# Baseline Expression of α4β2* Nicotinic Acetylcholine Receptors Predicts Motivation to Self-administer Nicotine

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**Background:** Marked interindividual differences in vulnerability to nicotine dependence exist, but factors underlying such differences are not well understood. The midbrain α4β2* subtype of nicotinic acetylcholine receptors (nAChRs) has been implicated in mediation of the reinforcing effects of nicotine responsible for dependence. However, no study has been performed evaluating the impact of interindividual differences in midbrain nAChR levels on motivation to self-administer nicotine.

**Methods:** Baseline levels of α4β2* nAChRs were measured using 2-[$^{18}$F]fluoro-A-85380 (2-FA) and positron emission tomography (PET) in five squirrel monkeys. Motivation to self-administer nicotine (number of lever presses) was subsequently measured using a progressive-ratio (PR) schedule of reinforcement.

**Results:** Greater motivation to self-administer nicotine was associated with lower levels of midbrain nAChRs.

**Conclusions:** The results suggest that level of expression of nAChRs is a contributing factor in the development of nicotine dependence. Similarly, it has been previously shown that low levels of dopamine $D_2$ receptors (DRD2) are associated with a higher preference for psychostimulant use in humans and nonhuman primates. Together, results from these PET studies of dopaminergic and nicotinic cholinergic transmission suggest that an inverse relationship between the availability of receptors that mediate reinforcement and the motivation to take drugs exists across different neurotransmitter systems.

**Key Words:** In vivo binding, nicotine self-administration, nicotinic receptors, nonhuman primates, positron emission tomography

The α4β2* subtype of nicotinic acetylcholine receptors (nAChRs), located in the midbrain area, has been implicated in mediating the reinforcing effects of nicotine (1). Animal models using genetically modified rodents implicate the involvement of these receptors in the development of nicotine dependence, since β2 subunit deletion decreases sensitivity to nicotine's reinforcing effects and α4 subunit modifications that increase sensitivity to nicotine result in increased sensitivity to nicotine reinforcement (2,3). Together, these results suggest the hypothesis that a high baseline level of midbrain nAChRs will be associated with a high motivation to self-administer nicotine. No studies have yet been performed to evaluate this hypothesis. Since those studies are not ethical in humans, to test this hypothesis we used adult male squirrel monkeys that had learned to self-administer nicotine (4,5). To determine the baseline level of midbrain nAChRs, we measure nondisplaceable binding potential ($BP_{ND}$) of 2-[$^{18}$F]fluoro-A-85380 (2-FA), a selective α4β2* nAChR positron emission tomography (PET) ligand. Nondisplaceable binding potential is proportional to the density of receptors available for radioligand binding in vivo.

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## Methods and Materials

### Subjects

Five adult drug-naive male squirrel monkeys (*Saimiri sciureus*), weighing 730 g to 950 g, were housed individually in a temperature- and humidity-controlled room and were maintained on a 12-hour light/dark cycle; the lights were on from 6:45 AM to 6:45 PM. Experiments were conducted during the light phase. Monkeys were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animals (AAALAC) and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, and the Guide for Care and Use of Laboratory Animals (6).

### PET Imaging Studies

**Radiochemistry.** [$^{18}$F]Fluoride was produced using an RDS111 negative ion cyclotron (CTI, Knoxville, Tennessee), and 2-FA was synthesized at the National Institute on Drug Abuse using a modified semiautomated method (7). The final product was formulated as a sterile and pyrogen-free isotonic solution. Radiochemical purity product was greater than 98% and specific activity was in the range from 275 GBq/μmol to 516 GBq/μmol (450 ± 130 GBq/μmol, average ± SD).

### PET and Magnetic Resonance Imaging Scanning Procedures

Data were acquired on a Siemens Exact ECAT HR+ tomograph (CTI) (63 slices, center to center spacing of 2.4 mm, with an in-plane reconstructed resolution, full-width at half maximum [FWHM], of 4.7 mm at the center of the field of view and reconstructed axial spatial resolution of 4.2 mm in three-dimensional [3-D] mode). Before each radioligand administration,

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transmission scans were obtained with three rotating germanium-68 ($^{68}$Ge)-gallium-68 ($^{68}$Ga) sources to be used to correct for photon attenuation by tissues and mask. Positron emission tomography images were reconstructed from the raw data with a standard filtered back-projection algorithm and a ramp filter.

