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Varenicline increases in vivo striatal dopamine $D_{2/3}$ receptor binding: an ultra-high-resolution pinhole [^{123}I]IBZM SPECT study in rats☆

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Abstract

Introduction: Ex vivo storage phosphor imaging rat studies reported increased brain dopamine $D_{2/3}$ receptor ($DRD_{2/3}$) availability following treatment with varenicline, a nicotinergic drug. However, ex vivo studies can only be performed using cross-sectional designs. Small-animal imaging offers the opportunity to perform serial assessments. We evaluated whether high-resolution pinhole single photon emission computed tomography (SPECT) imaging in rats was able to reproduce previous ex vivo findings.

Methods: Rats were imaged for baseline striatal $DRD_{2/3}$ availability using ultra-high-resolution pinhole SPECT (U-SPECT-II) and [^{123}I]IBZM as a radiotracer, and randomized to varenicline (n=7; 2 mg/kg) or saline (n=7). Following 2 weeks of treatment, a second scan was acquired.

Results: Significantly increased striatal $DRD_{2/3}$ availability was found following varenicline treatment compared to saline (time∗treatment effect): posttreatment difference in binding potential between groups corrected for initial baseline differences was 2.039 (P=.022), indicating a large effect size (d=1.48).

Conclusions: Ultra-high-resolution pinhole SPECT can be used to assess varenicline-induced changes in $DRD_{2/3}$ availability in small laboratory animals over time. Future small-animal studies should include imaging techniques to enable repeated within-subjects measurements and reduce the amount of animals.

Keywords: [^{123}I]IBZM SPECT; Varenicline; Dopamine receptor; Neuroimaging; Rat; Striatum

1. Introduction

Important advances are made in small animal imaging, including the introduction of submillimeter-resolution pinhole single photon emission computed tomography (SPECT) [1,2]. An example is the U-SPECT-II system [2]. Compared to ex vivo/in vitro assessments, imaging offers the possibility to measure receptor availability repeatedly, resulting in smaller numbers of animals needed. The U-SPECT-II system uses multiple focusing pinholes, resulting in high reconstructed image resolution [2]. Moreover, due to its high sensitivity and stationary detectors, relatively short acquisition time frames can be used to acquire images. Indeed, a recent study reported on its use in monitoring tracer dynamics for occupancy of dopamine (DA) transporters by cocaine in mice [3].

Varenicline is a partial α4β2 nicotinic acetylcholine receptor agonist currently used for the treatment of nicotine dependence [4]. Previous rat studies performed by our

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group, using ex vivo storage phosphor imaging with the selective DA $D_{2/3}$ receptor ($DRD_{2/3}$) antagonist [^{123}I]IBZM, have shown downstream effects of varenicline treatment on the DAergic system [5,6]. More specifically, 2-week treatment with 2 mg/kg varenicline led to approximately 14% higher striatal $DRD_{2/3}$ availability compared to saline-treated rats [5,6].

Given the clear advantages of repeated imaging, the goal of the present study was to evaluate whether pinhole SPECT is able to show similar effects of varenicline on in vivo $DRD_{2/3}$ availability in rats.

2. Methods and materials

Fourteen male Wistar rats (Charles River, the Netherlands), weighing 317±16 g, were randomized to varenicline treatment (n=7; 2 mg/kg body weight) or saline (n=7). Baseline $DRD_{2/3}$ availability was measured using U-SPECT-II with the validated radiotracer [^{123}I]IBZM. Starting the morning after the baseline scan, study medication (0.50±0.05 mg varenicline in 0.40±0.05 ml solution, or 0.40±0.05 ml saline) was injected subcutaneously once a day (between 09:00 a.m. and 11:00 a.m.) for 14 consecutive days, after which a second IBZM SPECT scan was acquired on the evening of the 14th day (between 5:00 p.m. and 8:00 p.m.). All experimental procedures were carried out in accordance with the Dutch Law on Protection of Animals and were approved by the Animal Ethics Committee of the University Medical Center Utrecht, where imaging was conducted.

The 2-mg/kg body weight dose for 14 consecutive days was chosen in correspondence with previous papers by Crunelle et al. [5,6] that showed a significant effect on ex vivo striatal $D_{2/3}$ binding following varenicline treatment. Additionally, nicotine self-administration in rats is still suppressed dose-dependently until 3-mg/kg doses [7].

