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Characterization of Optically Resolved 9-fluoropropyl-dihydrotetrabenazine as a Potential PET Imaging Agent Targeting Vesicular Monoamine Transporters

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Abstract

Labeling derivatives of dihydrotetrabenazine (DTBZ) with F-18 ($T_{1/2} = 110$ min) instead of C-11 ($T_{1/2} = 20$ min), would improve their utility and availability for imaging vesicular monoamine transporters (VMAT2) in clinical settings. The successful synthesis, reported previously, of two novel 9-fluoroalkyl(±)-DTBZ ligands prompted us to study the optically resolved active ligand 9-fluoropropyl-(+)-DTBZ (FP-(+)-DTBZ), which may have more promising characteristics. The inhibition constant (K_i) estimated for FP-(+)-DTBZ (using [^3H](±)-DTBZ as the labeled ligand in rat striatal homogenates) showed a lower value as compared to the racemic FP-(±)-DTBZ (0.10 ± 0.01 vs 0.19 ± 0.04 nM). The inactive isomer, FP-(−)-DTBZ, displayed a much lower binding affinity with a K_i value >3000 nM. Biodistribution studies in mice after an iv injection of [^{18}F]FP-(+)-DTBZ exhibited a ratio of striatum (ST, target) to cerebellum (CB, background) of 4.51 at 30 minutes post-injection, which is a higher value than previously obtained with the racemic ligand [^{18}F]FP-(±)-DTBZ (ST/CB = 2.95). Brain extraction at 30 minutes after the tracer injection in mice showed that $>95\%$ of the radioactivity corresponded to the parent, non-metabolized, compound remaining in the striatum, suggesting that the tracer has an excellent *in vivo* stability. Furthermore, localization of the tracer in the brain examined with *ex vivo* autoradiography displayed a typical distribution pattern consistent with VMAT2 sites. The highest labeling was observed in monoaminergic neuron regions (caudate putamen, olfactory tubercle, nucleus accumbens, substantia nigra, dorsal raphe and locus coeruleus). We also tested the selective labeling of this tracer at the dopamine neurons in unilateral-lesioned mice (treated with 6-hydroxydopamine). When [^{18}F]FP-(+)-DTBZ and [^{125}I]IPT ((N-(3'-iodopropen-2'-yl)-2-beta-carbomethoxy-3-beta-(4-chlorophenyl)tropane, a selective marker for dopamine transporters in dopaminergic neurons) were simultaneously injected into lesioned mice, we observed an excellent correlation ($r = 0.95$) for these tracers. From these findings, we conclude that [^{18}F]FP-(+)-DTBZ is a sensitive and selective tracer for VMAT2 binding sites and it may be useful for *in vivo* evaluation of diseases relating to changes of monoamine neuronal integrity.

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Introduction

The vesicular monoamine transporter type II (VMAT2) is an integral part of the mechanism for vesicular packaging and storage of monoamine neurotransmitters in the synapses of the brain. In contrast to the situation on the synaptic membrane, where there are specific and distinct transporters for active reuptake of three monoamines (dopamine, serotonin and norepinephrine), a common ATP-dependent transporter, VMAT2, is responsible for the movement of all three of the monoamines from the cytosol into the vesicular lumen. Therefore, imaging VMAT2 in the brain can provide a measurement of the integrity (total number) of all three types of monoaminergic neurons [1,2].

Degeneration of nigrostriatal neurons plays a central role in Parkinson's disease (PD). Increasing evidence suggests that positron emission tomography (PET) and single photon emission tomography (SPECT) imaging agents may provide useful clinical tools for diagnosis of PD [3–8]. There is a significant scientific need to develop probes for monitoring the integrity of dopamine neurons by PET and SPECT imaging [4,5]. In the past ten years, a plethora of dopamine transporter (DAT) imaging agents have been developed, most of which are tropane (or cocaine) derivatives which have varying degrees of affinity to serotonin and norepinephrine transporters [9–12].

