SUPPLEMENT

Developmental Neurotoxicity of Tobacco Smoke Directed Toward Cholinergic and Serotonergic Systems: More than Just Nicotine

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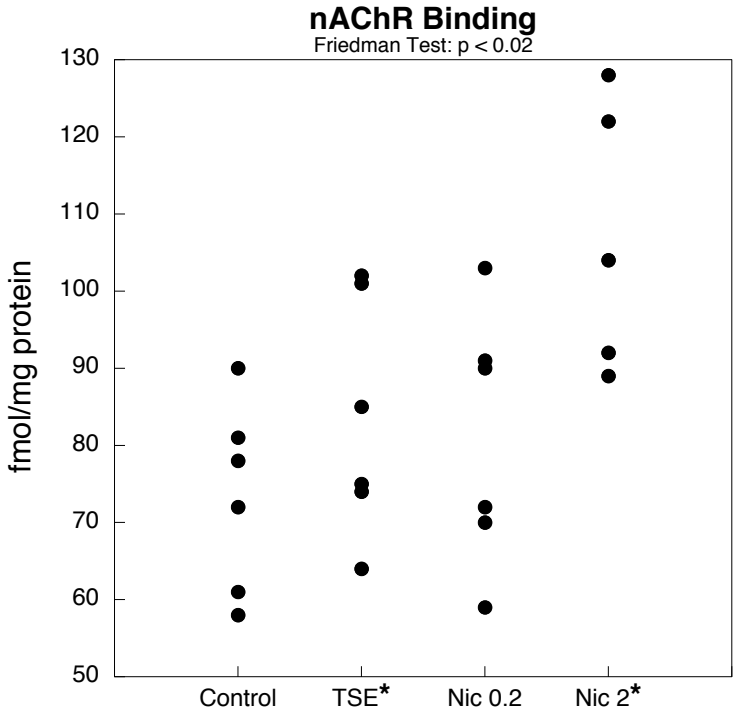

FIGURE S1

Non-pregnant, adult female rats were implanted with minipumps delivering TSE, nicotine (Nic) 0.2 mg/kg/day or nicotine 2 mg/kg/day, and cerebrocortical nAChR binding was evaluated after four weeks. Statistical comparisons were conducted non-parametrically because the distribution of values in each group was non-Gaussian (no central cluster). The Friedman test was conducted by matching the highest value in each group, then the next highest, then the third highest, etc. Asterisks denote treatment groups that differ significantly from control (post-hoc Wilcoxon signed-rank test). There was no significant difference between TSE and Nic 0.2.

TABLE S1: Body and Brain Region Weights (mean ± SE)

