Measurement of [123I]FP-CIT binding to the dopamine transporter (DAT) in healthy mouse striatum using dedicated small animal SPECT imaging: feasibility, optimization and validation.

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Notes

- **Congress**

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Abstract

Aim In vivo imaging of dopamine transporter (DAT), a reliable marker of degeneration of nigrostriatal dopaminergic innervation, has gained increasing interest in preclinical neurodegenerative research for studying disease mechanisms and testing new therapeutic strategies. We assessed the feasibility and the reliability of in vivo and ex vivo quantification of Methyl (3S,4S,5R)-8-(3-fluoropropyl)-3-(4-iodophenyl)-8-azabicyclo[3.2.1]octane-4-carboxylate ([123I]FP-CIT) binding to striatal DAT sites in mouse brain. **Methods** Dedicated small animal single-photon emission computed tomography (SPECT) images of [123I]FP-CIT binding were obtained in 3 groups of healthy mice: untreated (n=6), pre-treated with lugol solution (n=4), and pre-treated with selective dopamine transporter uptake inhibitor GBR12909 (n=4). Ex-vivo autoradiography studies were performed at the end of SPECT studies with phosphor image system in 4 out of the 6 untreated mice and in all mice pre-treated with lugol. Regions of interest were defined over the striatum. The specific binding (SB) was calculated using the cerebral cortex as reference region. **Results** SPECT images in untreated mice showed high [123I]FP-CIT uptake in the striatum and extra-cerebral regions. Lugol pre-treatment improved striatal images quality decreasing salivary and thyroid glands uptake. SB was higher ($p<0.0001$) in mice pre-treated with lugol (5.97±0.60) than in untreated mice (2.25±0.28). Autoradiography showed similar SB findings in untreated (2.27±0.33) and lugol-treated (4.27±0.57) mice ($p<0.0001$). In vivo striatal ^{123}I-FP-CIT SB and ex-vivo striatal ^{123}I-FP-CIT SB were significantly correlated ($r= 0.87$; $P< 0.0001$). SPECT competition studies showed a significant ($p<0.0001$) reduction of [123I]FP-CIT SB in the striatum after GBR12909. **Conclusion** We demonstrated the feasibility of [123I]FP-CIT imaging of the normal mouse brain using small-animal SPECT without pinhole collimators. The reliability of quantitative measurement of striatal [123I]FP-CIT SB is supported by competition studies showing measurable inhibition of uptake induced by GBR12909 and by the strong correlation between in vivo and ex vivo striatal [123I]FP-CIT SB. Our data also demonstrate that pre-treatment with lugol might improve striatal [123I]FP-CIT SB in mice.

Keywords

Mouse striatum; SPECT; [123I]FP-CIT; Parkinson's animal models; DAT; autoradiography.

1. Introduction

Neurodegenerative diseases represent great challenges for basic science and clinical medicine because of their prevalence, pathologies, lack of mechanism-based treatments, and impacts on individuals. Translational research might contribute to the study of neurodegenerative diseases. The mouse has become a key model for studying disease mechanisms and testing therapeutic strategies because genetically engineered mouse models are available and might recapitulate in part some aspects of the corresponding human diseases (1). The mouse has also the advantage to be easily maintained in a research services facility in comparison to other animal species.

Noninvasive in vivo imaging in mice has gained increasing interest in preclinical research in the last years thanks to the availability of high-resolution single-photon emission computed tomography (SPECT) and positron emission tomography (PET) scanners and of highly specific radiolabeled biomarkers. Of particular interest is the in vivo imaging of dopamine transporter (DAT), a plasma membrane protein expressed exclusively in dopamine neurons. DAT is abundant in the striatal dopaminergic terminals and it is considered a reliable marker of degeneration for nigrostriatal dopaminergic innervation and analysis (2). Several PET and SPECT radioligands for DAT are currently available (2). Methyl (3S,4S,5R)-8-(3-fluoropropyl)-3-(4-iodophenyl)-8-azabicyclo[3.2.1]octane-4-carboxylate ([123I]FP-CIT) is a DAT radioligand widely used to evaluate and monitor in vivo with SPECT the integrity of the nigrostriatal dopaminergic projection in patients with Parkinson's disease (3-6). Methyl (3S,4S,5R)-8-(3-fluoropropyl)-3-(4-iodophenyl)-8-azabicyclo[3.2.1]octane-4-carboxylate is a reliable and reproducible marker to measure DAT levels in living human brain (4-6) and it has the advantage to be available for clinical use. In recent years, in vivo imaging of DAT in small animals has become a feasible approach with the development of high-resolution SPECT systems (7). Methyl (3S,4S,5R)-8-(3-fluoropropyl)-3-(4-iodophenyl)-8-