Imaging of the VMAT2 has been proposed as an alternative for following degeneration of monoaminergic (primarily dopaminergic) neurons in Parkinson's disease. In animal models, the loss of monoamine neurons has been shown to be directly correlated to the reduction of radioligand binding to VMAT2 in the terminal fields [13]. Furthermore, rapid and differential losses of *in vivo* DAT ($[^{11}\text{C}]$ methylphenidate) and VMAT2 ($[^3\text{H}](\pm)$ -DTBZ) radioligand binding was demonstrated in MPTP-treated mice suggesting that the VMAT2 binding sites are proportionally related to the existence of functional dopamine neurons [14,15]. Animal data furthermore showed that $[^{11}\text{C}](+)$ -DTBZ is less sensitive to drugs affecting chronic dopamine levels in the brain, and thus might serve to accurately reflect the concentration of viable monoamine neurons [16–19]. PET imaging with VMAT2 radioligands has also been suggested as a method for measuring selective degeneration of projection neurons in the striatum of Huntington's disease [17,18].

$[^{11}\text{C}]$ TBZ (tetrabenazine) and derivatives targeting VMAT2 have been successfully developed and tested in humans [20]. As TBZ was known to be rapidly reduced *in vivo* to the corresponding hydroxyl compound dihydrotetrabenazine (DTBZ) [21], radioligand development for PET imaging focused on synthesis of $[^{11}\text{C}]$ DTBZ itself [22]. The racemic $[^{11}\text{C}](\pm)$ -DTBZ consists of at least two enantiomers, one of which, $[^{11}\text{C}](+)$ -DTBZ, is the active isomer [22,23]. Finally, synthesis of the single optically resolved isomer, $[^{11}\text{C}](+)$ -DTBZ (labeled at the 9-MeO position), provided the most specific PET tracer for measuring VMAT2 sites in the brain [22,24].

To further enhance the availability of PET imaging for studying VMAT2 binding sites, we have been searching for ^{18}F ($T_{1/2} = 110$ min) labeled DTBZ analogs, which may significantly increase the application of these tracers in clinics that are equipped with PET scanners, but do not have sufficient resources to make $[^{11}\text{C}](+)$ -DTBZ ($T_{1/2} = 20$ min). It is likely that a ^{18}F labeled VMAT2 tracer could be readily manufactured and distributed by the same national network of radiopharmacies currently supplying $[^{18}\text{F}]$ FDG for PET imaging studies in the clinics.

Recently, we have successfully prepared and tested the first series of fluorinated ($\pm$)-DTBZ derivatives targeting VMAT2 binding sites of dopamine, serotonin and norepinephrine neurons in the brain [25]. Two racemic derivatives, 9-fluoropropyl (FP)- and 9-fluoroethyl (FE)-DTBZ, displayed high binding affinities for VMAT2, and syntheses of these compounds in fluorine-18

form have been accomplished. In rodents, the radiofluorinated ligands showed excellent *in vivo* properties with distinct target (striatum) to background (cerebellum) ratio after an iv injection [25]. The 9-fluoropropyl derivative, FP-(±)-DTBZ, was chosen for further evaluations based on its better *in vivo* biodistribution profile in mouse brain. One major focus of our studies was to evaluate the stereoselectivity of binding between various enantiomers. It is known that (+)-DTBZ is the active enantiomer. We expected that the corresponding 9-fluoropropyl derivative, FP-(+)-DTBZ, would also be the active enantiomer; while the inactive isomer would be FP-(−)-DTBZ. In this study we are reporting the comparative binding affinity of the enantiomers and the *in vivo* characterization of the radiolabeled optically resolved enantiomer, [¹⁸F]FP-(+)-DTBZ, in normal and lesioned mice. The resolved [¹⁸F]FP-(+)-DTBZ ligand, demonstrating comparable and even better properties than [¹¹C](+)-DTBZ targeting VMAT2, will be more desirable for clinical application.

