blood nicotine levels are shown for these two selected mouse lines with the sexes combined (top panel) and with the sexes separated (lower panels). Three-way analysis of variance showed a significant effect of sampling time, F(5,50)=18.318, p<0.0001. The LS and SS mouse lines did not differ in blood nicotine levels, F(1,50)=0.871, p=0.35. There is a trend for males to have slightly higher blood nicotine levels than females. Although this trend is not significant, it approaches significance, F(190)=3.165, p=0.08.

Receptor Binding Assays

Figure 5 shows the concentration of [3H]nicotine and Fig. 6 shows the concentration of [3H]BTX binding sites in eight brain regions of LS and SS mice. Statistical analyses revealed no significant differences for nicotine binding in seven of eight brain regions. A significant difference was found in nicotine binding in cortex in the single point assays, \( t(16)=3.23, p<0.01 \); however, no differences were found in cortical \( B_{max} \) or \( K_d \) as can be seen in Table 2. No differences in BTX binding were found in six of eight brain regions (see Fig. 6). LS mice had a higher concentration of BTX binding sites in the striatum, \( t(18)=3.06, p<0.01 \); SS mice had a higher concentration of BTX binding sites in the cerebellum, \( t(20)=3.09, p<0.01 \). No significant sex differences were found. Table 2 shows \( K_d \) and \( B_{max} \) values calculated from Scatchard plots of data.

DISCUSSION

The results reported here demonstrated that the LS and SS selected mouse lines differ in sensitivity to nicotine as measured by response to nicotine in four of the components of the battery of behavioral and physiological tests and as measured by sensitivity to nicotine-induced seizures. The LS and SS mice did not differ in response to nicotine for the respiration and startle tests. Nicotine did not affect startle response in either mouse line and stimulated respiration to a similar degree at equal doses in both mouse lines. The fact that these mouse lines do not differ in nicotine's effects on respiratory or startle response whereas they differ in response to other tests suggests that the two mouse lines differ in CNS sensitivity to nicotine. If the LS and SS differ in some nicotine-related pharmacokinetic parameter (i.e., ab-
Nicotine response and receptors.

**FIG. 5.** Binding of [3H]nicotine in eight regions of LS and SS mouse brain: Cx, cortex; M, midbrain; H, hindbrain; P, hippocampus; S, striatum; T, hypothalamus; Cb, cerebellum; and Col, colliculi. Each bar represents the mean ± SEM of 12 animals with both sexes combined. *p < 0.01.

**FIG. 6.** Binding of [3H]BTX in eight regions of LS and SS mouse brain: Cx, cortex; M, midbrain; H, hindbrain; P, hippocampus; S, striatum; T, hypothalamus; Cb, cerebellum; and Col, colliculi. Each bar represents the mean ± SEM of 12 animals with both sexes combined. *p < 0.01.

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>K_d (± SEM)</th>
<th>B_max (± SEM)</th>
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<tbody>
<tr>
<td>Nicotine</td>
<td>LS 11.43 ± 2.31 nM</td>
<td>SS 13.65 ± 4.57 nM</td>
</tr>
<tr>
<td>Binding</td>
<td>SS 13.65 ± 4.57 nM</td>
<td>SS 13.65 ± 4.57 nM</td>
</tr>
<tr>
<td>BTX</td>
<td>LS 0.514 ± 0.087 nM</td>
<td>SS 0.482 ± 0.084 nM</td>
</tr>
<tr>
<td>Binding</td>
<td>SS 0.482 ± 0.084 nM</td>
<td>SS 0.482 ± 0.084 nM</td>
</tr>
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absorption, distribution, metabolism), it would be anticipated that the two lines should differ similarly for all nicotine effects. It has been reported that there are subtle differences in the way that nicotine distributes to different regions in rat brain [37]. Therefore, it is possible that these two mouse lines differ in response to some nicotine effects and not others because of these subtle differences in nicotine distribution. Nevertheless, the observation that the two mouse lines have minimal differences in blood and brain nicotine content following IP injection is consistent with the assertion that the differences in response are due to CNS sensitivity differences. Clearly, however, follow-up studies are necessary to test whether subtle distributional differences to brain also play a role in the differential nicotine response of the LS and SS mouse lines.