	Age	Male				Female			
		Control	TSE	Nic 0.2	Nic 2	Control	TSE	Nic 0.2	Nic 2
Body Weight (g)	30	111 ± 3	110 ± 3	115 ± 4	112 ± 2	104 ± 1	102 ± 4	100 ± 2	99 ± 1
	60	376 ± 11	374 ± 11	364 ± 13	381 ± 9	243 ± 1	246 ± 6	234 ± 3	230 ± 6
	100	546 ± 11	534 ± 14	553 ± 14	542 ± 9	307 ± 11	296 ± 5	302 ± 9	283 ± 6
	150	654 ± 18	598 ± 8	653 ± 11	647 ± 15	344 ± 8	329 ± 6	336 ± 9	327 ± 3
Region Weight (mg)									
frontal/parietal cortex	30	245 ± 8	247 ± 7	221 ± 8	243 ± 6	236 ± 14	228 ± 3	250 ± 11	234 ± 7
	60	260 ± 6	263 ± 15	278 ± 11	277 ± 12	272 ± 11	252 ± 11	241 ± 11	260 ± 7
	100	288 ± 16	270 ± 12	290 ± 9	276 ± 6	266 ± 11	263 ± 11	273 ± 6	260 ± 14
	150	294 ± 11	275 ± 14	305 ± 9	276 ± 5	280 ± 11	269 ± 14	268 ± 11	264 ± 13
temporal/occipital cortex	30	166 ± 10	173 ± 9	177 ± 9	160 ± 12	173 ± 6	177 ± 15	175 ± 9	187 ± 10
	60	177 ± 16	203 ± 18	184 ± 15	186 ± 8	176 ± 12	179 ± 8	170 ± 7	181 ± 12
	100	196 ± 14	206 ± 7	208 ± 15	204 ± 10	179 ± 8	196 ± 9	177 ± 12	181 ± 10
	150	194 ± 13	183 ± 10	200 ± 8	178 ± 10	173 ± 12	179 ± 5	168 ± 6	173 ± 7
hippocampus	30	112 ± 8	122 ± 5	117 ± 6	127 ± 10	118 ± 7	114 ± 5	110 ± 7	114 ± 5
	60	144 ± 6	144 ± 6	145 ± 9	138 ± 5	134 ± 4	138 ± 4	135 ± 4	135 ± 5
	100	149 ± 5	144 ± 4	147 ± 5	146 ± 6	140 ± 7	142 ± 7	145 ± 7	140 ± 8
	150	153 ± 4	161 ± 3	159 ± 6	149 ± 11	143 ± 8	144 ± 2	139 ± 10	147 ± 8
striatum	30	68 ± 5	73 ± 7	78 ± 8	79 ± 10	75 ± 7	81 ± 9	91 ± 13	63 ± 6
	60	100 ± 7	108 ± 10	92 ± 5	111 ± 8	97 ± 3	92 ± 7	102 ± 9	103 ± 4
	100	124 ± 9	93 ± 6	107 ± 4	103 ± 9	102 ± 8	99 ± 7	100 ± 8	102 ± 10
	150	116 ± 9	122 ± 6	137 ± 6	114 ± 9	103 ± 7	113 ± 6	121 ± 7	125 ± 8
midbrain	30	295 ± 12	283 ± 8	282 ± 5	279 ± 12	276 ± 12	260 ± 14	261 ± 13	287 ± 10
	60	368 ± 10	333 ± 16	358 ± 12	329 ± 9	311 ± 22	324 ± 10	315 ± 15	297 ± 13
	100	336 ± 13	386 ± 20	387 ± 13	362 ± 15	331 ± 6	326 ± 14	322 ± 8	350 ± 24
	150	369 ± 5	366 ± 10	381 ± 7	367 ± 15	341 ± 11	359 ± 11	338 ± 10	362 ± 10
brainstem	30	163 ± 6	173 ± 15	174 ± 6	164 ± 5	156 ± 6	164 ± 8	162 ± 7	173 ± 8
	60	219 ± 5	222 ± 9	221 ± 5	213 ± 8	224 ± 19	212 ± 6	200 ± 10	209 ± 10
	100	255 ± 6	247 ± 17	260 ± 11	250 ± 8	226 ± 10	235 ± 7	231 ± 7	245 ± 6
	150	269 ± 14	274 ± 10	279 ± 9	259 ± 6	241 ± 11	244 ± 6	237 ± 6	254 ± 6

Data represent mean ± SE obtained from six animals in each treatment group for each age and sex. For body weight, three-factor ANOVA (treatment, age, sex) indicates a main effect of treatment ($p < 0.002$) but no interactions of treatment with the other factors. Hence, post-hoc comparisons were not conducted for individual ages or sexes. The TSE group had significantly lower weights compared to all other groups: $p < 0.0003$ vs. control, $p < 0.006$ vs. Nic 0.2, $p < 0.02$ vs. Nic 2. The average deficits were 4.5%, 4% and 3%, respectively.

For brain region weight, four-factor ANOVA (treatment, age, sex, region) indicates no significant treatment effects and no interactions of treatment with the other factors. Note that weights for frontal/parietal cortex and temporal/occipital cortex are for the right hemisphere only.

Abbreviations: Nic 0.2 = Nicotine 0.2 mg/kg/day; Nic 2 = Nicotine 2 mg/kg/day.

TABLE S2: ChAT Activity (mean ± SE)