Materials and Methods

General

Male ICR mice weighing 20–25 g were used for the *in vivo* studies. Mice lesioned unilaterally with 6-hydroxydopamine (6-OH-DA) were obtained from Taconic (Hudson, NY). [³H](±)-TBZ, (−)-TBZ and (+)-(2R,3R,11bR)-9-O-desmethyl-α-dihydrotetrabenazine were kindly provided by NIH under the chemical synthesis and drug supply program. [¹²⁵I]IPT [N-(3'-iodopropen-2'-yl)-2-beta-carbomethoxy-3-beta-(4-chlorophenyl)tropane] was prepared in our lab following the procedure described previously [26]. Chemical synthesis of the optically active isomer was essentially identical to that reported previously for racemic FP-(±)-DTBZ [25]. Resolved 9-O-desmethyl-(+)-DTBZ was similarly alkylated by reacting with 3-fluoropropyl-p-toluenesulfonate to yield the optically resolved fluoropropyl derivative, FP-(+)-DTBZ. The inactive enantiomer FP-(−)-DTBZ was prepared from the resolved (−)-TBZ through reduction, deblocking and finally alkylation, following procedures reported previously [25]. FP-(+)-DTBZ and FP-(−)-DTBZ, as well as the racemic FP-(±)-DTBZ, were analyzed by using a chiral AD column (4.6 x 250 mm, Chiral Technologies, Inc.) with hexanes/isopropanol = 9/1 as the eluting solvent at a flow rate of 1 mL/min. The corresponding mesylate precursor for labeling of the optically resolved enantiomer, [¹⁸F]FP-(+)-DTBZ, was prepared as described previously for the racemic mixture [25]. Similar labeling and purification procedures to prepare the racemic ligand were also applied to the resolved isomer, and [¹⁸F]FP-(+)-DTBZ was successfully obtained with a specific activity of 1,500–2,000 Ci/mmol at the end of synthesis.

Homogenate Binding

Frozen rat brains were purchased from Pel-Freez (Invitrogen, <http://www.invitrogen.com>). The basal forebrain tissues containing the striatal region of the brains were dissected and pooled. The brain tissue homogenates were prepared in 50 mM Hepes, pH 7.5 and 0.32 M sucrose.

The specific binding of [^3H]($\pm$)-TBZ was determined following the procedures described [27]. The total volume of reaction for the assay was 0.2 ml. In competition experiments, compounds at concentrations up to 10^{-5}M were examined for their abilities to compete for the binding of [^3H]($\pm$)-TBZ (1.0–1.5 nM). Incubations were carried out routinely at room temperature for 90 min. The samples were then filtered through glass fiber filters No. 25 (Schleicher and Schuell, Keene, NH) and the filters containing bound ^3H ligand were dissolved in 7 ml Ecolite(+) overnight and the radioactivity was counted the next day in a scintillation counter (Beckman) with 65% counting efficiency. Nonspecific binding was determined in the presence of 10 μM ($\pm$)-TBZ. The results of inhibition experiments were subjected to nonlinear regression analysis using equilibrium binding data analysis by which K_i values were calculated.

Organ Distribution in Normal Mice

After anesthetizing ICR mice (22–25 g male) with isoflurane, 0.15 mL of a 0.1% bovine serum albumin solution containing [^{18}F]FP-(+)-DTBZ (10–20 μCi) was injected directly into the tail vein. The mice ($n = 3$ for each time point) were sacrificed by cervical dislocation (under isoflurane anesthesia) at indicated time-points post injection. The organs of interest were removed and weighed, and the radioactivity was counted with an automatic gamma counter. The percentage dose per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material. The total activity of the blood was calculated under the assumption that it was 7% of the total body weight. The %dose/g of samples was calculated by comparing the sample counts with the count of the diluted initial dose. Different regions corresponding to striatum (ST), hippocampus (HP), cerebellum (CB), and cortex (CX) were dissected from the brain and counted to obtain the regional distribution of the tracer.

For the dual isotope experiment, four lesioned mice obtained from Taconic were injected with a mixture of 20 μCi each of [^{18}F]FP-(+)-DTBZ and [^{125}I]IPT (N-(3'-iodopropen-2'-yl)-2-beta-carbomethoxy-3-beta-(4-chlorophenyl)tropane, a dopamine transporter ligand). Thirty minutes after injection, the mice were sacrificed and the brains removed. Different regions corresponding to striatum (ST), hippocampus (HP), cerebellum (CB), and cortex (CX) were dissected. In particular, the lesioned region was separated from non-lesioned area of the striatal tissues. Samples were counted in a gamma counter with two different window settings for F-18 and I-125, respectively, to obtain the regional distribution of each tracer.