azabicyclo[3.2.1]octane-4-carboxylate has been applied in experimental studies in monkeys and rats (8-11). The use of SPECT for imaging DAT in mice brain is more difficult given the small size of the brain and requires high spatial resolution and sensitivity of the SPECT scanner. Recently, ^{123}I-FP-CIT pinhole SPECT for small animals or conventional SPECT camera equipped with pinhole collimators showed accurate evaluation and measurement of striatal DAT density in healthy mice and in mice models of Parkinson's disease (12-15). However, the feasibility, the validation and the optimization of imaging acquisition with dedicated small-animal SPECT cameras has not been extensively explored in healthy mice. This is a crucial step for further application in experimental models characterized by dysfunction and/or degeneration of nigrostriatal dopaminergic innervation.

In this study we assessed the feasibility of imaging and quantification of striatal DAT in mouse brain using [123I]FP-CIT and a small-animal SPECT camera. We also evaluated to which extent blockage of thyroidal and salivary glands iodine uptake may improve the analysis of striatal DAT binding. Validation of in vivo specific binding of striatal [123I]FP-CIT was assessed with ex-vivo data obtained in the same animals with phosphor imaging autoradiography of [123I]FP-CIT and competition studies with selective dopamine reuptake inhibitor.

2. Materials and methods

2.1. Animals

Animal studies were performed in accordance in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 or the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, or the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Before imaging, animals were randomized into 3 groups: 6 untreated mice; 4 mice pre-treated

with oral lugol solution (1 µl, one hour before tracer administration); and 4 mice without lugol were treated with 5mg/Kg of the selective dopamine transporter uptake inhibitor GBR12909 (Tocris Bioscience, Minneapolis, MN, USA) given via the tail vein 30 min prior and again intraperitoneally 15 min following tracer administration (8). Phosphor imaging autoradiography was performed in 10 animals: 4 untreated, 3 pre-treated with lugol, and 3 pre-treated with GBR.

2.2. SPECT studies

All mice received about 30 MBq (29.7±3.73 MBq) of [123I]FP-CIT (DaTSCAN, Amersham Buchler, Braunschweig, Germany) in <200 µl into the tail vein using a winged infusion needle set. During radiotracer administration and imaging procedures, mice were maintained under general anesthesia with 2% isoflurane in 100% oxygen at 0.8 L/min, with a constant monitoring of their body temperature, using an infrared lamp during imaging studies. Previous studies in rats showed that equilibrium of [123I]FP-CIT binding is reached early after the radiotracer injection and that the ratio of specific to non-specific striatal uptake remaining stable between 2 and 4 hours post-injection (8). Therefore, imaging started 2 hours after tracer administration and data acquisition lasted 90 min using a dedicated small animal SPECT scanner (YAP-(S)PET scanner-ISE, Pisa, Italy) with 2.7 FWHM axial resolution. Images were reconstructed with expectation maximization (10 iterations, matrix size 128 × 128, voxel size 0.5 × 0.5 × 2 mm^3) (16).

After reconstruction, for the quantification of [123I]FP-CIT binding a circular region of interest (ROI) of 66 mm^2 was manually drawn on both left and right striatum on two consecutive axial slices of 2 mm thickness using the proprietary software (16,17). To obtain a quantitative evaluation of the background, another ROI of the same dimension was drawn on the cerebral cortex. An index of striatal specific binding was calculated as follows: (mean left

and right striatal uptake) – (mean left and right cortical uptake)/mean left and right cortical uptake.