The radiotracer [^{123}I]IBZM (specific activity: 448±82 MBq/nmol; radiochemical purity >95%; GE Healthcare, Eindhoven, the Netherlands) was injected as a bolus (58.01±3.99 MBq, in a 0.30±0.05 ml solution; mass: 0.06±0.01 μg) in the tail vein, and scanning started 90 min later [8,9]. Rats were anesthetized by intraperitoneal injection of a mixture of ketamine (75 mg/kg), medetomidine (0.5 mg/kg) and atropine (0.04 mg/kg) starting just prior to the [^{123}I]IBZM injection, and were not allowed to wake up between injection and scanning. All animals received gas anesthesia (isoflurane 2%) as additional anesthesia during scanning in order not to wake up during scanning. In order to wake up quickly after anesthesia, 0.5 mg/kg Antisedan [intraperitoneal (ip)] was administered immediately after the scanning procedure. After completion of the second scan, rats were euthanized by ip injection with an overdose (2.5 ml) of pentobarbital.

For imaging, the U-SPECT-II system (MILabs B.V., Utrecht, the Netherlands) was used, with a cylindrical collimator containing 75 pinholes (each with an aperture of 1.0 mm) as described earlier [2]. During scanning, rats were fixed in a horizontally positioned plastic bed with tight-fitting head holder. Temperature and respiration rate were measured with BioVet Physiological monitoring system (M2M Imaging, Newark, NJ, USA). Two bed positions were used with a SPECT acquisition of 15 min per position. Therefore, animals were scanned from 90 to 105 min in one bed position in one frame and from 105 to 120 min in the other bed position in one frame, following injection of the radioligand. Photopeak and background energy windows were selected around 159 keV±7.5% and 186 keV±7.5%, respectively. Images were reconstructed on a 0.375×0.375×0.375-mm voxel grid using a pixel-based accelerated iterative ordered subset algorithm [1] based on maximum-likelihood expectation maximization. Images were reconstructed using a precalculated matrix [10] with six iterations using 16 subsets. Postreconstruction images were blurred using Gaussian smoothing on the PMOD software package (version 3.0, PMOD Technologies Ltd.), and volumes of interest (VOIs) were drawn for the rat striatum in both hemispheres using isocontour lines (counts per pixel) at a 50% threshold (Fig. 1). VOI statistics were read on the noisy (nonblurred) images. The striatum as a whole was analyzed, and mean counts per pixel were calculated for combined right and left striatal regions in the PMOD-selected VOI. VOIs of the cerebellum as region of nonspecific binding were drawn using a standard cube sphere in the cerebellar region of 0.32 cm^3 in all rats.

As the outcome measure, the binding potential (BP_{ND} [11]) was calculated as follows: (total striatal [^{123}I]IBZM binding minus cerebellar binding)/cerebellar binding.

2.1. Statistics

Baseline differences in [^{123}I]IBZM BP_{ND} were tested with the t test for independent samples. Treatment effects were assessed with linear regression analyses with change in [^{123}I]IBZM BP_{ND} between baseline and posttreatment as the dependent variable, and treatment and [^{123}I]IBZM BP_{ND} at baseline as independent variables. In this model, the regression coefficient for treatment equals the difference in [^{123}I]IBZM BP_{ND} change between treatment groups adjusted for baseline differences. Statistical analysis were performed with SPSS version 18 (SPSS Inc., Chicago, IL, USA) using the t test and general linear model modules. Additionally, standardized effect size was calculated as the difference between the means of the varenicline and saline conditions and divided by the pooled standard deviation of the difference (Cohen's d). All tests were done using a two-sided alpha of 0.05. All descriptive data are presented as mean±the standard deviation.