Region	Postnatal Age	Male (pmol/min per mg protein)				Female (pmol/min per mg protein)			
		Control	TSE	Nic 0.2	Nic 2	Control	TSE	Nic 0.2	Nic 2
frontal/parietal cortex	30	0.81 ± 0.04	0.84 ± 0.04	0.99 ± 0.05	0.87 ± 0.04	0.85 ± 0.02	0.82 ± 0.02	0.94 ± 0.04	0.92 ± 0.06
	60	1.06 ± 0.04	1.24 ± 0.06	1.25 ± 0.02	1.25 ± 0.04	1.08 ± 0.04	1.18 ± 0.06	1.32 ± 0.06	1.24 ± 0.04
	100	0.95 ± 0.04	0.94 ± 0.06	0.96 ± 0.06	0.96 ± 0.04	0.92 ± 0.03	1.01 ± 0.05	0.98 ± 0.06	0.98 ± 0.06
	150	0.82 ± 0.03	0.90 ± 0.05	0.93 ± 0.02	0.91 ± 0.05	0.98 ± 0.03	0.78 ± 0.03	0.96 ± 0.06	0.96 ± 0.06
temporal/occipital cortex	30	0.59 ± 0.03	0.56 ± 0.03	0.64 ± 0.04	0.63 ± 0.02	0.69 ± 0.05	0.64 ± 0.04	0.64 ± 0.05	0.64 ± 0.02
	60	0.67 ± 0.02	0.69 ± 0.05	0.72 ± 0.04	0.64 ± 0.03	0.68 ± 0.03	0.66 ± 0.03	0.71 ± 0.03	0.67 ± 0.03
	100	0.52 ± 0.02	0.55 ± 0.04	0.55 ± 0.03	0.57 ± 0.02	0.58 ± 0.02	0.57 ± 0.03	0.58 ± 0.03	0.59 ± 0.02
	150	0.76 ± 0.02	0.72 ± 0.04	0.80 ± 0.03	0.74 ± 0.03	0.77 ± 0.04	0.72 ± 0.05	0.86 ± 0.05	0.80 ± 0.04
hippocampus	30	0.66 ± 0.05	0.65 ± 0.03	0.65 ± 0.03	0.68 ± 0.03	0.66 ± 0.02	0.65 ± 0.02	0.66 ± 0.01	0.72 ± 0.03
	60	1.10 ± 0.07	1.11 ± 0.04	1.15 ± 0.03	1.19 ± 0.05	1.22 ± 0.07	1.21 ± 0.07	1.29 ± 0.07	1.21 ± 0.02
	100	0.85 ± 0.06	0.93 ± 0.03	0.91 ± 0.04	0.91 ± 0.02	0.86 ± 0.05	0.86 ± 0.04	0.95 ± 0.04	0.96 ± 0.02
	150	0.81 ± 0.05	0.85 ± 0.05	0.91 ± 0.03	0.84 ± 0.03	0.86 ± 0.05	0.88 ± 0.03	0.96 ± 0.04	0.90 ± 0.02
striatum	30	4.05 ± 0.26	4.18 ± 0.07	3.94 ± 0.08	4.54 ± 0.56	4.03 ± 0.72	4.87 ± 0.35	4.12 ± 0.50	4.07 ± 0.31
	60	3.77 ± 0.09	3.44 ± 0.32	3.55 ± 0.25	3.57 ± 0.20	3.85 ± 0.06	3.22 ± 0.19	3.76 ± 0.20	3.87 ± 0.18
	100	2.92 ± 0.19	3.32 ± 0.22	3.62 ± 0.27	3.59 ± 0.33	3.25 ± 0.22	3.08 ± 0.15	3.48 ± 0.22	3.31 ± 0.29
	150	2.96 ± 0.12	2.78 ± 0.12	3.28 ± 0.15	3.20 ± 0.20	3.24 ± 0.23	3.03 ± 0.16	3.65 ± 0.31	3.01 ± 0.15
midbrain	30	0.63 ± 0.03	0.73 ± 0.03	0.66 ± 0.03	0.70 ± 0.04	0.64 ± 0.03	0.75 ± 0.02	0.66 ± 0.03	0.69 ± 0.05
	60	0.67 ± 0.03	0.72 ± 0.01	0.80 ± 0.04	0.76 ± 0.04	0.76 ± 0.02	0.69 ± 0.02	0.79 ± 0.04	0.80 ± 0.04
	100	0.60 ± 0.02	0.65 ± 0.03	0.68 ± 0.05	0.65 ± 0.03	0.69 ± 0.03	0.70 ± 0.05	0.72 ± 0.04	0.72 ± 0.05
	150	0.52 ± 0.03	0.53 ± 0.03	0.55 ± 0.02	0.55 ± 0.03	0.59 ± 0.03	0.51 ± 0.01	0.58 ± 0.04	0.57 ± 0.02
brainstem	30	1.76 ± 0.05	1.72 ± 0.05	1.95 ± 0.03	1.81 ± 0.07	1.88 ± 0.05	1.74 ± 0.04	1.97 ± 0.04	1.85 ± 0.04
	60	1.10 ± 0.03	1.10 ± 0.03	1.18 ± 0.04	1.27 ± 0.05	1.19 ± 0.07	1.16 ± 0.03	1.15 ± 0.04	1.26 ± 0.02
	100	0.86 ± 0.01	0.94 ± 0.03	1.03 ± 0.03	0.99 ± 0.02	0.99 ± 0.03	0.93 ± 0.04	1.04 ± 0.05	1.01 ± 0.03
	150	1.13 ± 0.05	1.14 ± 0.06	1.21 ± 0.05	1.28 ± 0.08	1.21 ± 0.04	1.09 ± 0.02	1.30 ± 0.05	1.29 ± 0.05

Data represent mean ± SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages.
Abbreviations: Nic 0.2 = Nicotine 0.2 mg/kg/day; Nic 2 = Nicotine 2 mg/kg/day.

