

Striatal dopamine during sensorial stimulations: A [¹⁸F]FDOPA PET study in human and cats

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Abstract

Sensory stimulations of the forelimb in cats are known to increase dopamine release in the ipsilateral striatum and to decrease it in the homologous contralateral structure. Using positron emission tomography in both humans and cats, the present study shows that such sensory stimulations greatly reduce [¹⁸F]FDOPA accumulation ipsilateral to the stimulation (by 40.4% and 26.4% in the human caudate and putamen, respectively, and by 33.3% in the cat striatum). This decrease in striatal [¹⁸F]FDOPA uptake suggests a reduced DA storage resulting from the increased amine release. No change was observed in the contralateral striatum in neither human or cat suggesting, in contrast, that [¹⁸F]FDOPA accumulation is not facilitated by decreased DA release. These results support the hypothesis that sensory stimulations activate a non-synaptic mode of dopamine release.

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It has been demonstrated that sensory stimulations of the forepaw in cats induce a long-lasting increase in dopamine (DA) release in the ipsilateral Caudate–Putamen complex. The opposite effect was observed in the contralateral structures [6,21,22]. These stimulus-induced alterations in DA release were originally measured as the efflux of [³H]DA during a local superfusion with [³H]tyrosine (the metabolic precursor of DA) through a push pull canula acutely implanted in the cat striatum. Although the physiological significance of this effect is still not fully understood, its biochemical mechanism was demonstrated to be mediated through a thalamo-cortico-striatal loop regulating DA in striatal terminals through local glutamate (GLU) afferents [1,20].

Indeed, local GLU afferents are well known to modulate DA release. This was extensively studied and referenced

in several reports [1,6,8,12,18–20]. A recent study using positron emission tomography (PET) and [¹¹C]raclopride, a DA D2-receptor antagonist, was undertaken to reveal this phenomenon in human. This study was paralleled in cat as a reference model. It was observed that sensory stimulations of the forelimb did not change striatal [¹¹C]raclopride binding in neither species [25]. The lack of post-synaptic effect of stimulation-induced DA overflow suggested that sensory stimulations may activate a tonic rather than phasic mode of DA release. These two different modes of DA release have been firstly proposed [9,12] and were also directly observed in vivo [24]. Specifically, phasic DA release is spike-dependent, occurs intrasynaptically, at high concentrations and is rapidly inactivated by reuptake. In contrast, tonic DA release is spike-independent, occurs extrasynaptically, at low concentrations and is regulated by corticostriatal glutamatergic afferents. An effect of forelimb stimulations on tonic rather than phasic DA release would involve too small changes in endogenous DA

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levels to be detected using PET and [^{11}C]raclopride [25] but might be characterized by an increased synthesis of the amine [19].

Considering the major role played by DA synthesis in the tonic release of DA, the aim of the present study was to evaluate the effect of forelimb stimulations on the striatal uptake of 6- ^{18}F fluoro-L-DOPA in both human and cat, using the same experimental paradigm that was tested using [^{11}C]raclopride [25]. The radioligand 6- ^{18}F fluoro-L-DOPA (^{18}F FDOPA) is uptaken into the nigrostriatal dopaminergic neurons where it is converted into 6- ^{18}F fluoro-L-dopamine (^{18}F FDA) by DOPA-decarboxylase (DDC) and it accumulates [4,11]. ^{18}F FDOPA uptake thus reflects DDC activity but also provides an index for the presynaptic storage capacity of ^{18}F FDA [3,7]. Interestingly, DDC activity is regulated by the degree of DA-induced autoreceptor occupancy [5,26]. In other words, an increased release of tonic DA could stimulate presynaptic DA autoreceptors which, in turn, would down-regulate DDC activity and lead to a decrease in ^{18}F FDOPA uptake. We therefore hypothesized that if the release of DA previously observed following forelimb stimulation in cats is tonic in nature, it should induce a reduction in ^{18}F FDA storage in nigrostriatal terminals together with a decrease in DDC activity, and lead to a reduction of ^{18}F FDOPA uptake.

Six normal volunteers were enrolled (mean age \pm S.D.: 45 ± 5.3 years; range: 34–51; three males/three females). All subjects except one were right handed. In addition, they were free of psychiatric or neurological disorders, had a normal physical examination and were free of any medication. All subjects underwent a standard brain MRI (Siemens Expert 1 T MRI system) with T1- and T2-weighted sequences prior to the PET scan to ensure that no lesion was present. The present study was performed after approval by the Lyon University Hospitals Ethics Committee. Prior participation to the study, all subjects were informed about the procedure to be followed in the study and signed an informed consent form according to the declaration of Helsinki.

