Pergamon

Pharmacology Biochemistry and Behavior, Vol. 47, No. 4, pp. 889–900, 1994

0091-3057/94 $6.00 + .00

Prenatal Nicotine Alters Nicotinic Receptor Development in the Mouse Brain

JENNIFER L. VAN DE KAMP AND ALLAN C. COLLINS[1]

Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309

Received 17 March 1993

VAN DE KAMP, J. L. AND A. C. COLLINS. *Prenatal nicotine alters nicotinic receptor development in the mouse brain.* PHARMACOL BIOCHEM BEHAV 47(4) 889–900, 1994.—Maternal smoking during pregnancy may affect development of the child, but little is known about potential mechanisms of these effects. Since chronic nicotine treatment alters brain nicotinic receptors in adults and also evokes tolerance which is regulated by genetic factors, pregnant mice of two inbred strains underwent chronic nicotine infusion to determine whether the developmental pattern of mouse brain nicotinic receptors would be altered. C3H/2ibg and C57BL/6ibg mice were infused SC with saline or 2.0 mg/kg/h nicotine during the last half of pregnancy. The developmental profiles of [^{3}H]nicotine and α-[^{125}I]bungarotoxin binding in seven brain regions obtained from the offspring were measured. Prenatal nicotine treatment increased levels of [^{3}H]nicotine binding at birth in the C3H hypothalamus, hippocampus, and possibly the cortex, and in the C57BL cortex. At later ages (20–30 days), [^{3}H]nicotine binding was elevated in the C3H hindbrain, hippocampus, striatum, midbrain, and possibly the cortex. The C57BL hindbrain, hippocampus, midbrain, and cortex also showed increased binding at 20–30 days. Little, if any, effect of prenatal nicotine treatment was observed on the development of the α-[^{125}I]bungarotoxin binding site. Since upregulated [^{3}H]nicotine binding returns to control levels in adult animals within seven days following termination of chronic nicotine infusion, it is unlikely that simple upregulation is responsible for the changes observed in 20–30-day-old mouse brains.

Nicotine Nicotinic receptors Development Genetics

MATERNAL smoking may result in pregnancy complications, a higher perinatal mortality rate, low birth weight, and long-term effects on the physical, emotional, and intellectual growth of the child (5,36). Learning difficulties and hyperactivity in children have been associated with maternal smoking (5,11,13,14,35). Several mechanisms have been proposed to explain these deleterious effects, including poor nutritional status of the mother (12,44), carbon monoxide exposure (4, 19,22), restriction of the blood flow to the placenta (34,40), and direct effects of nicotine on the developing fetus (24,25).

Nicotine is the most potent psychoactive component of tobacco, and many studies have reported that chronic treatment of adult animals with nicotine results in increases in brain high-affinity nicotinic receptor binding [see (52) for a recent review]. Similarly, human smokers display elevated levels of brain nicotinic receptors when compared with age-matched nonsmokers (2). The mammalian brain contains a minimum of two major subclasses of nicotinic receptors, as defined by ligand binding. One of these is measured by high-affinity agonist binding ([^{3}H]nicotine, [^{3}H]acetylcholine, [^{3}H]cytisine, or [^{3}H]methylcarbamylcholine) (1,38,42,47), and the other major class is measured using α-[^{125}I]bungarotoxin as the ligand (6,28,33). Both of these sites are upregulated by chronic nicotine treatment, but higher doses are generally required to evoke changes in the α-[^{125}I]bungarotoxin binding site (26,29,30,31).

Recently, several studies using the rat have demonstrated that chronic treatment of pregnant rats results in increases in fetal and neonatal rat brain nicotinic receptors, as measured by [^{3}H]nicotine binding (18,48,49). One of these studies (49) noted that alterations in binding persisted as long as 30 days postnatally in the cerebellum, a brain region that normally has very low levels of [^{3}H]nicotine binding in the adult rodent brain (28,33). In other brain regions (cortex, midbrain, hindbrain) upregulation was transient, and by 30 days of age no differences were seen between control and nicotine-treated animals. Slotkin et al. (49) argued that these changes in binding were not due to the same mechanisms that produce upregulation of nicotinic receptors in adult animals, but were due to nicotine-induced changes in cellular development and that the

[1] Requests for reprints should be addressed to Dr. Allan C. Collins, Institute for Behavioral Genetics, Campus Box 447, University of Colorado, Boulder, CO 80309.

cerebellum is particularly sensitive to these teratological actions.

Our laboratory group has been actively studying the effects of chronic nicotine treatment on adult mouse brain nicotinic receptors (26,27,29,30,31). These studies have demonstrated that chronic nicotine treatment, generally by intravenous infusion, results in dose-dependent increases in [^{3}H]nicotine binding that vary somewhat among brain regions. The increase in binding is reversible in that levels of binding return to control approximately seven days after chronic infusion is stopped (29). Chronic nicotine administration also results in tolerance to many of nicotine's behavioral and physiological effects, but this tolerance differs dramatically among inbred mouse strains. Some strains, such as the C57BL/6, develop marked tolerance at low infusion doses, and other strains, such as the C3H, develop minimal tolerance only after infusion with much larger doses (27,30,32). Curiously, these same inbred mouse strains do not differ in the dose-dependent upregulation of [^{3}H]nicotine binding (27).

The studies reported here are an attempt to determine whether chronic prenatal nicotine treatment produces upregulation of [^{3}H]nicotine binding and/or α-[^{125}I]bungarotoxin in the neonatal mouse brain and whether chronic nicotine treatment alters the developmental profiles for these two binding sites. Because our studies with adult C57BL/6 and C3H inbred mice indicate that these two strains differ markedly in their ability to develop tolerance to chronic nicotine treatment (27), both strains were used in an attempt to determine whether any effects of prenatal nicotine treatment are regulated by genetic factors. Because adult animals of these two inbred strains differ minimally, if at all, in agonist-induced upregulation of these nicotinic receptors (27), this strain comparison may also facilitate determining whether changes in nicotinic receptor binding are due to simple upregulation of the receptor or to a teratological action of nicotine.

METHODS

Animals

Mice of the C3H/2ibg and C57BL/6ibg strains were used in this study. These mouse strains have been maintained in the breeding colony at the Institute for Behavioral Genetics for at least 20 generations. The mice were kept on a 12-h light/dark cycle (lights on from 0700 to 1900) with free access to food (Wayne Sterilizable Lab Blox) and tap water, in a room maintained at 21 ± 2°C and 50% relative humidity.

Matings were carried out using 221 C3H/2ibg females mated with 38 C3H/2ibg males and 188 C57BL/6ibg females mated with 30 C57BL/6ibg males. Females were between 90 and 120 days of age when mated. Each female had previously given birth to and successfully reared one litter. Males were between 60 and 90 days of age when mated. Each male mated with 2–6 females.

Chronic Treatment

The females were checked daily for the presence of a vaginal plug (day 1 of pregnancy). Upon the observance of a vaginal plug, the females were separated from the males in groups of two to four per cage until day 11 of pregnancy. At this time, they were randomly assigned to one of three treatment groups: control, saline-infused, or nicotine-infused.

Females assigned to the saline- and nicotine-infused treatment groups were anesthetized with 80 mg/kg of pentobarbital, injected IP. A cannula made of silastic tubing was surgically implanted SC at the nape of the neck. Thirty minutes after surgery the mice were placed in individual cages. The cannulas were attached to thermoplastic tubing connected to a 1.0-ml glass syringe mounted on a Harvard Instruments infusion pump. The saline-infused group received saline at a flow rate of 35 μl/h (0.84 ml/day) until parturition (usually gestational day 20 or 21). The nicotine-infused group received saline at the same flow rate until gestational day 13. At this time, these mice began receiving L-nicotine base in a saline vehicle (flow rate, 35 μl/h, 0.84 ml/day) at an initial rate of 0.5 mg/kg/h. Each day thereafter, the dose was increased by 0.5 mg/kg/h until a dose of 2.0 mg/kg/h was achieved. This dose of nicotine was used because many earlier studies from our laboratory have shown that IV infusion of a 2.0-mg/kg/h dose of nicotine results in consistent upregulation of [^{3}H]nicotine binding in the adult mouse brain (30). The 2.0-mg/kg/h dose is a much higher dose than has been used in the rat, but the mouse metabolizes nicotine much more rapidly than does the rat; the $t_{1/2}$ for nicotine in the mouse is about 5–10 min (39), whereas in the rat the $t_{1/2}$ is more like 120 min (41). Consequently, the dose required in the mouse to produce pharmacological and toxicological actions is higher than that used in other species such as the rat. The 2.0-mg/kg/h dose was maintained until parturition. Preliminary results indicated that higher doses would result in very few pregnancies maintained until term, but this dose is sufficient to elicit upregulation in the number of receptors in both mouse strains (27). Nicotine was infused during the last half of gestation, since a pilot study indicated that earlier treatment resulted in interference in implantation. Mice assigned to the control group were placed in isolation cages beginning gestational day 11 through parturition. On the day of parturition, the females and their litters were placed in fresh cages.