TABLE S3: HC3 Binding (mean ± SE)

Region	Postnatal Age	Male (fmol/mg protein)				Female (fmol/mg protein)			
		Control	TSE	Nic 0.2	Nic 2	Control	TSE	Nic 0.2	Nic 2
frontal/parietal cortex	30	15.4 ± 1.3	13.2 ± 0.7	17.0 ± 1.2	15.5 ± 1.6	15.6 ± 2.0	14.2 ± 1.2	16.3 ± 1.6	14.2 ± 1.6
	60	15.4 ± 0.9	15.9 ± 1.3	18.8 ± 0.9	16.4 ± 1.3	17.3 ± 1.1	15.7 ± 1.6	19.7 ± 2.2	16.2 ± 0.6
	100	18.5 ± 1.1	15.7 ± 1.0	16.3 ± 0.8	16.1 ± 1.7	16.8 ± 1.4	16.4 ± 0.6	17.6 ± 1.0	17.8 ± 0.9
	150	15.0 ± 1.5	14.5 ± 1.0	15.3 ± 1.0	13.6 ± 0.7	16.4 ± 1.1	12.7 ± 0.6	15.4 ± 1.6	16.9 ± 1.6
temporal/occipital cortex	30	12.7 ± 0.6	11.0 ± 0.5	13.0 ± 0.7	10.6 ± 0.9	13.2 ± 0.8	12.0 ± 1.3	12.4 ± 0.7	11.0 ± 1.3
	60	11.4 ± 0.7	10.7 ± 1.0	12.8 ± 0.7	10.3 ± 0.4	12.7 ± 0.9	10.2 ± 0.5	11.9 ± 1.1	10.6 ± 0.5
	100	11.1 ± 0.3	10.8 ± 0.6	11.5 ± 0.7	10.6 ± 0.9	10.5 ± 0.2	11.0 ± 0.6	12.3 ± 1.0	12.6 ± 0.8
	150	11.8 ± 0.6	9.7 ± 0.6	11.9 ± 0.5	9.9 ± 0.6	11.5 ± 0.5	9.4 ± 0.4	11.7 ± 0.8	11.3 ± 0.4
hippocampus	30	19.6 ± 1.9	14.6 ± 0.4	17.5 ± 1.4	16.2 ± 2.0	15.4 ± 1.3	16.1 ± 0.9	15.7 ± 1.7	13.1 ± 1.3
	60	16.8 ± 0.6	13.8 ± 0.9	17.2 ± 1.6	14.6 ± 0.7	17.0 ± 0.7	14.9 ± 1.2	17.1 ± 2.5	14.4 ± 0.7
	100	13.6 ± 0.6	12.1 ± 1.0	13.6 ± 0.8	14.2 ± 1.4	14.5 ± 1.2	12.7 ± 1.2	14.3 ± 1.0	14.7 ± 0.7
	150	14.6 ± 0.6	12.3 ± 0.7	14.1 ± 0.4	13.4 ± 0.8	13.0 ± 0.3	12.7 ± 0.5	14.5 ± 0.6	13.4 ± 0.7
striatum	30	81 ± 11	76 ± 8	89 ± 11	72 ± 10	76 ± 6	88 ± 5	73 ± 7	62 ± 6
	60	91 ± 4	65 ± 6	97 ± 8	78 ± 7	84 ± 4	58 ± 4	85 ± 8	71 ± 9
	100	75 ± 3	79 ± 7	99 ± 10	99 ± 11	78 ± 6	79 ± 8	97 ± 8	90 ± 9
	150	66 ± 5	64 ± 7	77 ± 6	77 ± 4	75 ± 5	63 ± 5	78 ± 7	77 ± 2
midbrain	30	13.0 ± 1.1	12.6 ± 1.3	14.0 ± 1.2	12.1 ± 1.4	13.2 ± 0.8	13.4 ± 1.1	12.9 ± 0.8	11.3 ± 1.0
	60	11.6 ± 0.7	10.2 ± 0.4	12.0 ± 1.1	11.4 ± 0.6	9.1 ± 0.6	9.1 ± 0.9	10.8 ± 0.5	10.7 ± 1.0
	100	12.0 ± 0.5	11.6 ± 0.8	11.3 ± 0.7	11.8 ± 0.5	13.2 ± 1.0	12.2 ± 1.2	12.3 ± 0.9	11.6 ± 0.3
	150	11.2 ± 0.6	10.7 ± 0.7	10.9 ± 0.5	11.7 ± 1.1	11.8 ± 0.6	10.2 ± 0.4	10.8 ± 0.4	11.7 ± 0.7
brainstem	30	10.7 ± 0.5	10.9 ± 0.8	12.6 ± 0.6	10.4 ± 0.7	11.2 ± 0.6	11.6 ± 1.0	11.8 ± 0.9	12.1 ± 1.6
	60	6.8 ± 0.3	6.4 ± 0.4	6.9 ± 0.4	7.0 ± 0.4	6.3 ± 0.3	5.9 ± 0.2	6.6 ± 0.3	6.3 ± 0.4
	100	6.9 ± 0.2	5.8 ± 0.2	6.6 ± 0.3	6.4 ± 0.4	6.3 ± 0.2	5.6 ± 0.2	6.3 ± 0.3	6.8 ± 0.4
	150	6.1 ± 0.4	6.2 ± 0.6	5.7 ± 0.3	5.7 ± 0.4	5.5 ± 0.2	5.5 ± 0.3	6.3 ± 0.3	5.8 ± 0.4