Ex vivo Autoradiographic Studies

Both normal ICR mice and lesioned mice were injected with 200–400 μCi [^{18}F]FP-(+)-DTBZ and sacrificed 30 minutes post-injection. The brains were then removed immediately and frozen with powdered dry ice. Coronal sections of 20 μm thickness were cut on a cryostat microtome, thaw-mounted onto Fisher Superfrost Plus slides and dried at room temperature. The dried tissue sections were then exposed to Kodak Biomax MR film for ^{18}F tracer (overnight) in an autoradiographic cassette together with 20 μm thick ^{125}I standards (Amersham, Arlington Heights, IL).

Metabolic Stability in Mouse Brain

Two mice were injected with 200 μCi [^{18}F]FP-(+)-DTBZ and sacrificed at 30 minutes post-injection. The brains were removed and the striatal regions were dissected out. The striatal homogenates (prepared in PBS, 10 % w/v) were extracted with 0.5 ml of ethyl acetate in the presence of a small amount of carrier (10 μg of FP-(+)-DTBZ). Extraction was repeated three times and the ethyl acetate layers and the buffer layers were counted separately to determine the percent extracted. After condensing the ethyl acetate layers, the samples were analyzed on TLC (Silica 254F plate with the developing solvent of chloroform/ethanol/con.ammonium = 8/2/drop) for metabolite identification. A control sample ([^{18}F]FP-(+)-DTBZ mixed with normal mouse striatal homogenates in situ) was carried out in parallel for comparison.

Results and Discussion

We have recently reported racemic 9-fluoroalkyl derivatives of DTBZ as potential PET imaging agents targeting VMAT2 sites in the brain [25]. Due to the stereoselective binding of this series of compounds, we undertook the synthesis and evaluation of their enantiomers to identify the active isomer responsible for VMAT2 binding. The chemical synthesis and drug supply program of NIH kindly provided us with the optically resolved precursors, i.e. 9-O-desmethyl-(+)-DTBZ and (–)-TBZ as the starting material to prepare the desired enantiomers, FP-(+)-DTBZ and FP-(–)-DTBZ, respectively. The same reactions used for preparing racemic FP-(±)-DTBZ were used for the optically resolved derivatives.

Enantiomeric separation of 9-desmethyl-DTBZ has been reported previously by Kilbourn et al. [22]. That paper provided several useful starting points for our current work. Theoretically, we expected that racemic (±)TBZ would have four isomers. However, due to geometric constraints of the tetrahydroisoquinoline ring system and the steric bulk of the 3-*t*-butyl group, the synthesis of (±)TBZ normally produces only one pair of enantiomers, i.e. (3R, 11bR)-TBZ and (3S, 11bS)-TBZ (Fig. 1). After reduction of the keto group at the 2-position, the (±)-TBZ should produce four different isomers of (±)-DTBZ: (2R, 3R, 11bR), (2S, 3S, 11bS), (2R, 3S, 11bS) and (2S, 3R, 11bR). Chiral column liquid chromatography and enantiospecific enzymatic hydrolysis have been utilized to separate the enantiomers of (±)-DTBZ [22]. Kilbourn et al. found that an X-ray crystal structure analysis of (–)-α-9-O-desmethyl-dihydrotetrabenazine showed an absolute configuration of this compound as the (2S, 3S, 11bS)-isomer: when subsequently converted to (–)DTBZ this was identified by *in vitro* binding studies as the inactive isomer. Therefore, it was concluded that (+)-DTBZ has the absolute configuration of 2R, 3R, 11bR. The major obstacle for the enantiomer identification of (±)-DTBZ derivatives is the separation of these four potential isomers, particularly, the two major enantiomers (the active isomer: 2R, 3R, 11bR vs. the inactive isomer: 2S, 3S, 11bS) [22]. It was expected that FP-(±)-DTBZ, a 9-fluoropropyl-substituted derivative, would present very similar requirements for separation of the corresponding four possible enantiomers. Our goal was to identify the specific isomer that was responsible for high affinity binding to the VMAT2 sites. We carefully confirmed the optical resolution of the enantiomers by using a HPLC system equipped with a chiral column-AD Chiracel. As expected, the racemic FP-(±)-DTBZ yielded a HPLC profile with four distinct peaks. They showed retention times of 12.5 (peak 1), 18.3 (peak 2), 21.6 (peak 3) and 30.4 min (peak 4), respectively (Fig. 1). The ratio of combined peaks 3 and 4 to combined peaks 1 and 2 was approximately 5 to 1. This indicated that the pair of enantiomers, peak 3 (active enantiomer) and peak 4 (the inactive enantiomer) are the predominant pair as compared to the minor pair enantiomers peak 1 and peak 2. The optical resolution of FP-(+)-DTBZ (peak 3), as shown in Fig. 1, was greater than 98%, and only a trace amount of one other enantiomer (peak 2, <2%) was detected in the sample. When a sample of FP-(–)-DTBZ was examined on the same AD column, it showed two peaks, 1 and 4, with a 1 to 9 ratio. The HPLC profiles demonstrated that we could clearly separate FP-(+)-DTBZ and FP-(–)-DTBZ on the chiral AD column. All of the data indicated that we had successfully obtained enantiomers with high enantiomeric purities.