For the PET scans, monkeys were initially anesthetized with 1.5 mg/kg alfadolone and alfaxolone acetate (Saffan, Arnolds Veterinary Products, Shropshire, United Kingdom), given intramuscularly. Anesthesia was then maintained by 1.5% to 2.5% isoflurane. An individually molded thermoplastic face mask was secured to a custom-made monkey head-holder attached to a backboard.

Acquisition of dynamic PET scans ($n$ = 5) started with the injection of 2-FA as a bolus (47 ± 15 MBq/kg injected intravenously in approximately 1 mL of saline over 20 sec) and continued for 5 hours.

Anatomical magnetic resonance imaging (MRI) brain images were acquired on a 3.0 Tesla Siemens Magnetom Allegra MRI unit (Siemens Medical Solutions, Erlangen, Germany) using continuous intravenous infusion of 8 mg/kg to 11 mg/kg of Saffan per hour (Arnolds Veterinary Products) to maintain anesthesia.

Vital signs, including heart rate, electrocardiogram (ECG) (during PET studies), respiration rate, end-tidal carbon dioxide ($ET_{CO2}$), and blood oxygen saturation (always maintained above 95%), were continuously monitored during the studies.

### PET Data Analysis

Regions of interest (ROIs) for thalamus, midbrain, and cerebellum were drawn on the individual T1 MRI images co-registered to PET images, with reference to a stereotaxic atlas. Regions of interest for the muscles were placed on the back of the neck, in the area of the semispinalis cervicis, splenius capitis, and obliquus capitis muscles. Nondisplaceable binding potential values were calculated using a four-parameter reference tissue model (PMOD v. 2.75 [PMOD Technologies Ltd., Zurich, Switzerland]). For receptor quantification using muscles as a reference region, $BP_{ND}$ values were corrected for differences between brain tissue $V_{ND}$ and muscle $V_T$ using equation 6 from (8).

Nondisplaceable binding potential parametric images were generated using PMOD v. 2.75 (Gunn method) and cerebellum as the reference region. After co-registration of $BP_{ND}$ parametric maps to individual MRI images used to obtain the average MRI image, $BP_{ND}$ parametric maps were superimposed on the average MRI image without spatial normalization.

**Intravenous Nicotine Self-Administration.** Several days after obtaining PET data, acquisition sessions were initiated during which the monkeys were allowed to self-administer nicotine intravenously under a fixed-ratio (FR) schedule of reinforcement. Subsequently, they were switched to a progressive-ratio schedule of reinforcement (see [5] and Supplement 1 for details). Since there was an inverted U-shaped dose-effect curve under this schedule, we focused on responding maintained by a 30 μg/kg per injection dose of nicotine. This dose produces peak levels of injections per session, peak breaking point values, and a near maximal nicotine intake per session (5).

## Results

Contrary to our initial hypothesis, animals with low baseline levels of midbrain α4β2* nAChRs (low $BP_{ND}$, Figure 1A) exhibited a higher motivation to self-administer nicotine, as assessed by a number of responses made under the progressive-ratio (PR) schedule (Figure 1B and 1C). There was a significant negative correlation between the number of responses and midbrain $BP_{ND}$ values calculated using either cerebellum or muscle as reference regions (rank correlations for both, rho = −1, $p < .05$). The fact that similar results were obtained using either cerebellum or muscle as reference region suggests that any confounding role of nicotinic receptor expression within the cerebellum in these measurements was likely inconsequential. We should mention that it was not possible to analyze $BP_{ND}$ values in other brain areas that may be involved in reinforcement, such as striatum, because of the low receptor density and therefore unreliable specific binding signal obtained with 2-FA in those areas (8). The thalamus is a brain area that displays high levels of α4β2* nAChRs that can be measured by 2-FA. We found no significant correlation between the number of responses and thalamus $BP_{ND}$ values (Figure 1 in Supplement 1). This may reflect the lack of involvement of the thalamus in nicotine reinforcement processes. A trend was noted toward an inverse correlation between thalamus $BP_{ND}$ values and number of