Data represent mean ± SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages. Abbreviations: Nic 0.2 = Nicotine 0.2 mg/kg/day; Nic 2 = Nicotine 2 mg/kg/day.

TABLE S4: HC3/ChAT ratio (mean ± SE)

Region	Postnatal Age	Male				Female			
		Control	TSE	Nic 0.2	Nic 2	Control	TSE	Nic 0.2	Nic 2
frontal/parietal cortex	30	19.3 ± 2.0	15.9 ± 0.5	17.3 ± 1.0	17.1 ± 2.0	18.2 ± 2.1	17.3 ± 1.2	17.4 ± 1.5	15.9 ± 2.0
	60	14.6 ± 0.8	13.7 ± 0.7	14.7 ± 0.6	13.1 ± 0.8	15.6 ± 0.8	13.3 ± 1.1	15.0 ± 1.6	13.0 ± 0.3
	100	19.5 ± 1.1	16.8 ± 1.0	17.2 ± 0.9	17.0 ± 2.1	18.3 ± 1.2	16.4 ± 0.6	18.1 ± 1.1	18.3 ± 0.6
	150	19.7 ± 2.3	16.1 ± 1.0	16.5 ± 1.3	14.9 ± 0.5	16.7 ± 0.7	16.4 ± 0.9	16.3 ± 2.0	17.5 ± 1.1
temporal/occipital cortex	30	21.3 ± 2.1	19.9 ± 1.5	21.2 ± 2.2	17.3 ± 2.0	20.0 ± 1.9	18.9 ± 1.8	19.5 ± 0.7	17.1 ± 2.0
	60	17.1 ± 1.0	15.5 ± 0.5	18.0 ± 1.0	16.1 ± 0.6	18.6 ± 0.7	15.5 ± 0.7	17.2 ± 2.0	15.8 ± 0.9
	100	21.5 ± 1.3	20.2 ± 2.1	20.9 ± 0.9	18.6 ± 1.3	18.2 ± 0.8	19.3 ± 0.8	21.4 ± 1.5	21.1 ± 1.0
	150	15.5 ± 0.7	13.5 ± 1.1	15.0 ± 1.0	13.5 ± 0.8	15.0 ± 0.3	13.3 ± 1.0	13.8 ± 1.3	14.2 ± 0.6
hippocampus	30	29.7 ± 1.8	22.7 ± 1.3	27.2 ± 1.8	23.6 ± 2.5	23.4 ± 2.1	25.0 ± 1.8	23.8 ± 2.4	18.4 ± 1.9
	60	15.8 ± 1.5	12.1 ± 0.9	15.0 ± 1.5	12.3 ± 0.4	13.6 ± 0.7	12.4 ± 1.0	13.0 ± 1.4	11.9 ± 0.6
	100	16.4 ± 1.1	13.2 ± 1.3	15.0 ± 1.2	15.7 ± 1.6	17.0 ± 1.6	15.1 ± 2.0	15.0 ± 1.0	15.3 ± 0.8
	150	18.4 ± 1.5	14.5 ± 0.4	15.7 ± 0.8	16.0 ± 0.8	15.4 ± 0.9	14.5 ± 0.7	15.1 ± 0.6	15.1 ± 0.7
striatum	30	18.3 ± 3.3	16.3 ± 2.2	20.9 ± 1.4	12.7 ± 2.5	21.4 ± 3.1	18.5 ± 1.4	17.6 ± 2.2	16.6 ± 3.1
	60	24.2 ± 1.4	19.0 ± 0.5	28.6 ± 2.1	21.7 ± 1.2	22.1 ± 1.1	18.2 ± 1.2	23.6 ± 2.3	20.2 ± 1.3
	100	27.0 ± 1.8	23.7 ± 1.5	27.4 ± 1.9	29.8 ± 3.8	24.0 ± 1.0	24.4 ± 3.1	28.1 ± 2.2	27.3 ± 1.3
	150	22.3 ± 1.4	22.8 ± 1.8	23.5 ± 1.5	24.4 ± 1.7	23.5 ± 1.6	20.7 ± 0.6	21.4 ± 1.0	25.7 ± 1.2
midbrain	30	20.8 ± 1.6	17.8 ± 2.4	21.3 ± 1.5	17.3 ± 1.7	21.2 ± 2.0	17.8 ± 1.3	19.8 ± 1.6	16.5 ± 1.5
	60	17.1 ± 1.2	14.2 ± 0.6	15.3 ± 1.8	15.4 ± 1.4	12.7 ± 0.5	13.1 ± 1.0	13.9 ± 0.9	13.5 ± 1.4
	100	19.8 ± 0.4	17.8 ± 0.7	17.8 ± 0.7	18.3 ± 1.0	19.3 ± 0.9	18.4 ± 1.5	17.1 ± 0.8	17.4 ± 0.3
	150	21.2 ± 1.4	21.4 ± 0.8	20.0 ± 1.3	21.2 ± 1.0	20.1 ± 0.6	19.9 ± 0.3	19.1 ± 1.6	20.6 ± 0.9
brainstem	30	6.1 ± 0.1	6.4 ± 0.5	6.5 ± 0.2	5.8 ± 0.5	6.0 ± 0.5	6.7 ± 0.7	6.0 ± 0.5	6.6 ± 0.8
	60	6.2 ± 0.3	5.9 ± 0.4	5.9 ± 0.4	5.6 ± 0.4	5.4 ± 0.4	5.0 ± 0.2	5.7 ± 0.3	5.0 ± 0.3
	100	8.1 ± 0.2	6.2 ± 0.3	6.4 ± 0.2	6.6 ± 0.6	6.4 ± 0.3	6.1 ± 0.3	6.2 ± 0.4	6.6 ± 0.5
	150	5.5 ± 0.5	5.3 ± 0.6	4.8 ± 0.3	4.6 ± 0.5	4.6 ± 0.3	5.1 ± 0.3	4.9 ± 0.4	4.5 ± 0.2

Data represent mean ± SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages. Abbreviations: Nic 0.2 = Nicotine 0.2 mg/kg/day; Nic 2 = Nicotine 2 mg/kg/day.

TABLE S5: nAChR Binding (mean ± SE)