Materials

L-[^{3}H]Nicotine (*N*-[methyl^{3}H], initial specific activity 78.4 Ci/mmol) was obtained from Amersham Corporation (Arlington Heights, IL). α-[^{125}I]bungarotoxin (Tyr-^{125}I, initial specific activity 130.7–140.8 Ci/mmol) was obtained from New England Nuclear Corporation (Newton, MA). [^{3}H]Nicotine was repurified by thin-layer chromatography to reduce nonspecific binding and to eliminate what appears to be a low-affinity binding artifact (43). It was stored frozen with a fourfold excess of mercaptoacetic acid (42).

L-Nicotine, bovine serum albumin, polyethylenimine, tris-(hydroxymethyl)aminomethane (Tris HCl), and mercaptoacetic acid were purchased from Sigma Chemical Company (St. Louis). L-Nicotine was periodically redistilled to remove oxidation byproducts. Glass fiber filters and *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (HEPES) were purchased from Boehringer-Mannheim (Indianapolis). Scintillation fluid (Safety Solve) and polypropylene scintillation vials were obtained from Research Products International (Mount Prospect, IL). Inorganic compounds were reagent grade.

Tissue Preparation

Some of the C3H and C57BL females that had undergone the treatment previously described were sacrificed on the day of parturition. Male and female offspring generated by the three maternal treatment groups were randomly assigned to be sacrificed on postnatal days 0, 5, 10, 20, 30, or 60. The animals were sacrificed by cervical dislocation. The brains were removed, rinsed with distilled water, and dissected on ice into seven brain regions: cerebellum, hypothalamus, hind-

brain (pons medulla), hippocampus, striatum, midbrain (tissue remaining after removal of all other areas; contains primarily thalamus), and cortex. Due to the small size and low protein content of the brain tissue from the younger animals, brain regions from same-sex, same-age animals were pooled in the following manner: 10–12 animals for day 0, 5–6 animals for day 5, 2–3 animals for day 10, and 1–2 animals for day 20. An effort was made to pool as many animals per litter as possible; however, at the youngest ages, it was necessary to combine multiple litters. The tissue pieces were placed in 10 volumes of ice-cold Krebs–Ringer's HEPES buffer (NaCl, 118 mM; KCl, 4.8 mM; $CaCl_2$ 12.5 mm; $MGSO_4$ 1.2 mM; HEPES, 20 mM; pH adjusted to 7.5 with NaOH). The samples were homogenized with a glass–Teflon homogenizer. The tissue preparation method was essentially that of Romano and Goldstein (42). The particulate fraction was collected by centrifugation for 20 min at 18 000 × *g*. The buffer was discarded and the pellet was resuspended in 20 volumes of distilled water and incubated on ice for 60 min. After this incubation, the samples were centrifuged for 20 min at 18 000 × *g*. The buffer was discarded and the pellet was resuspended in 10 volumes of Krebs–Ringer's HEPES and centrifuged for 20 min at 18 000 × *g*. The buffer was discarded and 10 volumes of fresh buffer were added. The samples were then frozen at −70°C until assay. On the day each sample was assayed, it was resuspended and centrifuged for 20 min at 18 000 × *g*. The buffer was discarded and 20 volumes of distilled water were added. The tissue was again resuspended, then centrifuged 20 min at 18 000 × *g*. The water was discarded and 10 volumes of fresh distilled water were added to each sample. Prior to each centrifugation, the samples were incubated at 37°C for 5 min to promote the dissociation of drugs with which the animals had been treated from the tissue. This method has been shown to remove nicotine from mouse brain tissue (26). On the day of radioligand binding assay, representatives from each treatment group were selected for assay.

L-[^{3}H]Nicotine Binding

The binding of L-[^{3}H]nicotine was measured using a modification of the method of Romano and Goldstein (42) as described previously (28,33). Binding was measured using 100–600 μg of protein. Final incubation volume was 250 μl. HEPES-buffered Krebs–Ringer's solution was used as the buffer. In addition, 500 mM Tris (pH 7.5 at 37°C) was included to reduce the nonspecific binding. The binding was conducted in 12 × 75-mm polypropylene tubes at 4°C. The reaction was initiated by the addition of labeled ligand. Incubation time was 90 min. The binding was terminated by addition of 3 ml of ice-cold buffer followed immediately by filtration of the samples onto glass fiber filters that had been soaked in buffer containing 0.5% polyethylenimine to reduce nonspecific binding (42,47). The filters were subsequently washed four more times with 3-ml aliquots of ice-cold buffer. The vacuum was −50 to −100 torr. All filtrations and washes were conducted in a 4°C cold room using apparatus cooled to 4°C. Blanks were obtained by including 10 μM L-nicotine in the incubations. A single concentration of radiolabeled nicotine (approximately 5 nM) was used in all brain regions except the cortex. Binding to cortex was measured at five additional concentrations of ligand to calculate K_D and B_{max} from Scatchard plots.

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

α-[^{125}I]Bungarotoxin Binding

The binding of α-[^{125}I]bungarotoxin was measured as described previously (28). Binding was measured using 50–300 μg of protein in a final volume of 500 μl of HEPES-buffered Krebs–Ringer's solution. The reaction was initiated by the addition of α-[^{125}I]bungarotoxin and continued for 3 h at 37°C. At the completion of the incubation, samples were diluted with 3 ml of ice-cold buffer and filtered on glass fiber filters that had been soaked in buffer containing 0.5% polyethylenimine to reduce nonspecific binding. The filters were then washed four times with 3-ml aliquots of ice-cold buffer. Vacuum pressure was −50 to −100 torr. Samples containing 1 mM L-nicotine served as the blanks. A single concentration of α-[^{125}I]bungarotoxin (approximately 1.2 nM) was used in all of the brain regions except the cortex. Binding to cortex was measured at five additional concentrations of ligand to calculate K_D and B_{max} from Scatchard plots.

After the samples were washed, the glass fiber filters were placed in 12 × 75-mm borosilicate glass tubes. Radioactivity was determined on a Packard Minaxi Auto-Gamma 5000 series Gamma counter at 80% efficiency.

Protein Assay

Protein was measured using the method of Lowry et al. (23) with bovine serum albumin as the standard.

Data Analysis

The results of the maternal single ligand concentration binding assays were analyzed by analysis of variance (ANOVA) with two between-subjects factors (strain and treatment). The data were then sorted by strain and a second ANOVA, with one between-subjects factor (treatment), was performed.

The binding constants (K_D and B_{max}) were calculated for each ligand by linear regression analysis of Scatchard plots of the binding data in cortex. Similar two-way and one-way ANOVAs were performed, treating K_D and B_{max} as dependent variables.

The results of the single ligand concentration binding assays in the offspring were initially analyzed by three-way ANOVA, with strain, treatment, and day of age as between-subjects factors. The data were then sorted by strain and day of age and analyzed by one-way ANOVA, with treatment as the only between-subjects factor. Significance was set at the $p < .05$ level. Post hoc Newman–Keuls analyses were done if a significant treatment effect was observed by the one-way ANOVA. The binding data were also analyzed using a two-way ANOVA to assess whether Ligand × Treatment interactions occur. These were done separately for both strains in each brain region.

The binding constants were calculated for each ligand by linear regression analysis of Scatchard plots of the binding data in the cortex. Similar three-way and one-way ANOVAs were performed, treating K_D and B_{max} as dependent variables.

RESULTS

L-[^{3}H]Nicotine Binding

The effects of chronic saline and nicotine treatment on the number of L-[^{3}H]nicotine binding sites in seven brain regions

obtained from the dams are presented in Fig. 1 in the left two panels. In general, there were no significant strain differences in the amount of binding in any of the brain regions except the cortex, $F(1, 38) = 7.46$, where C3H mice exhibited significantly more binding than C57BL mice.

Significant main treatment effects due to dramatic increases in binding in most brain regions were observed in the two-way ANOVA. In the one-way ANOVAs, nicotine-infused C3H mice exhibited significant increases in binding in all seven brain regions, cerebellum, $F(2, 25) = 12.02$; hypothalamus, $F(2, 25) = 9.70$; hindbrain, $F(2, 25) = 16.63$; hippocampus, $F(2, 25) = 6.46$; striatum, $F(2, 25) = 5.83$; midbrain, $F(2, 25) = 11.14$; and cortex, $F(2, 25) = 23.74$. Significant increases were observed in four brain regions in C57BL mice, hypothalamus, $F(2, 13) = 6.74$; hippocampus, $F(2, 13) = 6.54$; midbrain, $F(2, 13) = 7.29$; and cortex, $F(2, 13) = 4.90$. The nicotine-infused C3H and C57BL dams exhibited an increase in the total number of cortical nicotine binding sites, B_{max}, $F(2, 12) = 9.03$, with no change observed in K_D.