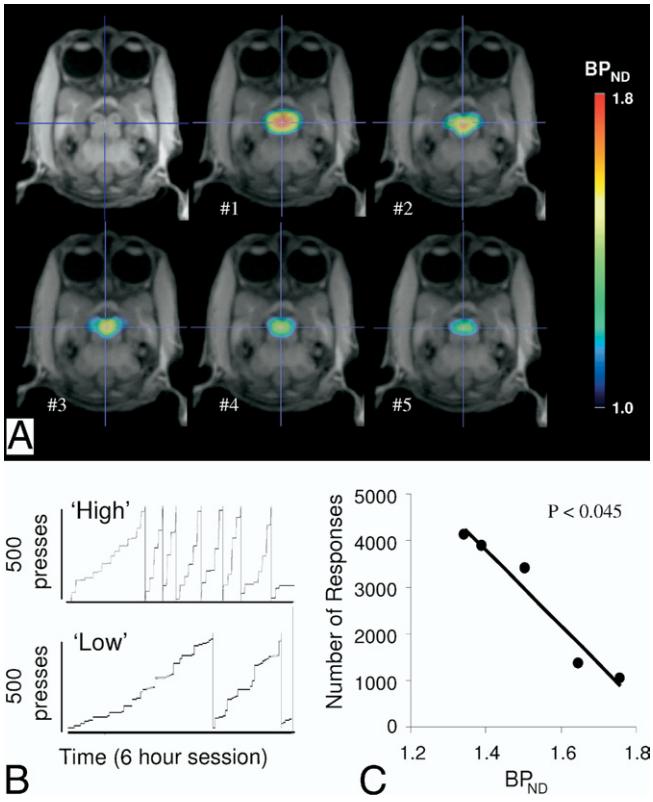


**Figure 1. (A)** α4β2* nAChRs ($BP_{ND}$ of 2-FA) at the level of the midbrain. Upper left image—an average T1 brain MRI from all five squirrel monkeys; #1 to #5 $BP_{ND}$ images from each individual monkey superimposed on the average MRI and sorted by the number of responses during the progressive-ratio schedule of reinforcement. Note that only voxels with $BP_{ND}$ values > 1 are shown for clarity. **(B)** Typical cumulative-response records of responding on the active lever under the progressive-ratio schedule of reinforcement maintained by 30 μg/kg injection dose of nicotine. Representative records are shown for a monkey with high (#5) and a monkey with low (#1) motivation to self-administer nicotine. **(C)** Inverse correlation between $BP_{ND}$ values measured in the midbrain (correlation shown here was obtained using cerebellum as a reference region) and number of responses made by the monkeys on the active lever during the progressive-ratio schedule of intravenous nicotine self-administration. $BP_{ND}$, nondisplaceable binding potential; 2-FA, 2-[$^{18}$F]fluoro-A-85380; MRI, magnetic resonance imaging; nAChR, nicotinic acetylcholine receptors.

responses under the PR schedule (Figure 1 in Supplement 1), which might be due to a correlation between the levels of α4β2* nAChRs in the two structures (Figure 2 in Supplement 1), but this correlation did not reach significance.

Data obtained during the acquisition phase under the FR schedule of reinforcement were also analyzed, since responses during acquisition may also reflect motivational effects of nicotine. There was a significant negative correlation between the number of days needed to reach the final fixed-ratio 10 (FR-10) response requirement and the number of nicotine injections self-administered per session under the final FR-10 schedule ($p < .05$). However, this result should be viewed with caution, due to the limited number of animals and the absence of strict criteria for increasing ratio requirements during the acquisition phase. We found no significant correlation between $BP_{ND}$ values calculated using either cerebellum or muscle as reference regions and the number of nicotine injections under the FR-10 schedule (all $p > .23$). This may reflect either different motivational aspects of nicotine taking assessed by FR and PR schedules or a limitation of 1-hour access to nicotine under the FR schedule to detect such correlation.