3. Results

Due to paravasal injection of the radioligand in one rat, analyses were performed on data from seven animals in the saline-treated group and six animals in the varenicline-

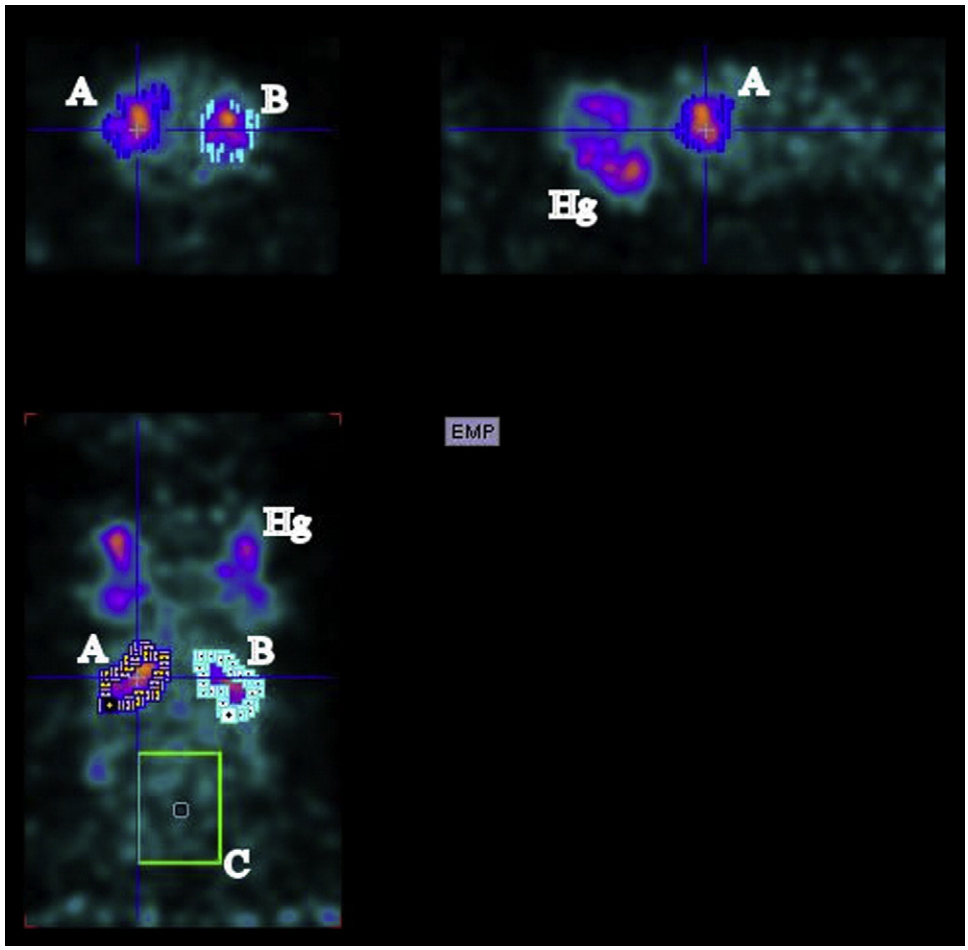

Fig. 1. A typical smoothed postreconstruction image with VOIs drawn for the rat striatum in left (A) and right (B) hemispheres using isocontour lines at a 50% threshold. VOIs of the cerebellum (C) were drawn using a standard cube sphere in the cerebellar region. Images were acquired 90 min after injection of 60.75 MBq [^{123}I]IBZM in a 0.30-ml solution (mass: 0.06 μg). Anesthesia included a mix of ketamine (75 mg/kg), medetomidine (0.5 mg/kg) and atropine (0.04 mg/kg) prior to scanning, and isoflurane 2% during scanning. Images were acquired using two bed positions with an acquisition time of 15 min per position. Uptake in the Harderian glands (Hg) is also noticeable.