The developmental profiles of L-[^{3}H]nicotine binding in each of the seven brain regions are presented for C3H and C57BL untreated (control) offspring and offspring treated in utero with saline or nicotine in Figs. 2 and 3. In the three-way ANOVA, there was a significant overall strain effect in the number of binding sites in hippocampus, $F(1, 337) = 4.06$, due to the overall higher levels of binding exhibited by the C57BL mice compared to C3H mice. Robust main effects of day of age were observed in all seven brain regions, cerebellum, $F(5, 303) = 131.78$; hypothalamus, $F(5, 301) = 28.18$; hindbrain, $F(5, 303) = 114.23$; hippocampus, $F(5, 302) = 20.84$; striatum, $F(5, 302) = 21.28$; midbrain, $F(5, 303) = 33.58$; cortex, $F(5, 303) = 11.94$), with Strain × Day of Age interaction terms significant in the hindbrain, $F(5, 303) = 3.80$; hippocampus, $F(5, 302) = 3.31$; and midbrain, $F(5, 303) = 4.23$. The age effects fell into three categories: regions which displayed extremely high levels of binding at birth and then declined rapidly to adult levels (cerebellum, hypothalamus, and hindbrain, which begins decreasing earlier in C3H than in C57BL mice); regions exhibiting lower than adult levels at birth, and then slowly climbing throughout development to reach adult levels (striatum and cortex); and regions with adult binding levels at birth, climbing rapidly to a peak level at around 10 days of age and then declining to adult levels (the hippocampus, which peaks higher in C57BL mice than in C3H mice, and the C57BL midbrain). C3H midbrain appeared to fall generally into the third category, although binding was slightly higher (30%) at birth than in adulthood.

Significant overall effects of treatment were observed in the hippocampus, $F(2, 302) = 10.27$; striatum, $F(2, 302) = 4.69$; midbrain, $F(2, 303) = 3.58$; and cortex, $F(2, 303) = 6.91$. In the hippocampus, both Strain × Treatment and Day of Age × Treatment interaction terms were significant, $F(2, 302) = 6.08$ and $F(10, 302) = 2.02$, respectively. In addition, the Strain × Day of Age × Treatment interaction term in the striatum was significant, $F(10, 302) = 3.16$.

When the data were sorted by strain and day of age, neonatal (day 0) C3H offspring prenatally treated with nicotine displayed significant increases in nicotine binding in the hypothalamus, $F(2, 19) = 4.26$, and hippocampus, $F(2, 19) = 4.39$, as well as a similar tendency in the cortex, $F(2, 20) = 3.25$, $p = .059$. Neonatal C57BL offspring treated in utero with nicotine also showed significantly increased levels of nicotine binding in the cortex, $F(2, 28) = 7.77$.

In the Scatchard plots generated in the cortex, no changes

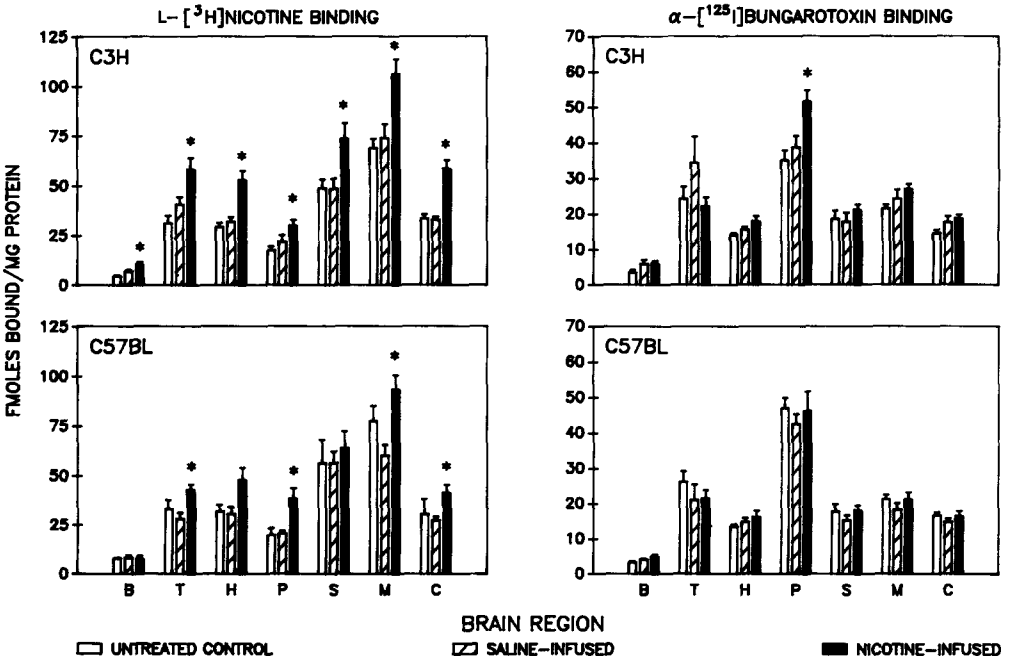

FIG. 1. The effect of no treatment (control), saline, or nicotine infusion during the last half of pregnancy on maternal L-[^{3}H]nicotine and α-[^{125}I]bungarotoxin binding in C3H and C57BL females in seven brain regions: cerebellum (B), hypothalamus (T), hindbrain (H), hippocampus (P), striatum (S), midbrain (M), and cortex (C). Each bar represents the mean ± SEM of four to nine animals. Significant treatment effects are indicated with asterisks (*$p < .05$).

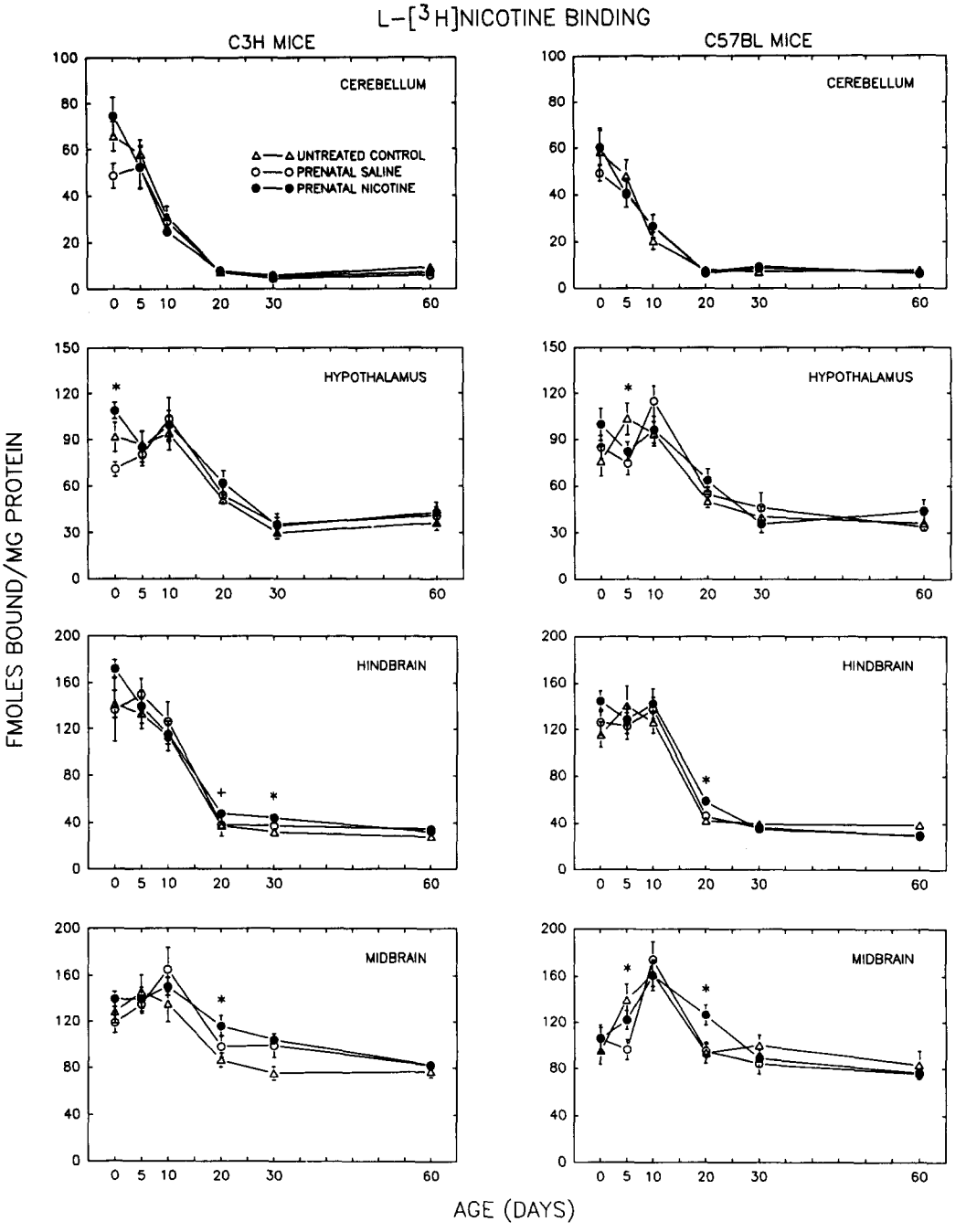

FIG. 2. The effect of no treatment (control), saline, or nicotine infusion in utero on the development of the L-[^{3}H]nicotine binding site in the cerebellum, hypothalamus, hindbrain, and midbrain of C3H and C57BL offspring. Each point represents the mean ± SEM of 6–14 samples. Significant treatment effects are indicated with asterisks (*$p < .05$). Tendencies toward significance are indicated with pluses (+$p < .07$).

