Supplementary Methods

Statistical analyses

Running-wheel data for animals in the 2 and 24 hrs day^{-1} exercise groups were analysed using three-way repeated measures ANOVAs for the habituation and following treatment period, with factors 'treatment', 'exercise' and 'time'. Withdrawal data were normalised by constraining data between 0–100 before calculating a composite total withdrawal symptom. Withdrawal data were analysed using a Kruskal-Wallis non-parametric test followed by Mann-Whitney *U* multiple comparisons with Dunn's corrected α-level. Two-way ANOVA for factors 'treatment' and 'exercise' was conducted for the analysis of autoradiographic binding data for each brain region. Two-way ANOVA for the factors 'treatment' and 'exercise' was used for comparison of BDNF levels in each brain region analysed and corticosterone measurements in plasma. All ANOVAs were followed by Bonferroni *post-hoc* comparisons when significance was reached (p<0.05).

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Quantitative receptor autoradiography

$\alpha4\beta2$ nAChR binding*: Sections were pre-incubated for 10 mins in 50 mM Tris-HCl buffer containing 0.9 % w/v NaCl, 5 mM KCl, 2.4 mM $CaCl_2$ and 1mM $MgCl_2$, pH 7.4 at room temperature, to remove endogenous ligands. Binding was carried out in 50 mM Tris-HCl pre-incubation buffer, pH 7.4 at room temperature, in the presence of 100 pM [^{125}I]epibatidine (specific activity 2200 Ci mmol^{-1}) for 2 hrs. Two series of adjacent sections were used from each mouse to measure total [^{125}I]epibatidine binding (no competing ligand) and [^{125}I]epibatidine binding in the presence of 20 nM cytisine. An additional set of adjacent sections were used to determine NSB in the presence of 300 µM nicotine salt for 2 hrs.

Incubations were terminated after rinsing slides twice in ice-cold (4°C) 50 mM Tris-HCl buffer, pH 7.4 for 10 mins and a final rinse in ice cold RO water.

α7 nAChR binding: Slides were pre-incubated for 30 mins in 50 mM Tris-HCl pre-incubation buffer, containing 0.5 % w/v BSA, pH 7.4 at room temperature. Pre-incubation of NSB slides included 1mM nicotine hydrogen tartrate. Binding was carried out in the same buffer in the presence of 3 nM [^{125}I]α-Bgtx (specific activity 108.8 Ci mmol^{-1}) for 3 hrs. NSB was again determined in adjacent sections in the presence of 300 µM nicotine salt for 2 hrs. Incubations were terminated after rinsing slides three times in ice-cold (4°C) 50 mM Tris-HCl buffer (no BSA), pH 7.4, for 10 mins using RO water between each rinse.

µ receptor binding: Slides were pre-incubated for 30 mins in 50 mM Tris-HCl pre-incubation buffer, containing 0.9 % w/v NaCl, pH 7.4 at room temperature. The slides were then incubated in 50 mM Tris-HCl buffer, pH 7.4 at room temperature in the presence of 4 nM [^{3}H]DAMGO (specific activity 51.5 Ci mmol^{-1}) for 60 mins. Non-specific binding was determined in adjacent sections in the presence of 1 µM naloxone. Incubation was terminated by rapid rinses (3 x 5 mins) in ice-cold 50 mM Tris-HCl buffer, pH 7.4 at room temperature, and distilled water (3 x 5 mins), then rapidly cool-air dried.

D_2 receptor binding: Slides were pre-incubated for 20 mins in 50mM Tris-HCl buffer, containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$ plus 1mM $MgCl_2$, pH 7.4, at room temperature. The slides were then incubated in pre-incubation buffer in the presence of 4nM [^{3}H]raclopride (specific activity 60 Ci mmol^{-1}) for 60 mins. Non-specific binding was determined in the presence of 10 µM sulpiride. Incubation was terminated by rapid rinses (6 x 1 min) in ice-cold 50mM Tris-HCl buffer and a final rinse in ice cold RO water.

Following binding, sections were rapidly dried under cold air for 2 hours, and dried for up to 7 days using anhydrous calcium sulphate (BDH Chemicals, Poole, UK). Adjacent total and non-

specific labelled sections were apposed to Kodak BioMax MR-1 film alongside autoradiographic microscale standards of known concentration. [^{125}I]epibatidine and [^{125}I]α-Bgtx bound sections were exposed to film for 24 hrs and 7 days, respectively, with a set of ^{14}C microscale standards which had been cross-calibrated to iodinated standards (Baskin & Wimpy, 1989; Miller & Zahniser, 1987). [^{3}H]-bound sections were exposed to film with ^{3}H microscale standards for a period of 6 weeks for D_2 receptors and 10 weeks for μ receptors.