2.3. Ex vivo autoradiography

For phosphor imaging autoradiography of [123I]FP-CIT animals were sacrificed at the end of imaging studies by cervical dislocation. The brain was surgically removed and immediately frozen in liquid nitrogen. Thereafter, consecutive 20 µm coronal brain sections were cut on a cryostat set to –20°C and sections were immediately apposed to a MultiSensitive Phosphor Screens (PerkinElmer, Waltham, MA, USA) for 15 minutes at room temperature. The screens were then removed from the cassettes under dim lighting and scanned at 600 dpi resolution using a Cyclone storage phosphor system. In vitro autoradiography was performed on 20-µm coronal brain sections. The amount of binding was calculated in digital light units [DLU/mm2] using the Optiquant v4.00 software (18,19). Semiquantitative analysis was performed within two ROI, one inside each striatum and one outside (the cerebral cortex). Striatum-cortex/cortex ratios were calculated to evaluate specific binding. Right and left striatum were averaged, like in vivo.

2.4. Statistical analysis

Statistical analysis was performed using Graphpad Prism 6.0. Results were expressed as the mean ± SD. Unpaired Student's *t* test was used to compare mean values. A p value <0.05 was considered statistically significant. The relationship between right and left striatal [123I]FP-CIT bindings analyzed *in vivo* by SPECT and *ex vivo* by autoradiography was assessed using the Pearson's correlation test.

3. Results

SPECT images in untreated mice showed high [123I]FP-CIT uptake in the striatum and also in extra-cerebral regions like the salivary and thyroid glands (Fig. 1 a and c). Lugol pre-treatment improved striatal images quality decreasing salivary and thyroid glands tracer uptake as well as cerebral background (Fig. 1 b and d). Semiquantitative analysis revealed that specific binding was significantly higher in mice pre-treated with lugol solution than in untreated mice (5.97±0.60 vs. 2.25±0.28; $p<0.0001$) (Fig. 2).

Similar findings were found at autoradiography (Fig. 3 a and b). Striatal [123I]FP-CIT specific binding was significantly higher in pre-treated (4.27±0.57) than in untreated mice (2.27±0.33) ($p<0.0001$). Right and left striatal [123I]FP-CIT specific binding values measured in vivo at SPECT were significantly correlated to those measured ex-vivo by autoradiography ($r=0.87$; $p<0.0001$) (Fig. 4). Competition studies showed a 50% reduction of [123I]FP-CIT specific binding in the striatum after GBR12909 administration (2.92±0.32) in comparison to untreated mice (5.97±0.60) ($p<0.0001$) (Fig. 5).

4. Discussion

The results of this study demonstrate the feasibility of [123I]FP-CIT SPECT imaging of the normal mouse brain using small-animal SPECT cameras without pinhole collimators. Despite the lower spatial resolution and sensitivity in comparison to the ultra-high resolution pinhole SPECT devices, our results suggest that it is possible to evaluate a reliable index of striatal [123I]FP-CIT specific binding. The reliability of our in vivo measurement is supported by the results of competitive studies showing that pre-injection of a selective dopamine reuptake

inhibitor significantly reduce the striatal binding of ^{123}I-FP-CIT and by ex vivo autoradiography showing significant correlation between in vivo and ex vivo striatal [123I]FP-CIT specific binding. We also demonstrate that a significant improvement of visual and quantitative analysis of striatal [123I]FP-CIT binding can be obtained with a lugol pre-treatment.

Only few studies evaluated [123I]FP-CIT binding with SPECT in mice (12-15). To our knowledge none of these studies assessed the effect of lugol pre-treatment on visual and quantitative analysis of SPECT images in comparison with untreated mice. In mice, the salivary glands and thyroid are close to the brain. Therefore, to prevent spillover from external activity and to better delineate the brain activity, a group of mice were pretreated with oral lugol solution. The metabolism of [123I]FP-CIT results not only in polar but also in lipophilic metabolites, which might contribute to the accumulation of ^{123}I in harderian glands, salivary glands but also to the cerebral background (14). In the present study we showed that there was a clear and expected improvement of striatal [123I]FP-CIT SPECT images with lugol pre-treatment. This pre-treatment provided a better view of the striatum with a reduction of the background activity. Noteworthy, we could quantify this improvement as an increase of more than 100% of the specific binding ratio values.