Four European male cats weighing about 3 kg and obtained from Iffa-Credo (France) were used. Studies were realized by licensed investigators in accordance with French (87–848, Ministère de l'Agriculture et de la Forêt) and European Economic Community (86–609, EEC) guidelines for care of laboratory animals. They were also approved by the regional ethical animal use committee. Animals were anesthetized by constant insufflation of halothane (1.5%) in medical air through a respiratory mask. Carbon dioxide concentration in expired gases, heart rhythm, and body temperature were continuously controlled all along the PET experiments. A head fixation system consisting in a stereotaxic holding device made with Plexiglas where ear-bars, orbital and hard palate pieces was used to secure a fixed and reproducible position of the cat's head during the PET measurements. A canula was inserted in the cephalic vein for radiotracer injection.

In human subjects, a stimulation of the right/left median nerve was applied at the wrist. Stimulation (pulse duration: 0.5 ms; frequency: 1 Hz) was delivered by a constant-voltage

stimulator. The intensity of the stimulus was adjusted just above the threshold for contraction of the right abductor pollicis brevis. The stimulation was perceived by the subject but remained absolutely non-noxious. In cats, the sensory stimulation (pulse duration: 0.5 ms; frequency: 0.2 Hz) was delivered through subcutaneously pad-implanted thin needles in one forelimb. As was done in humans, intensity was adjusted just above the threshold for muscular contraction. In both species, the stimulation was applied at 60 min post-radioligand injection and lasted for 30 min.

Each subject was examined with ^{18}F FDOPA on two separate occasions. On one occasion, subjects were scanned at control conditions and on the other occasion, they were scanned under stimulation conditions. The order between scan conditions was counterbalanced from one subject to the other. For each subject (humans and cats), the time interval between scans was at least one week.

PET studies were performed on a Siemens ECAT Exact HR+ used in three-dimensional mode. The system covers an axial distance of 15.5 cm. The transaxial resolution of the reconstructed images is about 4.1 mm full widths at half-maximum in the center. Transmission scans were acquired with three rotating ^{68}Ge - ^{68}Ga sources and used to correct the emission scans for the attenuation of 511 keV photon rays through tissue and head support.

^{18}F FDOPA was synthesized according to a recently developed method [13,17] involving the use of chiral phase transfer catalyst and a Schiff base [2]. ^{18}F FDOPA was purified by HPLC on a semi-preparative Uptisphere column. The chemical purity was checked on an analytical Lichrosorb C8. The enantiomeric excess was monitored by HPLC analysis on an Aztec Chirobiotic T chiral column. This reliable synthesis affords enantiomeric purity (>97%), radiochemical purity (>98%), a specific activity of 72–185 GBq/mmol.

The mean \pm S.D. amount of radioactivity injected in human was 118 ± 16.3 MBq. All subjects received 50 mg Benserazide orally 1 h before the study to inhibit peripheral DOPA decarboxylation. Cats received 74 MBq ^{18}F FDOPA in 2–3 ml, 30 min after a pretreatment with 2 mg/kg benserazide and followed by a flush with 2 ml saline.

The 3D emission data were reconstructed by 3D filtered back projection (Hanning filter; cut-off frequency, 0.5 cycles/pixel), giving a transaxial resolution of 6.5 mm full width at half maximum. Radioactivity was measured in a series of sequential time frames of increasing duration (from 30 s to 10 min) corrected for decay and plotted versus time. The total time for measurement of radioactivity in the brain was 90 min in both humans and cats.

In human subjects, one elliptical region of interest (ROI) of 2.5 ± 0.3 cm 2 was placed along the axis of each putamen and one circular ROI of 1.3 ± 0.1 cm 2 was positioned on each head of caudate nucleus. These ROIs were placed on five consecutive planes according to a human brain atlas. Non-specific level of radioactivity was averaged from a single elliptical ROI of 5 ± 0.5 cm 2 drawn over the occipital cortex on two consecutive planes. In cats, ROIs for the right and left

caudate nuclei and the cerebellum were drawn on the reconstructed PET images according to a cat brain atlas. Due to the small area of occipital cortex in the reconstructed images, the cerebellum was preferred as reference.

The influx rate constant of [^{18}F]FDOPA ($K_{\text{FDOPA}}^{\text{ACC}}$) was calculated in humans and cats using the following method. Radioactivity originating from striatum was reduced by that issued from the region of reference (occipital cortex in humans and cerebellum in cats). Values obtained were normalized by dividing by the reference value and plotted against time. Calculation was of the following type: (Striatum – Occ.)/Occ. = $F(t)$ in humans and (Striatum – Cerebellum)/Cerebellum = $f(t)$ in cats. The slope of the resulting plot was calculated using the data acquired between 60 and 90 min post-radioligand injection. This time period chosen for the stimulation (60–90 min) correspond to the one with a linear influx rate constant.

The non-noxious nature of the stimulation made it suitable as a model to be used in human subjects. We already performed PET experiments in awake animals [14], however, since the model was originally described in anesthetized cats (1.5% halothane), the same situation was presently maintained in the experiments done in cats.