The observation that a low level of baseline midbrain nAChRs was associated with a high motivation to self-administer nicotine is consistent with previous findings obtained with dopamine receptors and psychostimulant addiction. For example, low levels of dopamine $D_2$ receptor ($DRD_2$) expression are associated with a higher preference for psychostimulant use in humans (9) and a greater motivation to self-administer cocaine in monkeys (10). Furthermore, similar to blockade of $DRD_2$ reducing the reinforcing effects of psychostimulant drugs (11), blockade of nAChRs reduces the reinforcing effects of nicotine (12,13). It should be noted that in the above referenced $DRD_2$ PET studies, as well as in the present study, $BP_{ND}$ was used as an in vivo measure of receptor density. Since $BP_{ND}$ is directly proportional to the density of receptors available for radioligand binding (receptors not occupied by an endogenous transmitter), the observed intersubject variations in $BP_{ND}$ for $DRD_2$ and nAChRs may reflect potential differences in levels of endogenous dopamine and acetylcholine, respectively. Taken together, those studies of dopaminergic and nicotinic cholinergic transmission suggest that an inverse relationship between the availability of receptors that mediate reinforcement and the motivation to take drugs exists across different neurotransmitter systems.

## Discussion

Although these results rely on a limited sample size and should be duplicated with a larger group of animals, this report is the first to show that baseline level of nAChRs may affect the motivation to self-administer nicotine. A low level of α4β2* nAChRs could be one of the predisposing factors for nicotine dependence and measuring levels of nAChRs or exploring genetic factors that control baseline levels of α4β2* nAChRs may allow an assessment of individual risk of developing tobacco dependence, which remains the leading preventable cause of death in developed countries.

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*Supplementary material cited in this article is available online.*

1. Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, *et al.* (2005): Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* 436:103–107.
2. Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, *et al.* (1998): Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature* 391:173–177.
3. Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, *et al.* (2004): Nicotine activation of alpha4* receptors: Sufficient for reward, tolerance, and sensitization. *Science* 306:1029–1032.
4. Spealman RD, Goldberg SR (1982): Maintenance of schedule-controlled behavior by intravenous injections of nicotine in squirrel monkeys. *J Pharmacol Exp Ther* 223:402–408.
5. Le Foll B, Wertheim C, Goldberg SR (2007): High reinforcing efficacy of nicotine in non-human primates. *PLoS ONE* 2:e230.
6. National Academy of Sciences (1996): *Guide for the Care and Use of Laboratory Animals*. Washigton, DC: National Academy Press.
7. Horti AG, Scheffel U, Koren AO, Ravert HT, Mathews WB, Musachio JL, *et al.* (1998): 2-[18F]Fluoro-A-85380, an in vivo tracer for the nicotinic acetylcholine receptors. *Nucl Med Biol* 25:599–603.
8. Le Foll B, Chefer SI, Kimes AS, Shumway D, Goldberg SR, Stein EA, *et al.* (2007): Validation of an extracerebral reference region approach for the quantification of brain nicotinic acetylcholine receptors in squirrel monkeys with PET and 2-18F-fluoro-A-85380. *J Nucl Med* 48:1492–1500.
9. Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Gifford A, *et al.* (1999): Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. *Am J Psychiatry* 156:1440–1443.
10. Nader MA, Morgan D, Gage HD, Nader SH, Calhoun TL, Buchheimer N, *et al.* (2006): PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys. *Nat Neurosci* 9:1050–1056.
11. Wise RA (2004): Dopamine, learning and motivation. *Nat Rev Neurosci* 5:483–494.
12. Goldberg SR, Spealman RD, Goldberg DM (1981): Persistent behavior at high rates maintained by intravenous self-administration of nicotine. *Science* 214:573–575.
13. DeNoble VJ, Mele PC (2006): Intravenous nicotine self-administration in rats: Effects of mecamylamine, hexamethonium and naloxone. *Psychopharmacology (Berl)* 184:266–272.