The racemic FP-(±)-DTBZ and the resolved enantiomer FP-(+)-DTBZ and FP-(–)-DTBZ were subjected to a competition binding study with [³H](±)-TBZ in rat striatal homogenates. We observed a dramatic difference in binding potency between FP-(+)-DTBZ and FP-(–)-DTBZ (Table 1). The active enantiomer FP-(+)-DTBZ was 30,000 fold more potent than the inactive enantiomer FP-(–)-DTBZ showing K_i values of 0.1 ± 0.04 and >3000 nM, respectively. Consistently, the optically resolved enantiomer FP-(+)-DTBZ displayed a slightly higher binding affinity compared to that of the racemic FP-(±)-DTBZ ($K_i = 0.10 \pm 0.04$ vs 0.19 ± 0.04 nM). These results agree with the previous work on (+)-DTBZ in which the active isomer had a K_i value of 0.97 nM, while the other inactive enantiomer, (–)DTBZ, showed a very low

affinity ($K_i = 2200$ nM) [28]. Our results clearly confirmed the stereoselectivity of FP-DTBZ binding to VMAT2, with the same enantiomeric preference as that of DTBZ. The active enantiomer, FP-(+)-DTBZ showed an excellent binding affinity and would be a useful VMAT2 imaging agent. In contrast, the binding affinity of the inactive enantiomer FP-(-)-DTBZ to VMAT2 was very low.

Radiolabeled [^{18}F]FP-(+)-DTBZ was obtained by following the same procedures reported for the preparation of racemic [^{18}F]FP-($\pm$)-DTBZ [25]. A similar biodistribution in mice with a good brain penetration (4.82% dose/g at 2 min) was observed for the optically resolved ligand [^{18}F]FP-(+)-DTBZ (Table 2). The brain distribution clearly indicated the selective localization of the ligand in regions of brain enriched with monoamine neurons, i.e. it was the highest in striatum region (ST) for dopamine neurons followed by hypothalamus region (HP) for serotonin and/or norepinephrine neuronal terminals. Using the cerebellum (CB) as the background region (a nontarget region containing a minimal concentration of VMAT2), the ST/CB ratio peaked between 30 to 60 minutes (4.5–4.7), and the HP/CB ratio was 3.5 to 2.7 in the same period (Table 2). This is an improved ST/CB ratio (target vs. non-target) for the optically resolved [^{18}F]FP-(+)-DTBZ as compared to the ratio reported previously for the racemic [^{18}F]FP-($\pm$)-DTBZ (4.51 vs. 2.95 at 30 minutes post-injection, respectively) [25]. Clearly, the fluorinated derivative of DTBZ, especially the optically resolved enantiomer [^{18}F]FP-(+)-DTBZ, displayed a higher and better target vs. non-target ratio. Signal to noise ratios were improved in comparison with the value reported for the parent ligand [^{11}C]-(+)-DTBZ (ST/CB= 3.0 at 20 minutes post-injection) [23]. We also extracted the tracer from brain tissues at 30 minutes after an iv injection, and found less than 5% degradation of this tracer (data not shown). We, therefore, conclude that [^{18}F]FP-(+)-DTBZ is stable in the mouse brain. The *in vivo* metabolic stability is important because it suggests that the specific signals in regions of the brain are the direct result of binding association between the tracer and VMAT2 binding sites. These desirable characteristics (high selective contrast between target vs. non-target and enhanced brain stability) indicate that [^{18}F]FP-(+)-DTBZ is a suitable probe for *in vivo* imaging of VMAT2 in the brain. Initial non-human primate PET imaging studies with [^{18}F]FP-($\pm$)-DTBZ and [^{18}F]FP-(+)-DTBZ have been carried out (Kilbourn, unpublished data). The results, which are encouraging, will be published in a separate paper in the near future.