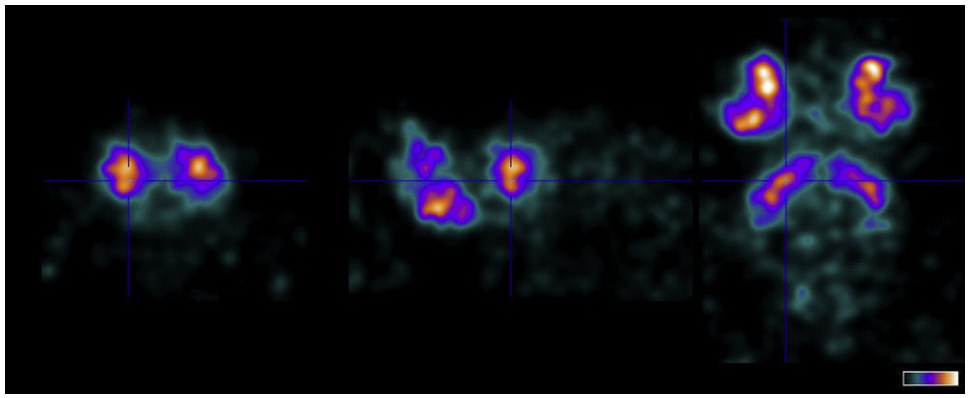

Fig. 2. High-resolution in vivo imaging of [^{123}I]IBZM binding to striatal $DRD_{2/3}$ receptors in rat, with the U-SPECT-II system, scanned 90 min after injection of 58.13 MBq in a 0.30-ml solution (mass: 0.06 μg) using anesthesia by intraperitoneal injection of ketamine (75 mg/kg), medetomidine (0.5 mg/kg) and atropine (0.04 mg/kg) prior to the radiotracer injection, and isoflurane 2% during scanning. Images were obtained using two bed positions with a SPECT acquisition time of 15 min per position. Shown are coronal (left panel), sagittal (mid panel) and horizontal slices (right panel). A cross is positioned at the level of the striatum. Also, nonspecific uptake of the radiotracer in the Harderian glands is clearly visualized.

treated group. As expected, IBZM binding was clearly and symmetrically visualized in the striatum of all animals, and cerebellar binding was much lower but could be delineated accurately (Fig. 2).

Baseline striatal $DRD_{2/3}$ BP_{ND} did not differ significantly between saline- and varenicline-treated animals (saline 4.94±2.03 vs. varenicline 3.92±1.13; d=0.62; P=.30). Following treatment, varenicline-treated animals showed statistically significantly greater changes in striatal $DRD_{2/3}$ BP_{ND} (posttreatment BP_{ND}: 6.14±1.44; 57% higher than baseline) compared to saline-treated rats (4.14±1.01; 16% lower than baseline). The posttreatment difference in BP_{ND} between groups corrected for initial baseline differences was 2.039 (S.E.=0.752, t=−2.713, P=.022; 95% CI 0.36–3.72) (Fig. 3), indicating a large effect size (d=1.48).

4. Discussion

Using ultra-high-resolution pinhole SPECT, we were able to measure in vivo significant changes in striatal $DRD_{2/3}$ availability after 2 weeks of varenicline treatment compared to saline treatment in a within-subject design.

Acute varenicline administration increases in vivo dopamine release in mice and rats [12,13], and one may therefore expect a down-regulation of $DRD_{2/3}$ receptors, which are localized predominantly postsynaptically. However, the effects of *sustained* administration of varenicline on in vivo dopamine release have, to our knowledge, not yet been determined. Sustained administration might cause the DAergic system to respond with decreased cell firing to DAergic cell bodies presynaptically (mainly located in the ventral tegmental area, projecting to the ventral striatum), thereby inducing up-regulating of the postsynaptic $DRD_{2/3}$ receptors (for a more detailed hypothesis on the working mechanism of varenicline treatment on $DRD_{2/3}$ up-regulation, see Refs. [6] and [14]).

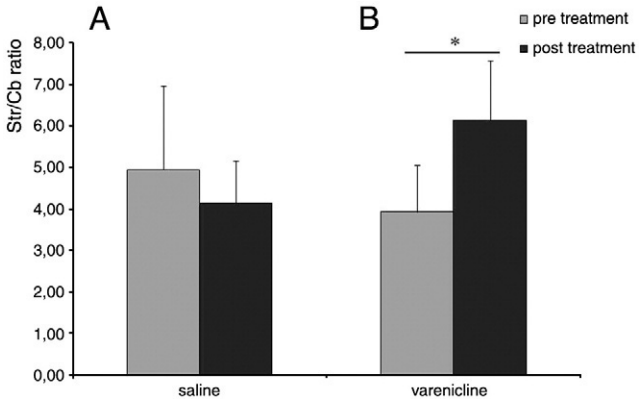

Fig. 3. Specific striatal to nonspecific [^{123}I]IBZM BP_{ND} following injection of [^{123}I]IBZM at baseline and after 14 days of subcutaneous saline (A) or varenicline (B) treatment in rats. Both baseline and posttreatment ratios are presented as mean±SD. *P<.05.