Inspection of the data regarding levels of nicotine in blood following nicotine injection indicates that the differential response of the LS and SS mice cannot be explained by differences in nicotine metabolism. At no time did these two selected lines differ in blood nicotine levels indicating that the two lines can metabolize the drug at roughly equivalent rates. However, the two mouse lines may differ in distribution of the drug to highly perfused tissues as is indicated by the slightly higher brain nicotine levels in the LS mice. These mouse lines tend to differ in brain nicotine levels when these levels are assessed across all time points via analysis of variance. However, at individual times these differences are not great and are only statistically significant at the 2 min time point (2 and 5 min in females). Nevertheless, the LS mice have significantly higher brain nicotine levels than SS mice. This is not surprising since the LS mice have slightly higher total body fat than SS mice [24]. It is expected that the drug would be distributed to highly perfused areas (i.e., brain) before less perfused areas (i.e., body fat). Since nicotine was administered on the basis of total body weight, the higher brain nicotine levels of the fatter LS mice are not surprising.

The finding that males tend to have higher brain nicotine levels than females was unexpected. Although these differences are small, they are statistically significant nonetheless. This is interesting because sex differences were not found in response to nicotine or in levels of nicotinic binding sites.

The differential response of these mouse lines in the test battery is comparable to differences we have previously reported in inbred mouse strains [31, 36]. Recently we have screened 19 inbred strains for test battery nicotine response (manuscript in preparation). Comparison of the LS and SS data with data from these 19 strains reveals that the LS mice are among the most nicotine sensitive while SS mice are among the least nicotine sensitive of all mice tested in our laboratory. At higher doses, these two selected mouse lines also differ in sensitivity to nicotine; specifically, these mice differ in sensitivity to nicotine-induced seizures. This differ-
ential response is comparable to that which we have seen previously in C3H and DBA inbred mice [42–46]. Additionally, these differences are among the greatest seen for any of the 19 inbred strains tested thus far [41]. Interestingly, LS mice are more sensitive than SS mice to both low dose nicotine effects (i.e., test-battery effects) and high dose nicotine effects (i.e., seizure sensitivity). This is in contrast to our findings in C3H and DBA mice. Although C3H mice are more sensitive than DBA mice to nicotine-induced seizures, they are less sensitive to low dose effects as measured in our test battery [31, 36, 41, 44]. Apparently, low and high dose effects of nicotine are controlled by different mechanisms, possibly by the two sites labeled by the nicotinic ligands: nicotine and BTX, respectively.

Consistent with our findings in inbred strains of mice, we found no differences in binding of [3H]nicotine in seven of eight brain regions examined. We found a difference in cortical nicotine binding using a single ligand concentration assay, but this finding was not replicated by the more reliable determinations of cortical Bmax and KB. Unlike our findings in DBA and C3H mice [42–44], we found no differences in levels of BTX binding in the hippocampus of LS and SS mice. This finding is surprising considering our previous findings in inbred mice of a positive correlation between nicotine-induced seizure sensitivity and hippocampal BTX binding [41]. The present finding in LS and SS mice indicates that something other than, or in addition to, hippocampal BTX binding must regulate sensitivity to nicotine-induced seizures. The differential binding of BTX in cerebellum of these two selected mouse lines may be important with regard to their differential sensitivity to nicotine-induced seizures. The cerebellum has been implicated in the propagation of audiogenic seizures [23] and therefore could conceivably be involved in the propagation of nicotine-induced seizures. However, since the less severe sensitive SS mice had greater cerebellar BTX binding than the more severe sensitive LS mice, and since this site is presumably excitatory, the significance of these binding differences may not be great. The significance of the differential BTX binding in the striatum of these two selected mouse lines is unknown at this time.

The results presented here clearly demonstrate that the LS and SS mice, which were selectively bred for differential response to a hypnotic dose of ethanol, also differ dramatically in their response to nicotine. The LS are among the most nicotine sensitive and the SS are among the least nicotine sensitive of any mice tested in our laboratory. This finding suggests that the same genes may regulate the response to both alcohol and nicotine. The difference in response to nicotine cannot be readily explained by differences in nicotine metabolism or distribution or by differences in the number or affinity of either of the brain nicotinic receptors. As noted previously, several studies [5, 56, 57] have demonstrated that ethanol stabilizes nicotinic receptors in the desensitized state. Possibly, the LS and SS mouse lines differ in rate of desensitization or re sensitization of nicotinic receptors. We will explore this possibility in future studies. Because the LS and SS mouse lines were selectively bred for differential sensitivity to ethanol, identifying the causes of the differential response of these two mouse lines to nicotine may ultimately prove to be useful in understanding why humans use alcohol and tobacco together.

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