The anatomical distribution of [^{18}F]FP-(+)-DTBZ in the mouse brain was further evaluated by *ex vivo* autoradiography (Fig. 2). Dopamine-enriched regions, i.e. the caudate putamen, accumbens, olfactory tubercle and substantia nigra as well as serotonin and norepinephrine neuron-enriched regions, i.e. the hypothalamic nucleus, dorsal raphe, median raphe and locus coeruleus showed distinctive labeling reflecting VMAT2 density. The low background and high signal to noise ratio observed by autoradiography were consistent with the data measured by regional brain tissue dissection method (Table 2).

Normally, a parkinsonian rat model would be used for testing the sensitivity of measurement of selective neuronal loss. The model is often produced by lesioning the forebrain bundle of the rat brain with 6-OH-DA, which disrupts dopaminergic neuron projection to the caudate putamen area [29]. In the present study, we used a group of mice that were lesioned on one-side of the brain by a direct injection of 6-OH-DA into striatum (obtained from Taconic). This mouse model is less predictable due to the uncertainty of a direct injection of 6-OH-DA into a specific brain region. However, we were able to purchase a few lesioned mice showing the expected lesioning of striatal neurons. Using this lesion-mouse model, [^{18}F]FP-(+)-DTBZ clearly differentiated the normal from the lesioned site. We observed highly dense labeling in the normal caudate putamen (CPu) and very low labeling on the lesioned side of CPu (Fig. 3). To accurately measure the degree of lesion, we performed a dual-isotope experiment comparing the striatal uptakes in lesioned vs. unlesioned sites using [^{18}F]FP-(+)-DTBZ. A well

characterized marker, [125 I]IPT (selective for dopamine transporters) was injected simultaneously. As expected, these mice showed a range of neuronal damage, from slight lesion to a more severe lesion, as evidenced by reduced binding of both radiotracers in the lesioned side (Table 3). Lower uptakes in the lesioned sites as measured by [18 F]FP-(+)-DTBZ (VMAT2 sites) correlated very well with the data obtained with [125 I]IPT (DAT sites) ($r = 0.95$). Our results with [18 F]FP-(+)-DTBZ are thus consistent with earlier studies showing reduced VMAT2 binding in rodent models of unilateral dopaminergic nerve destruction [13,30].

In conclusion, we are reporting a novel PET imaging agent for VMAT2 based on an optically resolved 9-fluoropropyl derivative of DTBZ, [18 F]FP-(+)-DTBZ. It exhibited high binding affinity, excellent *in vivo* kinetics and unmatched target-to-nontarget ratios. In a parkinsonian model, using 6-OH-DA lesioned mice, the novel agent displayed reduced uptakes in the lesioned side of caudate putamen, which is consistent with the loss of dopaminergic neurons. We are confident that [18 F]FP-(+)-DTBZ can be a useful PET imaging agent for neurodegenerative diseases associated with a reduction of monoamine neurons.

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Fig 1.

Separation of four enantiomers of FP-(±)-DTBZ on a chiral AD column with hexanes/isopropanol (9/1) as the eluting solvent (flow rate of 1 mL/min). The ratio of two major enantiomers (peak 3 & 4) to the minor ones (peak 1 & 2) is 5:1. The optical resolution of FP-(+)-DTBZ reached 98% (shown as peak 3) and the inactive isomer FP-(-)-DTBZ showed a major peak 4 (90%) with a small contamination of peak 1 (10%).