The results of the present study are in line with previous studies using storage phosphor imaging, in which we reported significantly higher striatal $DRD_{2/3}$ BP_{ND} after 2 weeks of varenicline treatment as compared to saline treatment in rats [5,6]. More specific, our ex vivo studies found significant increases after varenicline administration of 14% (95% CI: 3%–25%) and 13% (95% CI: 1%–25%) in the first [5] and second study [6], respectively. When we look more closely into the present data and compare the results of only the posttreatment scans between the two groups under study, we still found a statistically significant (P=.020; F=0.44; t=2.85; df=8.80) difference of 48% (95% CI: 12%–84%) in striatal $DRD_{2/3}$ availability between treatment groups. It should be noted that this difference looks rather big (48% vs. 14% and 13%), but the observed differences in the previous studies are still within the 95% CI of the current study (12%–85%). A possible explanation may be the relatively low baseline BP_{ND} in the saline condition in the current study, whereas the relatively broad 95% CI of the difference in BP_{ND} between the two treatment conditions in the current study may be related to the relatively large variation of the data (SDs approximately 29% in our current SPECT study compared to SDs of approximately 10% in the storage phosphor imaging studies [5,6]). The larger SD in the current study compared to the previous ex vivo studies could be attributed either to the smaller number of animals in the current study compared to the ex vivo studies or to the more reliable assessment of striatal $DRD_{2/3}$ binding with storage phosphor imaging (e.g., no partial volume artefacts). Additionally, in our current study, the saline group had decreases (although not statistically significantly) in $DRD_{2/3}$ binding over time, while the varenicline-treated group showed an opposite effect, an effect that can not been shown using a cross-sectional study [5,6]. Nevertheless, compared with a cross-sectional study design, a repeated imaging study design commonly enables the detection of statistically significant changes between groups with a lower number of animals per group.

Our previous storage phosphor studies and the current study show similarities by using same dosages of medication, identical rat strains and IBZM from the same supplier. However, in contrast with the former studies, animals were subjected to injections for 14 days followed by anesthesia and sacrificed, and rats might have been more stressed due to the baseline scanning and waking up before starting the injections for 14 days. Additionally, while ex vivo storage phosphor imaging was performed 24 h following the last injection of varenicline/saline, SPECT imaging occurred on the same day of the last injection (approximately 8 h following the last injection), which may have led to an greater difference in $DRD_{2/3}$ binding between groups. Also, the acquired SPECT images did not allow us to assess reliably $DRD_{2/3}$ binding in dorsal versus ventral striatal regions. Moreover, we did only measure BP_{ND}. In future studies, it may be of interest to account for possible changes in metabolism of IBZM by measuring parent compound in plasma in rats and to calculate BP_P. However, theoretically,

it is not likely that varenicline will influence the metabolism of IBZM. Also, we did not measure the total numbers (Bmax) of $DRD_{2/3}$ receptors in the rat brain in this or previous studies [5,6], which might have been interesting to test whether the presently observed increased IBZM binding reflects up-regulation of DA receptors. Finally, the radiotracer was injected as a 0.3-ml solution. The variability of the injected mass and the time lapse between injections of the first and last animal might also have affected, even simply, the variability in the data. Also, several anesthesia were used which are known to affect D2 binding. However, all animals were given the same amount of anesthesia in an identical time lapse in both groups and over both sessions, such that possible changes in receptor binding induced by anesthesia would not have affected varenicline-treated rats differently compared to saline-treated rats or between scanning sessions. We acquired static images at one time point after injection. In future studies, it would be of interest to acquire dynamic images to optimize the quantification (e.g., using the reference tissue method). However, previous pinhole SPECT studies have shown that, in rodents, specific striatal IBZM binding ratios are reached 80–90 min postinjection [8,9] and remains stable up to at least 120 min postinjection. Nevertheless, it is not addressed yet whether the presently calculated binding potential (BP_{ND}) under transient equilibrium conditions is in agreement with the calculation under true equilibrium conditions.

In conclusion, we provide evidence that ultra-high-resolution [^{123}I]IBZM SPECT can detect changes in brain $DRD_{2/3}$ availability induced by varenicline. Future studies should include imaging techniques in experiments in small animals to enable repeated within-subjects measurements, which may reduce consequently the amount of animals needed, although reproducibility studies are needed to calculate the power of this approach.

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