Region	Postnatal Age	Male (fmol/mg protein)				Female (fmol/mg protein)			
		Control	TSE	Nic 0.2	Nic 2	Control	TSE	Nic 0.2	Nic 2
frontal/parietal cortex	30	53 ± 7	58 ± 7	70 ± 7	63 ± 6	67 ± 7	52 ± 9	65 ± 5	60 ± 6
	60	56 ± 4	50 ± 6	62 ± 4	59 ± 5	51 ± 7	45 ± 5	56 ± 5	59 ± 2
	100	54 ± 2	52 ± 4	52 ± 3	53 ± 4	57 ± 3	51 ± 3	50 ± 3	52 ± 2
	150	55 ± 4	50 ± 2	51 ± 9	46 ± 4	45 ± 6	44 ± 3	43 ± 5	47 ± 7
temporal/occipital cortex	30	63 ± 9	68 ± 9	78 ± 10	69 ± 4	59 ± 7	66 ± 9	73 ± 6	65 ± 7
	60	63 ± 4	46 ± 10	67 ± 7	57 ± 5	52 ± 6	48 ± 6	54 ± 8	60 ± 3
	100	56 ± 3	58 ± 5	59 ± 6	61 ± 2	58 ± 3	56 ± 3	58 ± 2	64 ± 3
	150	57 ± 5	61 ± 2	60 ± 7	61 ± 3	54 ± 5	50 ± 5	65 ± 2	59 ± 6
hippocampus	30	53 ± 5	35 ± 9	53 ± 2	57 ± 8	45 ± 5	26 ± 9	45 ± 4	43 ± 4
	60	50 ± 5	43 ± 6	52 ± 4	48 ± 4	46 ± 7	44 ± 3	39 ± 7	52 ± 3
	100	30 ± 3	31 ± 2	25 ± 4	33 ± 2	33 ± 3	30 ± 3	33 ± 3	36 ± 2
	150	27 ± 2	31 ± 1	26 ± 4	31 ± 3	24 ± 2	29 ± 4	31 ± 2	26 ± 3
midbrain	30	78 ± 5	66 ± 10	82 ± 6	79 ± 4	64 ± 6	54 ± 10	81 ± 3	77 ± 5
	60	66 ± 4	59 ± 3	63 ± 4	64 ± 3	48 ± 6	52 ± 4	64 ± 4	61 ± 3
	100	55 ± 2	56 ± 1	60 ± 2	62 ± 2	62 ± 1	61 ± 2	60 ± 2	65 ± 2
	150	52 ± 4	51 ± 2	57 ± 7	55 ± 4	50 ± 3	48 ± 3	43 ± 5	47 ± 6
brainstem	30	34 ± 4	35 ± 5	42 ± 3	44 ± 2	34 ± 3	19 ± 6	37 ± 3	41 ± 2
	60	30 ± 1	26 ± 2	29 ± 2	31 ± 1	23 ± 4	25 ± 2	29 ± 2	28 ± 1
	100	27 ± 1	25 ± 1	28 ± 2	28 ± 1	28 ± 1	24 ± 1	26 ± 1	29 ± 2
	150	26 ± 1	26 ± 2	24 ± 4	25 ± 2	24 ± 1	23 ± 1	27 ± 1	24 ± 2

Data represent mean ± SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages. Abbreviations: Nic 0.2 = Nicotine 0.2 mg/kg/day; Nic 2 = Nicotine 2 mg/kg/day.

TABLE S6: 5HT Receptor Binding (mean ± SE)