in K_D were observed at any day of age in either mouse strain (Table 1). However, the total number of binding sites (B_{max}) was significantly increased in the neonatal nicotine-treated C57BL offspring, $F(2, 15) = 13.77$. In addition, there was a tendency toward increased B_{max} in the neonatal nicotine-treated C3H offspring, $F(2, 9) = 3.36$, $p = .081$. There were no other significant effects of treatment on B_{max} at any other age in either mouse strain.

At 5 days of age, both saline- and nicotine-infused C57BL offspring displayed decreased nicotine binding as compared to untreated control offspring in the hypothalamus, $F(2, 27) = 3.35$, and striatum, $F(2, 27) = 6.07$. In the midbrain, sa-

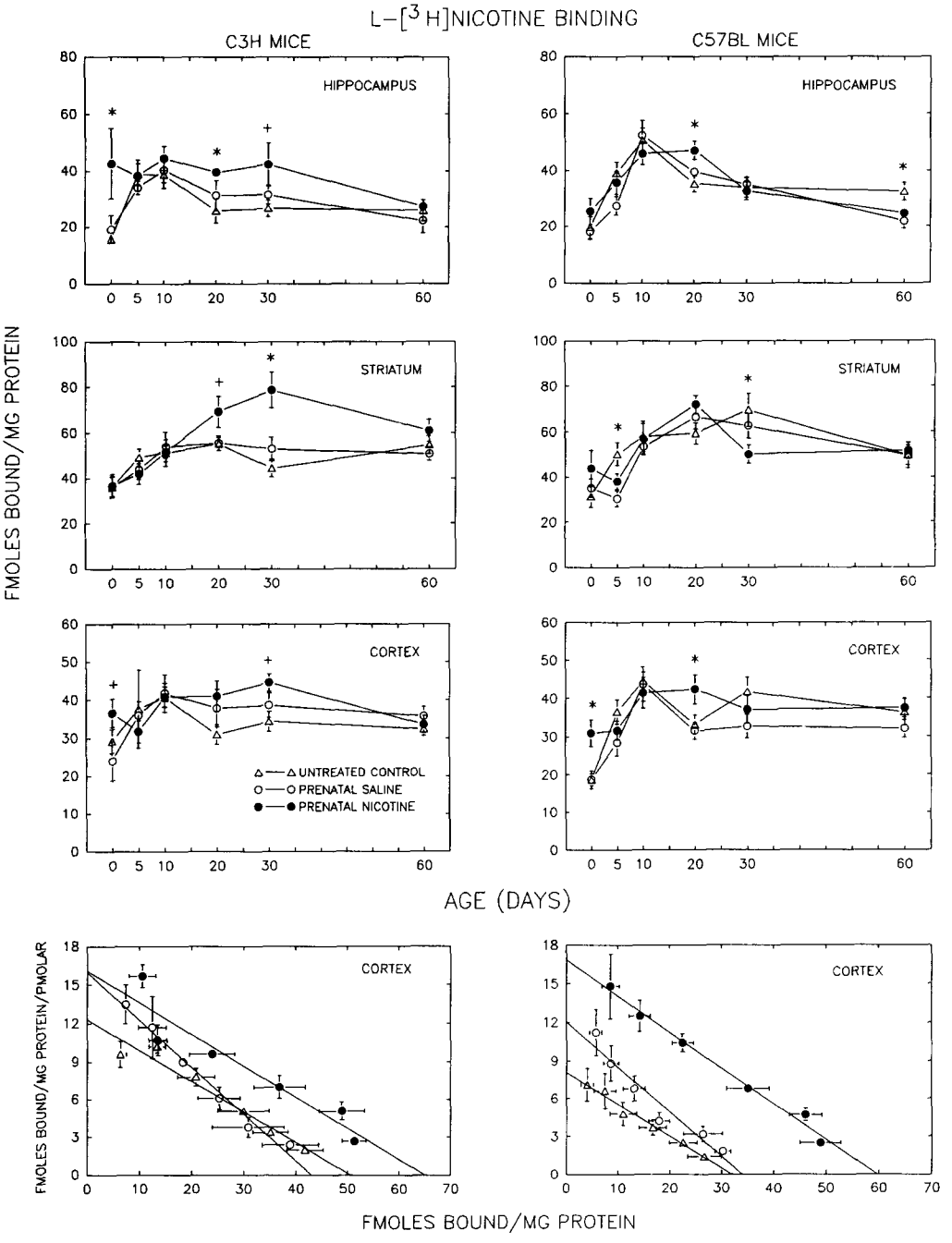

FIG. 3. The effect of no treatment (control), saline, or nicotine infusion in utero on the development of the L-[^{3}H]nicotine binding site in the hippocampus, striatum, and cortex of C3H and C57BL offspring. Each point represents the mean ± SEM of 6–14 samples. Significant treatment effects are indicated with asterisks (*$p < .05$). Tendencies toward significance are indicated with pluses ($+p < .07$). Sample Scatchard plots from cortices of day 0 animals are presented in the lower two figures. Each point represents the mean +/− SEM of 3–6 samples.

line-treated offspring had significantly less binding than either nicotine-treated or untreated offspring, $F(2, 27) = 4.15$.

At 20 days of age, significant treatment effects were observed in several brain regions in the nicotine-treated offspring of both mouse strains. C3H offspring treated prenatally with nicotine exhibited significantly higher levels of binding in the hippocampus, $F(2, 23) = 22.66$, and midbrain, $F(2, 23) = 3.42$, as well as similar trends in the striatum, $F(2, 23) = 3.18$, $p = .060$, and cortex, $F(2, 23) = 3.32$, $p = .054$. Nicotine-treated C57BL offspring displayed significant increases

TABLE 1

EFFECT OF MATERNAL NICOTINE TREATMENT ON CORTICAL [^{3}H]NICOTINE BINDING PARAMETERS (K_D AND B_{max}) IN OFFSPRING

	Control		Saline		Nicotine	
	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}
C3H						
Day 0	3.5 ± 0.4	46.3 ± 4.7	2.4 ± 0.1	40.7 ± 6.3	3.6 ± 0.4	61.1 ± 1.8
Day 5	3.6 ± 0.7	60.0 ± 5.1	3.8 ± 0.4	65.5 ± 8.9	2.4 ± 0.1	53.2 ± 4.5
Day 10	3.3 ± 1.0	61.9 ± 5.9	3.1 ± 0.1	68.8 ± 4.6	3.4 ± 0.6	65.1 ± 2.1
Day 20	3.8 ± 0.2	57.2 ± 4.6	4.6 ± 1.1	68.3 ± 15.1	3.5 ± 0.7	66.4 ± 5.7
Day 30	4.1 ± 1.1	69.6 ± 14.9	3.8 ± 1.2	73.8 ± 7.2	3.9 ± 0.4	69.4 ± 6.5
Day 60	4.2 ± 0.6	63.1 ± 5.2	2.8 ± 0.4	60.5 ± 3.3	4.4 ± 1.1	55.2 ± 4.5
C57						
Day 0	2.9 ± 0.4	32.6 ± 3.4	2.5 ± 0.5	31.0 ± 3.4	2.9 ± 0.2	53.6 ± 3.4
Day 5	2.8 ± 0.2	52.2 ± 7.1	2.9 ± 0.6	56.4 ± 7.6	3.6 ± 0.5	63.2 ± 4.3
Day 10	2.8 ± 0.4	66.0 ± 5.3	2.5 ± 0.2	60.1 ± 6.2	3.4 ± 0.7	73.9 ± 8.9
Day 20	4.5 ± 0.4	65.4 ± 4.2	4.7 ± 0.5	56.2 ± 4.5	3.7 ± 0.7	66.8 ± 3.9
Day 30	3.8 ± 0.6	74.9 ± 5.1	5.9 ± 2.3	67.8 ± 11.6	5.1 ± 0.9	60.8 ± 5.4
Day 60	3.0 ± 0.6	59.4 ± 6.5	2.8 ± 0.3	55.5 ± 5.2	3.4 ± 0.3	66.9 ± 7.5

Pregnant animals were infused with saline or 2.0 mg/kg/h nicotine, or remained untreated controls for the second half of gestation. Offspring were sacrificed postnatally at 0, 5, 10, 20, 30, or 60 days, and their cortices used for [^{3}H]nicotine binding assays. K_D values are nM. B_{max} values are fmol/mg protein. Values are means ± SEM of three to six separate determinations.

in nicotine binding in the hindbrain, $F(2, 28) = 5.25$; hippocampus, $F(2, 28) = 5.26$; midbrain, $F(2, 28) = 5.28$; and cortex, $F(2, 28) = 4.14$.