We showed that [123I]FP-CIT binds specifically to striatal mice DAT sites since GBR12909 dihydrochloride, a potent competitive inhibitor of dopamine uptake, reduced by 50% the striatal specific binding ratios. Only in vivo studies in rats and monkeys have reported the effects of blockade striatal [123I]FP-CIT by GBR12909 (8). Our findings are in agreement with the results of this study showing a reduction of about 50% of striatum to cerebellum ratios using a similar dose of GBR12909 injected 5 minutes prior to injection of ^{123}I-FP-CIT. We found that approximately 5 mg/kg GBR12909 (approximately 9.5 μmol/kg) is necessary to block in vivo approximately 50% of striatal [123I]FP-CIT binding relative to baseline in

mouse brain, suggesting a competition between tracer and drug. In fact, the in vitro affinity of GBR 12909 for the dopamine transporter is comparable to that of FP-CIT (12).

Our study also provides evidence of strong correlation between the in vivo SPECT and the ex vivo phosphoroimaging autoradiographic analysis of striatal [123I]FP-CIT in mice. Due to partial volume effects and the higher sensitivity of ex vivo DAT phosphor imaging than in vivo SPECT, we expected the ex vivo ratios to be higher than the SPECT ratios, instead we found DAT phosphor imaging values close to the SPECT data. Differences in the definition of the background ROI and the relatively low sensitivity of the phosphoroimager that we used in this study could in part account for these results. We did not assess the correlation between [123I]FP-CIT and immunohistochemistry of DAT because previous studies have demonstrated a highly significant correlation in control and MPTP-treated mice between in vivo [123I]FP-CIT binding and ex-vivo DAT expression (12).

5. Conclusions

Methyl (3S,4S,5R)-8-(3-fluoropropyl)-3-(4-iodophenyl)-8-azabicyclo[3.2.1]octane-4-carboxylate imaging with a dedicated small animal SPECT scanner provides an accurate quantitative measurement of DAT binding in the mouse striatum. As the nigrostriatal system is a region spatially well defined and does not suffer the interference of the uptake of other brain structures, SPECT could be a valuable system to study in vivo new animal models of disease and to test new therapeutic approaches. Future studies are required to assess the sensitivity of the method in detecting different degree of DAT changes.

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Title of Figures

Fig. 1. **[123I]FP-CIT SPECT of mice striatum with and without lugol solution.**

[123I]FP-CIT SPECT sagittal view in an untreated mouse (a and c) and in a mouse pre-treated with Lugol solution (b and d).

Fig.2. **Graph of ^{123}I-FP-CIT SPECT striatal specific binding.**

Striatal specific binding of [123I]FP-CIT at SPECT imaging in untreated and lugol pre-treated mice. *$p<0.0001$.

Fig. 3. **SPECT and autoradiography of mouse brain.**

Axial SPECT and corresponding autoradiographic images in an untreated mouse (a and b) and in a mouse pre-treated with Lugol solution (c and d). A clear enhancement of [123I]FP-CIT uptake in the striatum after lugol treatment due to a background reduction was confirmed by phosphoimager.

Fig. 4.

Graph of [123I]FP-CIT striatal specific binding of SPECT and autoradiography.

Relationship between [123I]FP-CIT striatal specific binding of right and left striatum in vivo with SPECT and ex-vivo with phosphor imaging autoradiography.

Fig. 5.

SPECT imaging with and without the use of the selective dopamine transporter uptake inhibitor. Striatal specific binding of [123I]FP-CIT at SPECT imaging in untreated and GBR12909 pre-treated mice. *$p<0.0001$.

