Baseline Expression of $\alpha_4\beta_2^*$ 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 $\alpha_4\beta_2^*$ subtype of nicotinic acetylcholine receptors (nAChRs) has been implicated in mediating 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 $\alpha_4\beta_2^*$ nAChRs were measured using 2-[18F]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 D2 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

**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.

**PET Imaging Studies**

**Radiochemistry.** [18F]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. 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,
transmission scans were obtained with three rotating germanium-68 (68Ge)-gallium-68 (68Ga) 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 alfadalone and alfaxalone acetate (Saffan, Arnolds Veterinary Products, Shropshire, United Kingdom), given intra-muscularly. 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 (ETco2), 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, BPND values were corrected for differences between brain tissue VND and muscle VT 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 BPND parametric maps to individual MRI images used to obtain the average MRI image, BPND 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 (Figure 1B and 1C), there was a significant negative correlation between the number of responses and midbrain BPND 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 BPND 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 BPND 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 BPND values and number of

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**Figure 1.** (A) α4β2* nAChRs (BPND 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 BPND 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 BPND 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 BPND 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. BPND, nondisplaceable binding potential; 2-FA, 2-[18F]fluoro-A-85380; MRI, magnetic resonance imaging; nAChR, nicotinic acetylcholine receptors.
levels of dopamine D2 receptor (DRD2) expression are associ-
receptors and psychostimulant addiction. For example, low
was associated with a high motivation to self-administer nicotine
number of nicotine injections under the FR-10 schedule (all
This may reflect either different motivational aspects of
The observation that a low level of baseline midbrain nAChRs
was associated with a high motivation to self-administer nicotine
the reinforcing effects of psychostimulant drugs (11), blockade of
should be noted that in the above referenced DRD2 PET studies,

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.

This study was supported by the Intramural Research Pro-
gram of the National Institute on Drug Abuse, National Institutes
of Health (NIH), U.S. Department of Health and Human Services
(DHHS).

The authors reported no biomedical financial interests or
potential conflicts of interest.

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online.

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