Similar effects were observed at 30 days of age in the prenatally nicotine-treated C3H offspring. Significant increases in nicotine binding were observed in the hindbrain, $F(2, 25) = 3.38$, and striatum, $F(2, 25) = 9.23$, while similar tendencies were seen in the hippocampus, $F(2, 25) = 3.08$, $p = .063$, and cortex, $F(2, 25) = 3.20$, $p = .057$. Nicotine-treated C57BL offspring, however, did not differ from controls in any brain region with the exception of the striatum, where they displayed a significant decrease in the level of nicotine binding sites at this age, $F(2, 24) = 3.52$.

In the adult C57BL offspring prenatally treated with nicotine or saline, significant decreases in hippocampal binding were observed when compared to untreated control offspring, $F(2, 25) = 4.75$.

The effects of chronic nicotine treatment represent changes in the number of L-[^{3}H]nicotine binding sites (Table 1). Changes in binding were due to changes in B_{max}. K_D values were not affected by prenatal nicotine treatment.

α-[^{125}I]Bungarotoxin Binding

The effect of chronic saline and nicotine treatment on the number of maternal α-[^{125}I]bungarotoxin binding sites in seven brain regions is presented in Fig. 1 in the right two panels. In general, no strain differences in bungarotoxin binding were observed, although C3H mice had significantly higher levels of binding in the midbrain than C57BL mice, $F(1, 44) = 8.79$.

The dramatic treatment effect observed for L-[^{3}H]nicotine binding was not seen for α-[^{125}I]bungarotoxin binding. An overall effect of treatment was seen for C3H mice in the hindbrain, $F(2, 26) = 3.59$; hippocampus, $F(2, 26) = 8.98$; midbrain, $F(2, 26) = 4.06$; and cortex, $F(2, 26) = 3.78$, but post hoc analyses indicated a significant effect of nicotine infusion only in the hippocampus. No effect of treatment was observed in any brain region in the C57BL mice. In the Scatchard plots generated in the cortex, a similar lack of effect was seen, with no changes in K_D or B_{max} observed in either mouse strain.

The development of α-[^{125}I]bungarotoxin binding in each of seven brain regions is presented for C3H and C57BL untreated (control) offspring and offspring treated in utero with saline or nicotine in Figs. 4 and 5. There was a significant overall strain effect, $F(1, 229) = 5.75$, in the three-way ANOVA in midbrain binding, with C57BL mice exhibiting overall lower levels of binding than C3H mice. Again, strong main effects of day of age were observed for every brain region, cerebellum, $F(5, 296) = 46.37$; hypothalamus, $F(5, 298) = 41.73$; hindbrain, $F(5, 298) = 179.05$; hippocampus, $F(5, 298) = 125.53$; striatum, $F(5, 299) = 52.26$; midbrain, $F(5, 299) = 175.39$; cortex, $F(5, 299) = 118.62$, with Strain × Day of Age interaction terms significant in the hypothalamus, $F(5, 298) = 2.44$; hippocampus, $F(5, 298) = 8.11$; striatum, $F(5, 299) = 11.17$; midbrain, $F(5, 299) = 2.32$; and cortex, $F(5, 299) = 3.45$. Again, the age effects fell into three categories: regions which displayed extremely high levels of binding at birth and then declined rapidly to adult levels (cerebellum and hindbrain); regions exhibiting higher than adult levels at birth which increase and peak at 10 days of age, then decrease to adult level (midbrain and cortex); and regions with adult binding levels at birth, climbing rapidly to a peak level at around 10 days of age and then declining to adult levels (hypothalamus, hippocampus, and striatum). In the latter two categories, C57BL offspring displayed peak binding levels which continued longer than C3H offspring. No significant overall effects of treatment were observed in any brain region; however, a significant Strain × Treatment interaction term was obtained in the cortex, $F(2, 229) = 3.39$.

When the data were sorted by strain and day of age, 10-day-old C3H offspring treated prenatally with nicotine displayed significantly lower levels of bungarotoxin binding in the cerebellum, $F(2, 30) = 4.54$, whereas 20-day-old C57BL

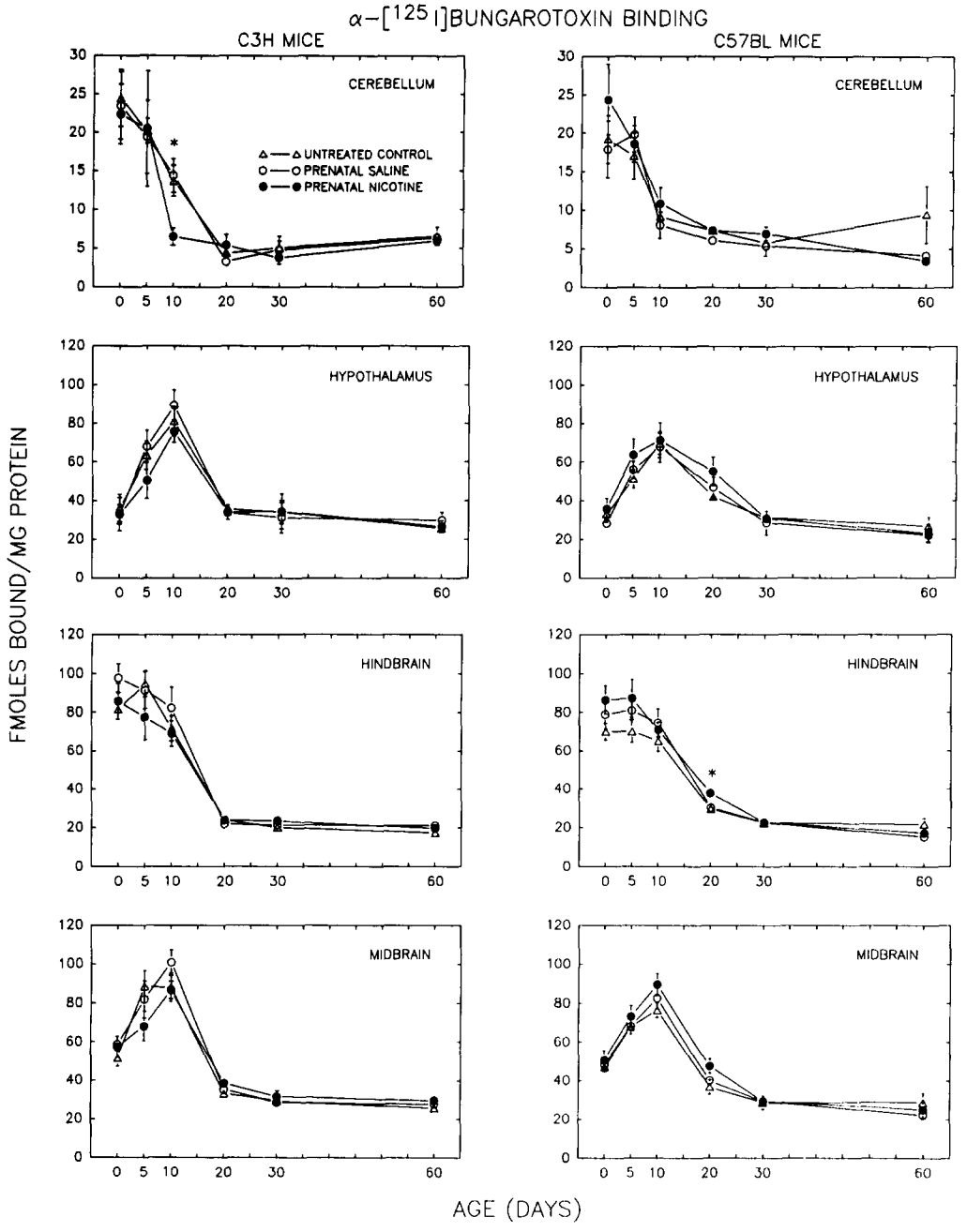

FIG. 4. The effect of no treatment (control), saline, or nicotine infusion in utero on the development of the α-[^{125}I]bungarotoxin binding site in the cerebellum, hypothalamus, hindbrain, and midbrain of C3H and C57BL offspring. Each point represents the mean ± SEM of 6–14 samples. Significant treatment effects are indicated with asterisks (*$p < .05$).

offspring treated in utero with nicotine exhibited significantly higher binding in the hindbrain, $F(2, 26) = 6.00$.