FIGURE 1

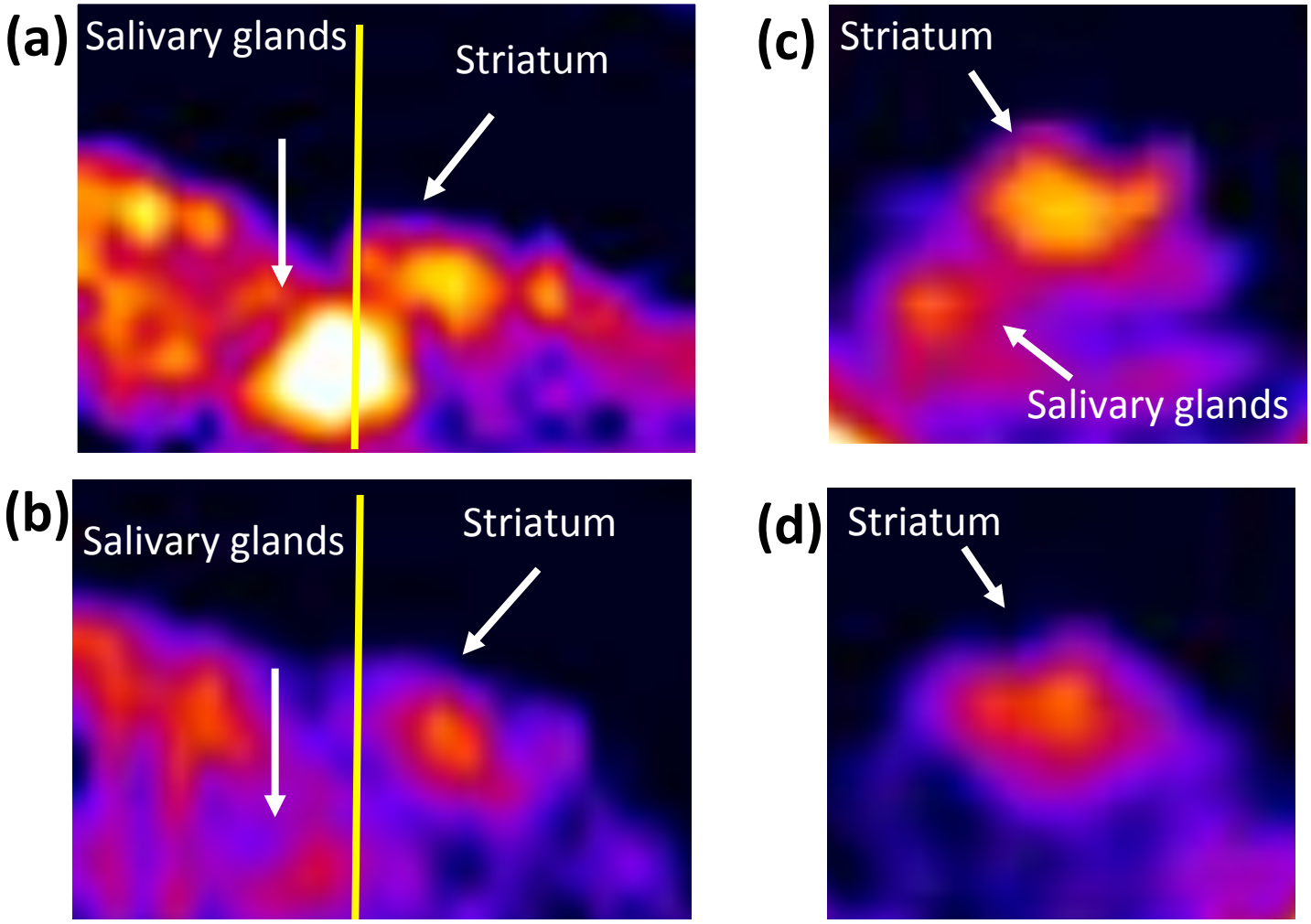

(a)
Salivary glands
Striatum
(b)
Salivary glands
Striatum
(c)
Striatum
Salivary glands
(d)
Striatum