Fig 2.

Ex vivo autoradiography of a normal mouse brain revealed the anatomical localization of VMAT sites labeled by [^{18}F]FP-(+)-DTBZ. In this experiment 500 μCi [^{18}F]FP-(+)-DTBZ was injected into a normal ICR mouse and sacrificed at 30 minutes post-injection. The high-density sites clearly reflected the regional distribution of monoaminergic neurons in the brain: CPu, caudate putamen; OT, olfactory tubercle; Ac, nucleus accumbens; Hy, hypothalamic nucleus; SN, substantia nigra; DR, dorsal raphe; MR, median raphe; LC, locus coeruleus.

Fig 3.

Ex vivo autoradiography (30 minutes postinjection) of [^{18}F]FP-(+)-DTBZ clearly differentiate the lesioned (L, indicated by an arrow) from the un-lesioned side (N) in the brains of 6-OH-DA unilaterally lesioned mice. 300 μCi [^{18}F]FP-(+)-DTBZ was injected and animals were sacrificed at 30 minutes post-injection.

Table 1

Inhibition constants (K_i , mean $\pm$ SEM) of FP-($\pm$)-DTBZ, FP-(+)-DTBZ and FP-(-)-DTBZ on [^3H]($\pm$)-TBZ binding to VMAT2 in rat striatal homogenates

Compound	K_i (nM)
FP-($\pm$)-DTBZ	0.19 ± 0.04
FP-(+)-DTBZ	0.10 ± 0.01
FP-(-)-DTBZ	>3000
($\pm$)-TBZ	1.3 ± 0.1

K_d value of 8.2 nM for [^3H]($\pm$)-TBZ was used for the calculation based on the K_i value reported previously [23]. The results are means $\pm$ SEM of three independent measurements done in duplicate.

Table 2Biodistribution of [^{18}F]FP-(+)-DTBZ in normal mice (avg of 3 mice $\pm$ SD)

Organ distribution (%dose/g)					
Organ	2 min	30 min	60 min	120 min	240 min
Blood	2.12 $\pm$ 0.17	1.73 $\pm$ 0.15	0.97 $\pm$ 0.04	0.57 $\pm$ 0.09	0.06 $\pm$ 0.02
Heart	5.47 $\pm$ 1.19	2.43 $\pm$ 0.22	1.67 $\pm$ 0.02	0.91 $\pm$ 0.14	0.12 $\pm$ 0.01
Muscle	0.78 $\pm$ 0.11	1.10 $\pm$ 0.12	0.73 $\pm$ 0.04	0.53 $\pm$ 0.05	0.06 $\pm$ 0.01
Lung	9.04 $\pm$ 4.71	3.17 $\pm$ 0.33	2.00 $\pm$ 0.05	1.26 $\pm$ 0.13	0.19 $\pm$ 0.01
Kidney	10.7 $\pm$ 2.02	5.81 $\pm$ 1.15	3.48 $\pm$ 0.34	2.28 $\pm$ 0.34	0.31 $\pm$ 0.02
Spleen	7.38 $\pm$ 1.62	3.69 $\pm$ 0.25	2.11 $\pm$ 0.05	1.40 $\pm$ 0.35	0.17 $\pm$ 0.02
Liver	24.6 $\pm$ 1.37	22.2 $\pm$ 1.98	14.5 $\pm$ 0.91	9.81 $\pm$ 0.94	1.58 $\pm$ 0.08
Skin	1.05 $\pm$ 0.12	1.42 $\pm$ 0.03	1.06 $\pm$ 0.04	0.67 $\pm$ 0.06	0.09 $\pm$ 0.01
Brain	4.82 $\pm$ 0.86	2.05 $\pm$ 0.37	1.10 $\pm$ 0.32	0.68 $\pm$ 0.11	0.16 $\pm$ 0.03
Bone	1.95 $\pm$ 0.30	4.88 $\pm$ 1.43	9.22 $\pm$ 0.48	12.7 $\pm$ 1.84	3.49 $\pm$ 0.35
Regional brain distribution (%dose/g)					
Region					
Cerebellum	4.67 $\pm$ 0.86	1.41 $\pm$ 0.23	0.89 $\pm$ 0.01	0.54 $\pm$ 0.09	0.41 $\pm$ 0.02
Striatum	7.28 $\pm$ 1.73	6.34 $\pm$ 1.01	4.24 $\pm$ 0.78	1.76 $\pm$ 0.33	0.46 $\pm$ 0.13
Hippocampus	4.54 $\pm$ 0.77	2.23 $\pm$ 0.59	1.18 $\pm$ 0.12	0.58 $\pm$ 0.15	0.41 $\pm$ 0.01
Cortex	4.54 $\pm$ 0.94	1.88 $\pm$ 0.63	0.89 $\pm$ 0.11	0.51 $\pm$ 0.02	0.36 $\pm$ 0.02
Hypothalamus	6.41 $\pm$ 1.11	4.95 $\pm$ 0.84	2.42 $\pm$ 0.52	1.02 $\pm$ 0.11	0.47 $\pm$ 0.01
Ratio (vs CB)					
Striatum	1.55 $\pm$ 0.09	4.51 $\pm$ 0.10	4.74 $\pm$ 0.91	3.27 $\pm$ 0.22	1.48 $\pm$ 0.32
Hippocampus	0.97 $\pm$ 0.02	1.59 $\pm$ 0.21	1.32 $\pm$ 0.14	1.08 $\pm$ 0.12	0.99 $\pm$ 0.02
Cortex	0.97 $\pm$ 0.06	1.31 $\pm$ 0.26	1.00 $\pm$ 0.12	0.98 $\pm$ 0.19	0.87 $\pm$ 0.03
Hypothalamus	1.37 $\pm$ 0.02	3.52 $\pm$ 0.07	2.71 $\pm$ 0.59	1.90 $\pm$ 0.15	1.15 $\pm$ 0.05