In the Scatchard plots generated in the cortex, significant interaction terms, Strain × Treatment, $F(2, 121) = 3.16$, and Day of Age × Treatment, $F(10, 121) = 2.42$, were observed in the three-way ANOVA for the K_D. When the data had been sorted by strain and day of age, the only significant effect of treatment on K_D was seen in neonatal C3H offspring, where nicotine treatment significantly decreased the K_D, $F(2, 8) = 4.86$. No overall effects of strain or treatment were observed for B_{max} in the three-way ANOVA; however, when sorted by strain and day of age, neonatal nicotine-treated C57BL mice exhibited an increase in B_{max}, $F(2, 10) = 4.60$. These data are summarized in Table 2.

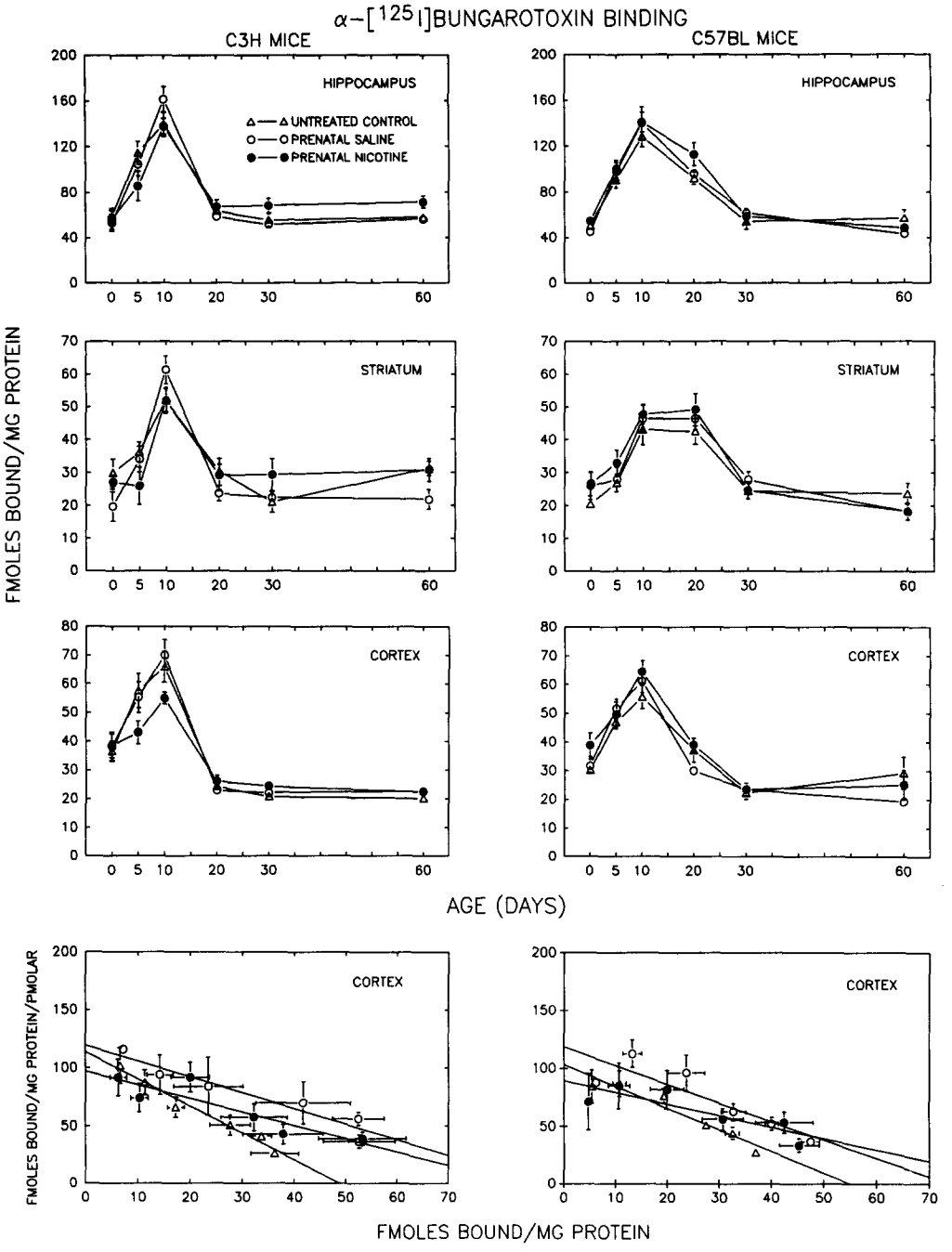

FIG. 5. The effect of no treatment (control), saline, or nicotine infusion in utero on the development of the α-[^{125}I]bungarotoxin binding site in the hippocampus, striatum, and cortex of C3H and C57BL offspring. Each point represents the mean ± SEM of 6–14 samples. No significant treatment effects were observed. Sample Scatchard plots from cortices of day 0 animals are presented in the lower two figures. Each point represents the mean ± SEM of two to six samples.

Selectivity of Drug Effect

The data reported to this point suggest that the L-[^{3}H]-nicotine binding site was affected by prenatal nicotine treatment in some brain regions, whereas the α-[^{125}I]bungarotoxin site was not. A two-way ANOVA of the data analyzed across days, separately by strain, detected significant ligand-treatment interactions for the C3H strain, but these effects were seen only in the striatum, $F(2, 315) = 4.25$, and cortex, $F(2, 316) = 3.14$. The interaction term obtained for

TABLE 2

EFFECT OF MATERNAL NICOTINE TREATMENT ON α-[^{125}I]BUNGAROTOXIN BINDING PARAMETERS (K_D AND B_{max}) IN OFFSPRING

	Control		Saline		Nicotine	
	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}
C3H						
Day 0	0.4 ± 0.1	47.4 ± 5.8	0.6 ± 0.1	73.7 ± 8.2	0.9 ± 0.2	80.9 ± 15.5
Day 5	0.5 ± 0.1	127.3 ± 21.8	0.7 ± 0.1	126.7 ± 32.2	0.4 ± 0.1	68.9 ± 7.7
Day 10	0.6 ± 0.1	129.5 ± 12.1	0.4 ± 0.0	114.6 ± 13.4	0.4 ± 0.1	95.1 ± 7.4
Day 20	0.7 ± 0.0	48.1 ± 2.1	0.5 ± 0.1	44.1 ± 7.2	0.8 ± 0.1	56.4 ± 8.8
Day 30	0.6 ± 0.1	39.0 ± 3.8	0.7 ± 0.2	42.4 ± 5.3	0.5 ± 0.1	42.1 ± 5.3
Day 60	0.7 ± 0.1	45.4 ± 4.4	0.7 ± 0.2	45.5 ± 4.9	0.4 ± 0.0	32.8 ± 3.3
C57BL						
Day 0	0.4 ± 0.1	48.2 ± 1.1	0.5 ± 0.1	58.9 ± 4.9	0.8 ± 0.2	69.5 ± 6.3
Day 5	0.4 ± 0.1	74.9 ± 7.2	0.7 ± 0.2	109.9 ± 13.4	0.7 ± 0.1	101.3 ± 19.7
Day 10	0.4 ± 0.1	107.2 ± 19.3	0.5 ± 0.0	124.3 ± 17.7	0.6 ± 0.1	114.9 ± 11.4
Day 20	0.5 ± 0.1	61.3 ± 6.8	0.8 ± 0.1	61.0 ± 2.8	0.6 ± 0.1	84.5 ± 9.4
Day 30	0.4 ± 0.1	43.3 ± 6.1	0.6 ± 0.2	50.2 ± 8.1	0.5 ± 0.1	46.9 ± 4.2
Day 60	0.6 ± 0.1	47.9 ± 7.7	0.8 ± 0.1	41.3 ± 5.9	0.7 ± 0.1	41.0 ± 3.2

Pregnant animals were infused with saline or 2.0 mg/kg/h nicotine, or remained untreated controls for the second half of gestation. Offspring were sacrificed postnatally at 0, 5, 10, 20, 30, or 60 days, and their cortices used for α-[^{125}I]bungarotoxin binding assays. K_D values are nM. B_{max} values are fmol/mg protein. Values are mean ± SEM of two to six separate determinations.

the C3H midbrain just missed significance, $F(2, 316) = 2.50$, $p = 0.08$.