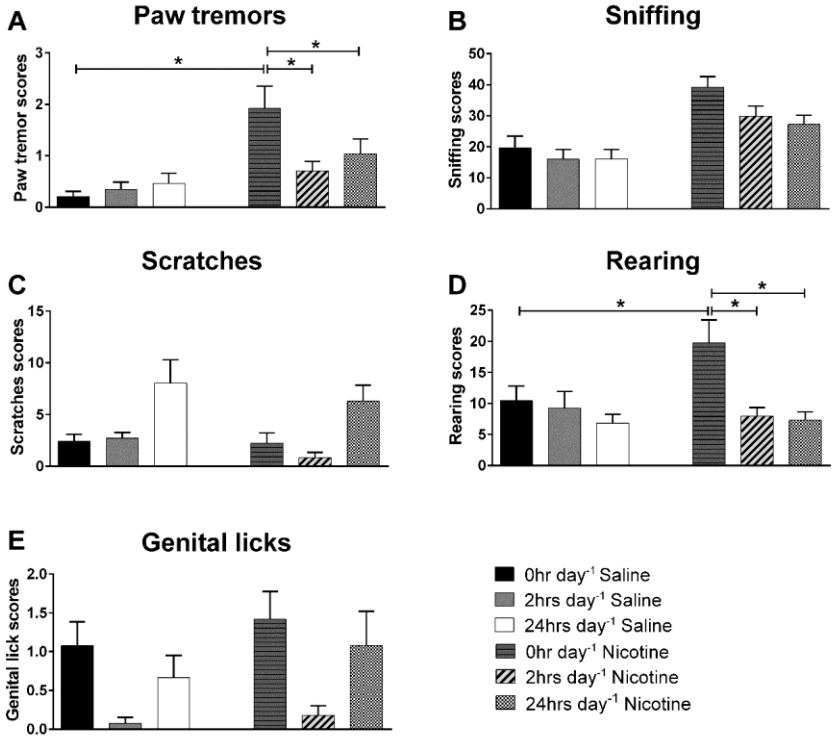
For development, films were covered with an aqueous solution of 50 % v/v Kodak D19 developer for 3 mins. The reaction was stopped by 1 min rinse in distilled water containing a drop of glacial acetic acid. Images were fixed by submersion in Kodak rapid fix solution for 5 mins. Films were then rinsed in distilled water and dried overnight in a fume cupboard.

Films were analysed by video-based densiometry using an MCID image analyser (Imaging Research, Canada) as previously described by Kitchen et al. (1997). In brief, fmol mg^{-1} tissue equivalents for receptor binding were derived from either ^{3}H or ^{14}C microscale standards, and the relationship between tissue radioactivity and optical density was calculated using MCID software, with appropriate adjustments to allow for radioactive decay of both the standards and the radioligands. Specific receptor binding was derived by subtraction of NSB from total binding for α7 nAChRs, μ and D_2 receptors. For α4β2* specific nAChRs, cytisine-resistant epibatidine binding was subtracted from total epibatidine binding to give cytisine-sensitive epibatidine binding.

For each region, quantified measures were taken from both left and right hemispheres, therefore receptor binding represents a duplicate determination for each brain region and the n values listed refer to the number of animals analysed. The following structures were analysed by sampling 5–20 times with a box tool: cortex (8 x 8 mm), olfactory tubercle (6 x 6 mm) and

hippocampus (5 x 5 mm). All other regions were analysed by free-hand drawing. Brain structures were identified by reference to the mouse atlas of Franklin and Paxinos (2001).

Supplementary Figures



Supplementary Figure S1. Effect of wheel-running exercise regimen on individual nicotine withdrawal symptoms. Mice underwent one of three exercise regimes: 0, 2 or 24 hrs day^{-1} running-wheel access. Withdrawal was precipitated by mecamylamine (3 mg kg^{-1}, s.c.) following 14 days of either saline or nicotine (24 mg kg^{-1} day^{-1}) treatment via subcutaneous minipumps. Abstinence signs were evaluated during a 30-min period after mecamylamine injection. (A) Front paw tremors were assigned a value of 0.5 for each episode. (B) Sniffing was assigned a value of 0.5 for each episode. (C) Scratches were assigned a value of 0.5 for each episode. (D) Rearing was assigned a value of 0.5 for each episode. (E) Genital licks were assigned a value of 1 for appearance within each 5-min bin. Data are presented as mean ± SEM. *p<0.05. Precise group sizes are reported in Table 1.