FIGURE 2

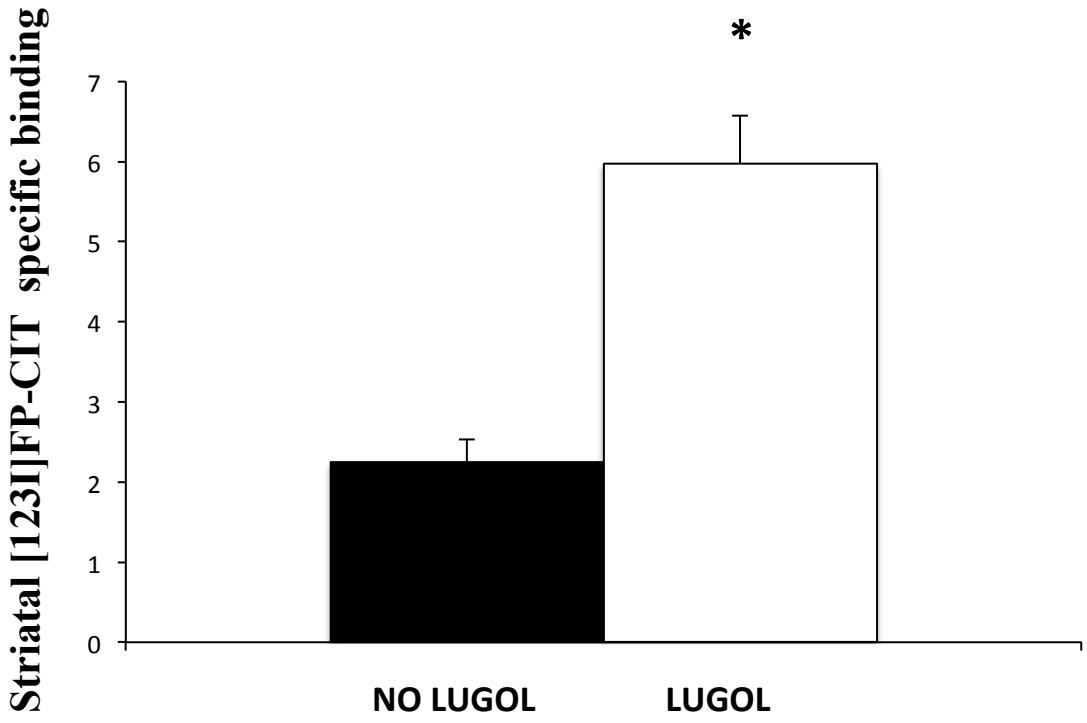
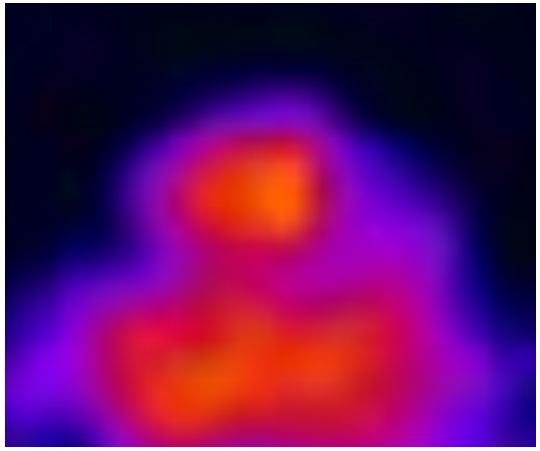
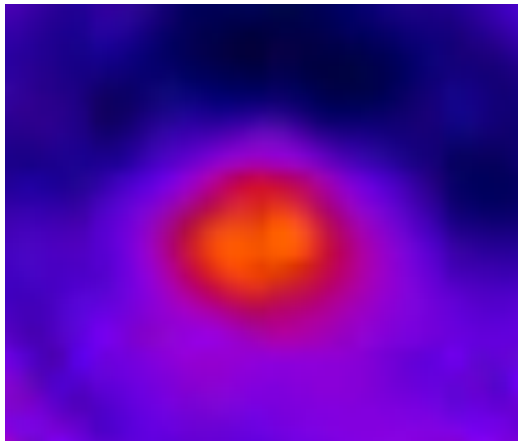

Striatal [123I]FP-CIT specific binding
*
7
6
5
4
3
2
1
0
NO LUGOL
LUGOL

FIGURE 3

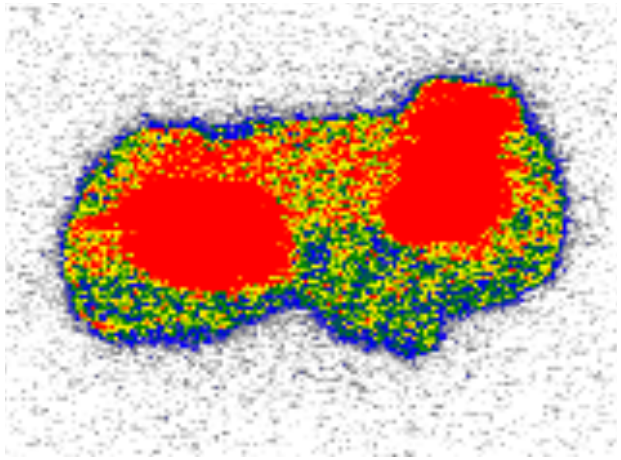
(a)



(c)



(b)



(d)