DISCUSSION

Consistent with the observations of previous studies (3,7-9,26,29,30,31,45,46), chronic nicotine treatment resulted in an increase in the number of [^{3}H]nicotine binding sites in the dams. Although the increases in L-[^{3}H]nicotine binding observed in maternal C3H mice in the present study are similar in magnitude to those described in earlier studies, the maternal C57BL mice displayed smaller increases in binding than those reported previously, possibly due to differences in the route of administration or induction of nicotine-metabolizing enzymes during pregnancy. In either case, larger doses may be required to achieve receptor increases of the same magnitude in the C57BL mice.

Maternal α-[^{125}I]bungarotoxin binding was virtually unchanged after chronic nicotine infusion in the present study, except for an increase in the level of binding in the hippocampus of C3H mice, replicating a similar finding by Collins and Marks (8). Other studies also indicate that chronic infusion of higher doses of nicotine are required to produce a change in the number of α-[^{125}I]bungarotoxin binding sites (26,27).

Chronic nicotine infusion appeared to disrupt the ontogeny of the [^{3}H]nicotine binding site. As in earlier studies in the rat (49), elevated binding was seen at birth in many brain regions. This effect quickly disappeared. At later time points, ages 20 to 30 days, [^{3}H]nicotine binding was increased in many of the brain regions (hindbrain, hippocampus, striatum, midbrain, and cortex) of the C3H offspring prenatally treated with nicotine. Nicotine-treated C57BL offspring exhibited similar increases at the later ages (20-30 days) in the hindbrain, hippocampus, midbrain, and cortex. In the rat, prolonged elevated levels are seen only in the cerebellum (49). The rat-mouse difference may be due to species differences, but we suspect that the more widespread changes seen in our study reflect the higher doses of nicotine used.

In the mouse, the ontogeny of the nicotinic receptor appears to vary by brain region. Our data, which agree with two earlier studies using the mouse (16,21), indicate that some regions display high levels of binding at birth and then decline rapidly to adult levels (cerebellum, hypothalamus, and hindbrain, which begins decreasing earlier in C3H than in C57BL mice); other regions exhibited lower than adult levels at birth, and then slowly climbed throughout development to reach adult levels (striatum and cortex); and other regions showed adult binding levels at birth, which increased rapidly to a peak level at around 10 days of age and then declined to adult levels (hippocampus, which peaks higher in C57BL mice than in C^{3}H mice, and C57BL midbrain). The type of developmental profile did not, however, serve as any predictor of the increased binding seen at 20-30 days of age.

The development of the [^{3}H]nicotine binding site in the rat is radically different in that a gradual rise in the number of binding sites is seen, with adult levels attained on postnatal days 28-30 (48,49,53). These results indicate that there is a species difference in the developmental profile of the [^{3}H]nicotine binding site. Species differences might explain why we saw elevated levels of [^{3}H]nicotine binding at 20-30 days in different brain regions than were seen in the rat.

Prenatal nicotine treatment seemed to have little effect on α-[^{125}I]bungarotoxin binding. The ANOVA generally did not detect significant treatment effects on α-[^{125}I]bungarotoxin binding, whereas significant treatment effects were seen for the L-[^{3}H]nicotine binding site in several brain regions. This argues that the L-[^{3}H]nicotine binding site may be uniquely sensitive to nicotine. The two-way ANOVA (Ligand × Treatment) of the data, obtained from the two strains analyzed separately, detected significant interactions only in the C3H strain and only in the striatum and cortex; the midbrain showed a trend. Thus, the C3H strain may be more sensitive

to prenatal nicotine effects on the [^{3}H]nicotine binding site. The failure to detect a significant interaction term in most of the brain regions presumably arose because nicotine treatment did not produce a change in the development of either ligand. There were, however, some modest strain differences in response to nicotine. Cerebellar levels of binding decreased more quickly in the nicotine-treated C3H offspring, achieving adult levels at an earlier age. C57BL offspring treated with nicotine, on the other hand, displayed increased toxin binding in the hindbrain at 20 days of age.

The developmental profile for mouse α-[^{125}I]bungarotoxin binding reported here is very similar to that reported by Falkeborn et al. (15) and Fiedler et al. (16). The two strains differed slightly in the onset of the decrease in binding in some brain regions, with C57BL mice maintaining the peak binding levels longer than the C3H mice.

The apparent increase in the number of nicotinic acetylcholine receptors at birth in some brain regions may represent a true upregulation of the receptor. However, the mechanism for the increases in binding observed at later ages in several brain regions is probably not the same as that seen in adults treated chronically with nicotine. Marks et al. (29) have demonstrated that [^{3}H]nicotine binding returns to control levels in the adult mouse brain in approximately 7 days following termination of nicotine infusion. Thus, the elevated levels of binding seen in the offspring 20–30 days after parturition are most probably due to a teratological effect of nicotine on development. If this is the case, the C3H strain may be slightly more sensitive to teratological actions of nicotine.

In neural tissue, there appears to be a relationship between the rapid loss of toxin binding sites and the maturation of cholinergic synapses, resulting in the suggestion that nicotinic receptors may guide incoming fibers for successful synaptogenesis (17,51). In mice, the decrease in α-[^{125}I]bungarotoxin binding coincides with the onset of EEG activity (20). A sharp rise in choline acetylcholinesterase in the rat striatum (a measure of cholinergic innervation) occurs at the same time as the peak in toxin binding (10). Thus, the loss of receptors appears to follow functional innervation.

If receptor formation is important in synaptogenesis, prenatal nicotine exposure could perturb this process. Choline acetyltransferase and choline uptake activities peak at the time of synaptogenesis in the rat (37). Prenatal nicotine treatment disrupted the development of these activities, and it was speculated that there may have been premature stimulation of some endogenous maturational signal. An early switchover from neurogenesis to differentiation would result in a deficit in brain cell number, and this effect has been observed in the cortices of rats treated prenatally with nicotine (50).

In conclusion, prenatal nicotine treatment altered the developmental profiles of [^{3}H]nicotine but not α-[^{125}I]bungarotoxin binding sites. Subtle differences in the developmental profiles that exist among the two inbred strains of mice and subtler differences between the strains in nicotine effects suggest that genetic factors regulate the maturation of the nicotinic receptor. This is important, since not all children whose mothers smoked during pregnancy are measurably affected.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Institute on Drug Abuse (DA-03194). A.C.C. is supported, in part, by a Research Scientist Development Award from NIDA (DA-00116) and J.L.V. was supported by a training grant from the National Institute on Child Health and Human Development (HD-07289).

REFERENCES

1. Abood, L. G.; Grassi, S. [^{3}H]Methylcarbamylcholine, a new radioligand for studying brain nicotinic receptors. Biochem. Pharmacol. 5:4199–4202; 1986.
2. Benwell, M. E. M.; Balfour, D. J. K.; Anderson, J. M. Evidence that tobacco smoking increases the density of (−)-[^{3}H]nicotine binding sites in human brain. J. Neurochem. 50:1243–1247; 1988.
3. Bhat, R. V.; Turner, S. L.; Selvaag, S. R.; Marks, M. J.; Collins, A. C. Regulation of brain nicotinic receptors by chronic agonist infusion. J. Neurochem. 56:1932–1939; 1991.
4. Bureau, M. A.; Monette, J.; Shapcott, D.; Pare, C.; Mathieu, J. L.; Lippe, J.; Blovin, D.; Berthiaume, Y.; Begin, R. Carboxyhemoglobin concentration in fetal cord blood and in blood of mothers who smoked during labor. Pediatrics 69:371–373; 1982.
5. Butler, N. R.; Goldstein, H. Smoking in pregnancy and subsequent child development. Br. Med. J. 4:573–575; 1973.
6. Clarke, P. B. S.; Schwartz, R. D.; Paul, S. M.; Port, C. B.; Pert, A. Nicotinic binding in rat brain: Autoradiographic comparison of [^{3}H]acetylcholine, [^{3}H]nicotine, and [^{125}I]α-bungarotoxin. J. Neurosci. 5:1307–1315; 1985.
7. Collins, A. C.; Marks, M. J. The effects of chronic nicotine administration on brain nicotinic receptor numbers. In: Martin, W. R.; Van Loon, G. R.; Iwamoto, E. T.; Davis, L., eds. Tobacco, smoking, and nicotine. New York: Plenum Press; 1987: 439–450.
8. Collins, A. C.; Marks, M. J. Progress towards the development of animal models of smoking-related behaviors. J. Addict. Disorders 10:109–126; 1991.
9. Collins, A. C.; Miner, L. L.; Marks, M. J. Genetic influences on acute responses to nicotine and nicotine tolerance in the mouse. Pharmacol. Biochem. Behav. 30:269–278; 1988.
10. Coyle, J. T.; Campochiaro, P. Ontogenesis of dopaminergic-cholinergic interactions in the rat striatum: A neurochemical study. J. Neurochem. 27:673–678; 1976.
11. Davie, R.; Butler, N.; Goldstein, H. From birth to seven: The second report of the National Child Development Study (1958 cohort). London: William Clowes & Sons; 1972.
12. Davies, D. P.; Gray, O. P.; Ellwood, P. C.; Abernethy, M. Cigarette smoking in pregnancy: Associations with maternal weight gain and fetal growth. Lancet 1:385–387; 1976.
13. Denson, R.; Nanson, J. L.; McWalters, M. A. Hyperkinesis and maternal smoking. Can. Psychiatr. Assoc. J. 20:183–187; 1975.
14. Dunn, H. G.; McBurney, A. K.; Ingram, S. Maternal cigarette smoking during pregnancy and the child's subsequent development: II. Neurological and intellectual maturation to the age of 6 1/2 years. Can. J. Public Health 68:43–50; 1977.
15. Falkeborn, Y.; Larsson, C.; Nordberg, A.; Slanina, P. A comparison of the regional ontogenesis of nicotine- and muscarine-like binding sites in mouse brain. Int. J. Dev. Neurosci. 1:289–296; 1983.
16. Fiedler, E. P.; Marks, M. J.; Collins, A. C. Postnatal development of two nicotinic cholinergic receptors in seven mouse brain regions. Int. J. Dev. Neurosci. 8:533–540; 1990.
17. Freeman, J. A.; Lutin, W. A. Use of α-bungarotoxin (α-BT) in the histochemical and electrophysiological identification of cholinergic synapses in the optic tectum of the toad *Bufo marinus*. Trans. Am. Neurochem. Soc. 6:143; 1975.
18. Hagino, N.; Lee, J. W. Effect of maternal nicotine on the development of sites for [^{3}H]-nicotine binding in the fetal brain. Int. J. Dev. Neurosci. 3:567–571; 1985.
19. Heron, H. J. The effects of smoking during pregnancy: A review with a preview. North Am. Med. J. 61:543–548; 1962.
20. Kobayashi, J.; Inman, O.; Buno, W.; Himwich, H. E. A multi-