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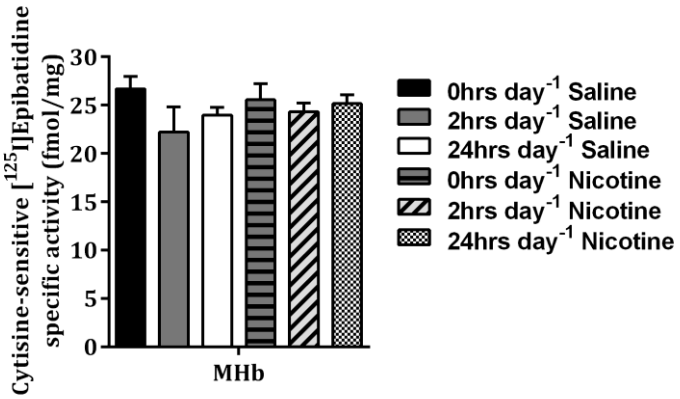

Supplementary Figure S2. Effect of exercise on α4β2* nAChR binding in saline- and nicotine-withdrawn mice. Cytisine-sensitive [^{125}I]epibatidine binding in saline- and nicotine-withdrawn mice undergoing different exercise regimes in the medial habenula. Data are presented as mean ± SEM. *p<0.05. Precise group sizes are reported in Table 1. *Abbreviations*: MHb, medial habenula.

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Supplementary Tables

Supplementary Table S1: Effect of exercise on α4β2* nAChR binding in saline- and nicotine-withdrawn mice

[^{125}I] epibatidine (fmol mg^{-1} tissue)												
Brain region	**0 hr day^{-1}**				**2 hrs day^{-1}**				**24 hrs day^{-1}**			
	Saline		**Nicotine**		**Saline**		**Nicotine**		**Saline**		**Nicotine**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
FrA	8.60	0.77	11.91	1.12	8.08	1.18	12.94	0.46	9.23	0.74	11.26	0.32
MtCx	9.19	0.79	11.81	0.96	8.08	1.20	11.21	0.50	8.25	0.54	10.11	0.85
SS	9.25	0.58	12.71	1.01	7.99	1.20	12.52	0.57	7.81	0.81	12.08	1.26
Pir	5.54	0.24	8.78	0.81	5.61	0.78	9.88	1.21	5.09	0.82	8.73	0.94
RS	9.66	0.92	12.68	0.79	9.52	1.37	10.93	1.44	8.82	1.02	11.32	1.05
CPu	9.67	0.81	11.77	1.25	8.61	1.37	10.72	0.31	8.66	0.76	10.99	0.97
MS	5.73	0.38	7.64	0.66	4.22	0.79	7.84	1.31	4.74	0.64	6.91	0.79
VDB	5.01	0.45	6.93	0.82	3.87	0.74	7.17	0.91	4.62	0.68	6.41	0.66
AuCx	7.69	0.58	12.44	1.11	6.91	0.58	13.12	0.97	7.69	1.17	10.14	1.32

Abbreviations: AuCx, auditory cotex; CPu, caudate-putamen; FrA, frontal association; MS, medial septum; MtCx, motor cortex; nAChR, nicotinic acetylcholine receptor; Pir, piriform cortex; RS, retrosplenial cotex; SEM, standard error of the mean; SS, somatosensory cortex; VDB, vertical limb of the diagonal band of Broca.

Supplementary Table S2: Effect of exercise on α7 nAChR binding in saline- and nicotine-withdrawn mice

[^{125}I]α-bungarotoxin (fmol mg^{-1} tissue)												
Brain region	**0 hr day^{-1}**				**2 hrs day^{-1}**				**24 hrs day^{-1}**			
	Saline		**Nicotine**		**Saline**		**Nicotine**		**Saline**		**Nicotine**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
FrA	20.30	2.28	21.88	1.75	22.24	1.64	24.32	2.01	19.91	2.57	23.64	1.55
CPu	30.26	1.65	33.46	2.35	27.58	2.70	27.38	3.58	27.50	2.47	32.45	2.61
ZI	47.81	3.35	51.29	3.65	44.55	4.03	53.44	3.84	53.62	2.60	54.18	3.45
VLG	31.58	2.44	29.95	1.95	29.28	1.27	34.93	2.52	31.25	2.15	36.12	0.54

Abbreviations: CPu, caudate-putamen; FrA, frontal association; SEM, standard error of the mean; VLG, ventral lateral geniculate; ZI, zona incerta.