Table 3Dual isotope experiment with [^{18}F]FP-DTBZ and [^{125}I]IPT in lesioned mice

Regional brain distribution (%dose/g) and [ratio vs CB] measured at 30 min post-injection								
¹⁸ F]FP-DTBZ	Mouse 1		Mouse 2		Mouse 3		Mouse 4	
Cerebellum	1.58	[1.00]	1.62	[1.00]	1.26	[1.00]	1.76	[1.00]
Striatum L	3.36	[2.13]	4.12	[2.54]	4.17	[3.30]	8.03	[4.57]
Striatum N	8.46	[5.37]	6.29	[3.87]	7.28	[5.76]	8.90	[5.07]
Hippocampus	2.95	[1.87]	3.00	[1.85]	2.24	[1.78]	3.00	[1.71]
Cortex	1.53	[0.97]	1.51	[0.93]	1.31	[1.04]	1.94	[1.10]
Remainder	2.30	[1.46]	2.24	[1.38]	2.06	[1.63]	2.45	[1.39]
Hypothalamus	5.19	[3.29]	5.04	[3.10]	4.01	[3.18]	5.68	[3.23]
% StrL/StrN	39.7		65.6		57.3		90.1	
¹²⁵ I]IPT								
Cerebellum	0.96	[1.00]	0.77	[1.00]	0.81	[1.00]	0.76	[1.00]
Striatum L	3.00	[3.14]	3.53	[4.55]	4.75	[5.90]	6.89	[9.07]
Striatum N	9.21	[9.63]	6.33	[8.17]	9.09	[11.3]	7.77	[10.2]
Hippocampus	0.87	[0.91]	0.70	[0.90]	0.81	[1.00]	0.62	[0.81]
Cortex	0.69	[0.72]	0.62	[0.80]	0.66	[0.81]	0.87	[1.15]
Remainder	1.34	[1.40]	1.16	[1.49]	1.57	[1.95]	1.16	[1.53]
Hypothalamus	1.28	[1.34]	1.22	[1.58]	1.42	[1.76]	0.99	[1.31]
% StrL/StrN	32.6		55.7		52.3		88.7	

Striatum lesioned side: StrL

Striatum normal side : StrN