disciplinary study of changes in mouse brain with age. Rec. Adv. Psychiatry 5:239–298; 1963.
21. Larsson, C.; Nordberg, A.; Falkeborn, Y.; Lundberg, P. A. Regional [^{3}H]-acetylcholine and [^{3}H]-nicotine binding in developing mouse brain. Int. J. Dev. Neurosci. 3:667–671; 1985.
22. Longo, L. D. Carbon monoxide effects on oxygenation of the fetus *in utero*. Science 194:523–525; 1976.
23. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
24. Luck, W.; Nau, H. Exposure of the fetus, neonate, and nursed infant to nicotine and cotinine from maternal smoking. N. Engl. J. Med. 311:672; 1984.
25. Manning, F. A.; Feyerabend, C. Cigarette smoking and fetal breathing movements. Br. J. Obstet. Gynecol. 83:262–270; 1976.
26. Marks, M. J.; Burch, J. B.; Collins, A. C. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. J. Pharmacol. Exp. Ther. 226:817–825; 1983.
27. Marks, M. J.; Campbell, S. M.; Romm, E.; Collins, A. C. Genotype influences the development of tolerance to nicotine in the mouse. J. Pharmacol. Exp. Ther. 259:392–402; 1991.
28. Marks, M. J.; Collins, A. C. Characterization of nicotine binding in mouse brain and comparison with the binding of α-bungarotoxin and quinuclidinyl benzilate. Mol. Pharmacol. 22: 554–564; 1982.
29. Marks, M. J.; Stitzel, J. A.; Collins, A. C. Time course study of the effects of chronic nicotine infusion on drug response and brain receptors. J. Pharmacol. Exp. Ther. 235:619–628; 1985.
30. Marks, M. J.; Stitzel, J. A.; Collins, A. C. Dose-response analysis of nicotine tolerance and receptor changes in two inbred mouse strains. J. Pharmacol. Exp. Ther. 239:358–364; 1986.
31. Marks, M. J.; Stitzel, J. A.; Collins, A. C. Influence of kinetics of nicotine administration on tolerance development and receptor levels. Pharmacol. Biochem. Behav. 27:505–512; 1987.
32. Marks, M. J.; Stitzel, J. A.; Collins, A. C. Genetic influences on nicotine responses. Pharmacol. Biochem. Behav. 33:667–678; 1988.
33. Marks, M. J.; Stitzel, J. A.; Romm, E.; Wehner, J. M.; Collins, A. C. Nicotinic binding sites in rat and mouse brain: Comparison of acetylcholine, nicotine, and α-bungarotoxin. Mol. Pharmacol. 30:427–436; 1986.
34. Mochizuki, M.; Maruo, T.; Masuko, K.; Ohtsu, T. Effects of smoking on the fetoplacental-maternal system during pregnancy. Am. J. Obstet. Gynecol. 149:413–420; 1984.
35. Naeye, R. L.; Peters, E. C. Mental development of children whose mothers smoked during pregnancy. Obstet. Gynecol. 64: 601–607; 1984.
36. Nasrat, H. A.; Al-Hachim, G. M.; Mahmoud, F. A. Perinatal effects of nicotine. Biol. Neonate 49:8–14; 1986.
37. Navarro, H. A.; Seidler, F. J.; Schwartz, R. D.; Baker, F. E.; Dobbins, S. S.; Slotkin, T. A. Prenatal exposure to nicotine impairs nervous system development at a dose which does not affect viability or growth. Brain Res. Bull. 23:187–192; 1989.
38. Pabreza, L. A.; Dhawan, S.; Kellar, K. J. [^{3}H]Cytisine binding to nicotinic cholinergic receptors in brain. Mol. Pharmacol. 39: 9–12; 1991.
39. Petersen, D. R.; Norris, K. J.; Thompson, J. A. A comparative study of the disposition of nicotine and its metabolites in three inbred strains of mice. Drug Metab. Dispos. 6:725–731; 1984.
40. Philipp, K.; Pateisky, N.; Endler, M. Effects of smoking on uteroplacental blood flow. Gynecol. Obstet. Invest. 17:179–182; 1984.
41. Plowchalk, D. R.; deBethizy, J. D. Interspecies scaling of nicotine concentrations in the brain: A physiologically based pharmakinetic study. In: Lippiello, P. M.; Collins, A. C.; Gray, J. A.; Robinson, J. H., eds. Biology of nicotine: Current research issues. New York: Raven Press; 1992:55–70.
42. Romano, C.; Goldstein, A. Stereospecific nicotine receptors on rat brain membranes. Science 210:647–649; 1980.
43. Romm, E.; Lippiello, P. M.; Marks, M. J.; Collins, A. C. Purification of L-[^{3}H]nicotine eliminates low affinity binding. Life Sci. 46:935–943; 1990.
44. Rush, D. Examination of the relationship between birth weight, cigarette smoking during pregnancy and maternal weight gain. J. Obstet. Gynecol. Br. Commonwealth 81:746–752; 1974.
45. Schwartz, R. D.; Kellar, K. J. Nicotinic cholinergic receptor binding sites in the brain: Regulation in vivo. Science 228:214–216; 1983.
46. Schwartz, R. D.; Kellar, K. J. In vivo regulation [^{3}H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs. J. Neurochem. 45:427–433; 1985.
47. Schwartz, R. D.; McGee, R., Jr.; Kellar, K. J. Nicotinic cholinergic receptors labeled by (^{3}H)-acetylcholine in rat brain. Mol. Pharmacol. 22:56–62; 1982.
48. Sershen, H.; Reith, M. E. A.; Banay-Schwartz, M.; Lajtha, A. Effects of prenatal administration of nicotine on amino acid pools, protein metabolism, and nicotine binding in the brain. Neurochem. Res. 7:1515–1522; 1982.
49. Slotkin, T. A.; Orband-Miller, L.; Queen, K. L. Development of [^{3}H]-nicotine binding sites in brain regions of rats exposed to nicotine prenatally via maternal injections or infusions. J. Pharmacol. Exp. Ther. 242:232–237; 1987.
50. Slotkin, T. A.; Orband-Miller, L.; Queen, K. L.; Whitmore, W. L.; Seidler, F. J. Effects of prenatal nicotine exposure on biochemical development of rat brain regions: Maternal drug infusions via osmotic mini-pumps. J. Pharmacol. Exp. Ther. 240: 602–611; 1987.
51. Sytkowski, A. J.; Vogel, Z.; Nirenberg, M. Development of acetylcholine receptor clusters on cultured muscle cells. Proc. Natl. Acad. Sci. U. S. A. 70:270–274; 1973.
52. Wonnacott, S. The paradox of nicotinic acetylcholine receptor upregulation by nicotine. Trends Pharmacol. Sci. 11:216–219; 1990.
53. Yamada, S.; Kagawa, Y.; Isogai, M.; Takayanagi, N.; Hayashi, E. Ontogenesis of nicotinic acetylcholine receptors and presynaptic cholinergic neurons in mammalian brain. Life Sci. 38:637–644; 1986.