Subtype and Region	Postnatal Age	Male (fmol/mg protein)				Female (fmol/mg protein)			
$5HT_{1A}$ Receptors		Control	TSE	Nic 0.2	Nic 2	Control	TSE	Nic 0.2	Nic 2
frontal/parietal cortex	30	84 ± 10	108 ± 3	106 ± 10	91 ± 11	100 ± 5	92 ± 10	102 ± 8	89 ± 6
	60	86 ± 3	91 ± 7	101 ± 6	88 ± 7	95 ± 3	78 ± 6	101 ± 7	92 ± 7
	100	97 ± 12	90 ± 14	91 ± 10	92 ± 13	98 ± 11	99 ± 9	101 ± 14	93 ± 10
	150	80 ± 5	77 ± 5	82 ± 5	72 ± 2	80 ± 4	76 ± 5	77 ± 8	85 ± 8
temporal/occipital cortex	30	102 ± 14	123 ± 2	124 ± 5	100 ± 10	114 ± 9	115 ± 10	118 ± 7	100 ± 6
	60	106 ± 5	114 ± 4	123 ± 3	105 ± 3	118 ± 5	103 ± 5	105 ± 12	109 ± 7
	100	106 ± 12	98 ± 16	125 ± 15	120 ± 17	113 ± 11	118 ± 11	132 ± 17	126 ± 18
	150	123 ± 7	126 ± 4	141 ± 6	128 ± 6	129 ± 7	99 ± 5	134 ± 9	145 ± 10
midbrain	30	68 ± 3	62 ± 6	69 ± 2	59 ± 9	66 ± 4	53 ± 5	67 ± 5	56 ± 8
	60	60 ± 3	53 ± 5	56 ± 3	61 ± 6	53 ± 3	44 ± 6	55 ± 2	54 ± 2
	100	52 ± 2	39 ± 5	46 ± 5	46± 5	52 ± 2	50 ± 2	59 ± 7	46 ± 4
	150	45 ± 2	44 ± 4	45 ± 3	56 ± 6	48 ± 2	44 ± 5	45 ± 3	52 ± 4
brainstem	30	34 ± 2	40 ± 2	40 ± 2	39 ± 4	36 ± 1	36 ± 2	42 ± 3	38 ± 3
	60	39 ± 2	41 ± 1	43 ± 2	42 ± 2	41 ± 2	35 ± 3	39 ± 3	40 ± 2
	100	29 ± 2	26 ± 3	30 ± 3	29 ± 2	29 ± 2	26 ± 3	30 ± 3	29 ± 4
	150	30 ± 2	30 ± 3	28 ± 1	29 ± 2	28 ± 1	26 ± 1	31 ± 1	30 ± 3
$5HT_2$ Receptors									
frontal/parietal cortex	30	55 ± 4	65 ± 5	65 ± 1	71 ± 4	54 ± 7	61 ± 2	56 ± 2	66 ± 1
	60	51 ± 2	63 ± 1	58 ± 1	58 ± 2	57 ± 3	55 ± 1	63 ± 2	61 ± 2
	100	56 ± 2	58 ± 2	57 ± 2	58 ± 2	59 ± 2	52 ± 2	59 ± 2	55 ± 4
	150	46 ± 1	54 ± 2	50 ± 2	49 ± 1	47 ± 2	48 ± 1	52 ± 1	50 ± 2
temporal/occipital cortex	30	28 ± 2	30 ± 2	33 ± 3	34 ± 2	34 ± 2	30 ± 1	31 ± 2	30 ± 2
	60	30 ± 1	34 ± 2	33 ± 1	31 ± 1	34 ± 1	31 ± 1	33 ± 1	35 ± 1
	100	30 ± 1	33 ± 1	32 ± 2	32 ± 1	32 ± 1	29 ± 1	33 ± 1	35 ± 2
	150	23 ± 1	29 ± 1	27 ± 1	27 ± 1	26 ± 1	27 ± 2	30 ± 1	28 ± 1
midbrain	30	9.4 ± 0.7	1.03 ± 0.5	10.3 ± 0.5	10.8 ± 1.0	9.7 ± 0.7	9.9 ± 0.3	10.2 ± 0.5	10.8 ± 0.4
	60	9.0 ± 0.2	8.9 ± 0.3	8.7 ± 0.4	9.3 ± 0.5	8.9 ± 0.3	8.7 ± 0.6	9.7 ± 0.3	9.6 ± 0.4
	100	9.1 ± 0.2	10.2 ± 0.5	10.5 ± 0.5	10.2 ± 0.5	11.1 ± 0.8	9.1 ± 0.6	11.6 ± 0.4	11.1 ± 0.8
	150	9.2 ± 0.3	9.7 ± 0.3	10.0 ± 0.3	10.8 ± 0.2	9.5 ± 0.4	9.3 ± 0.2	10.0 ± 0.4	10.4 ± 0.3
brainstem	30	7.5 ± 0.3	8.6 ± 0.3	9.1 ± 0.5	8.9 ± 0.3	8.9 ± 0.3	7.9 ± 0.4	9.9 ± 0.4	8.7 ± 0.3
	60	9.5 ± 0.3	10.2 ± 0.2	10.5 ± 0.4	10.9 ± 0.3	10.2 ± 0.3	9.3 ± 0.3	10.9 ± 0.4	10.4 ± 0.2
	100	8.9 ± 0.6	10.0 ± 0.8	10.8 ± 0.9	10.4 ± 0.6	10.4 ± 0.3	10.8 ± 0.9	9.4 ± 0.4	10.6 ± 0.9
	150	8.2 ± 0.3	8.8 ± 0.4	8.5 ± 0.5	8.7 ± 0.3	8.5 ± 0.4	8.5 ± 0.4	9.6 ± 0.5	9.1 ± 0.5

Data represent mean ± SE obtained from six animals in each treatment group for each age and sex. Results of multivariate ANOVA are provided in the main text. Note that the assays for each region and age were run in separate experiments, so absolute values cannot be compared strictly across ages or between regions. Accordingly, statistical comparisons in the main text were conducted on log-transformed data, which evaluates the treatment differences as a proportion to control values, rather than as an arithmetic difference. Representing the data as proportional differences (percent control) enables a full comparison of treatment effects and treatment interactions with all the other variables, even though absolute values for the controls cannot be compared across regions and ages. Abbreviations: Nic 0.2 = Nicotine 0.2 mg/kg/day; Nic 2 = Nicotine 2 mg/kg/day.