In each experiment, $K_{\text{FDOPA}}^{\text{ACC}}$ was calculated during the 60–90 min post-injection period in the left and right caudate and in putamen using occipital cortex as input function. When applied, the stimulation lasted for the time period used for $K_{\text{FDOPA}}^{\text{ACC}}$ calculation (60–90 min).

No significant difference was noted between the heart frequency before and after the beginning of the sensory stimulation. In contrast, sensory stimulation significantly reduced $K_{\text{FDOPA}}^{\text{ACC}}$ by 40% (from 0.0095 ± 0.002 to 0.006 ± 0.001 , $p < 0.01$) in caudate nucleus and by 26% (from 0.01 ± 0.004 to 0.008 ± 0.001 , $p < 0.05$) in the putamen ipsilateral to the stimulated limb (Table 1). No alteration in $K_{\text{FDOPA}}^{\text{ACC}}$ was detected in the contralateral structures.

The low intensity stimulation used and the presence of anesthesia in cats allowed considering the stimulation as non-noxious. No fluctuations in cardiac or respiratory rhythms were ever detected during the experiments. As observed in humans, a 33% ($p < 0.01$) decrease in $K_{\text{FDOPA}}^{\text{ACC}}$ was detected in the striatum ipsilateral to the stimulated forepaw (from

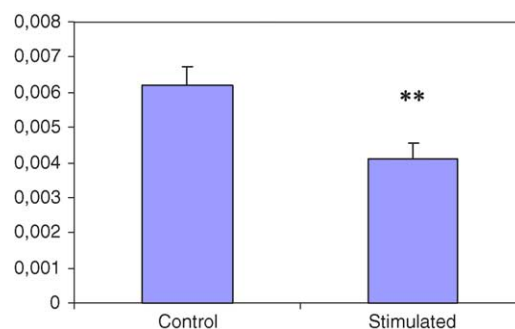


Fig. 1. Influx rate constant for [^{18}F]FDOPA ($K_{\text{FDOPA}}^{\text{ACC}}$) obtained in the cat striatum at control conditions (i.e. no stimulation was applied during the scan) and under stimulation conditions (i.e. a continuous lateralized sensory stimulation was applied from 60 to 90 min post-radioligand injection). The stimulation reduced $K_{\text{FDOPA}}^{\text{ACC}}$ by 33% ($p < 0.01$).

0.00618 ± 0.00056 to 0.00412 ± 0.00042 , Fig. 1). No alterations were detected in the contralateral striatum.

In both humans and cats, sensory stimulations induced significant reductions in [^{18}F]FDOPA accumulation in the ipsilateral striatum but induced no change in the contralateral structure. The reduction in [^{18}F]FDOPA accumulation gives information on the effects of sensory stimulations on DA metabolism in both human and cat. The $K_{\text{FDOPA}}^{\text{ACC}}$ is an index of both DA synthesis activity (DDC activity) and DA intraterminal accumulation [4,7]. The major reduction in $K_{\text{FDOPA}}^{\text{ACC}}$ observed in the striatum ipsilaterally to the stimulation is consistent with a reduction of DA synthesis and/or an increase of DA release. At first, the absence of activation of DA synthesis in response to an increased DA release could appear surprising. However, such an observation has already been reported after GLU application on synaptosomal preparations from rat striatum [8]. Indeed GLU neurotransmission in the striatum is known to induce complex alterations of DA release [1,12,19] and was observed to simultaneously increase the release and decrease the synthesis of DA [8]. We could have also expected the reduced DA release originally measured in the contralateral striatum following forelimb stimulation to induce an increased [^{18}F]FDOPA accumulation. This latter effect did not occur in our study. It is not surprising that a reduced release does not result in a change in DA synthesis since, in that

Table 1

[^{18}F]FDOPA accumulation constant ($K_{\text{FDOPA}}^{\text{ACC}}$) in normal human subjects, in the side ipsilateral to the stimulated forelimb with and without sensory stimulation

Subjects, number/age/sex	Caudate control	Caudate stimulated	Putamen control	Putamen stimulated
1/43/M	0.007	0.006	0.014	0.009
2/44/F	0.009	0.006	0.005	0.0065
3/51/F	0.0088	0.003	0.011	0.009
4/51/M	0.012	0.007	0.013	0.008
5/46/F	0.008	0.006	0.013	0.008
6/34/M	0.012	0.006	0.0075	0.006
Mean \pm S.D.	0.0095 ± 0.002	0.006 ± 0.001 ($p < 0.01$)	0.01 ± 0.004	0.008 ± 0.001 ($p < 0.05$)

$K_{\text{FDOPA}}^{\text{ACC}}$ was measured from 60 to 90 min post-radioligand injection. Individual values and mean \pm S.D. of $K_{\text{FDOPA}}^{\text{ACC}}$ are given at control conditions (i.e. no stimulation was applied during the scan) and under stimulation conditions (i.e. a continuous lateralized sensory stimulation was applied from 60 to 90 min post-radioligand injection). Values were significantly reduced in caudate ($p < 0.01$) and putamen ($p < 0.05$) by the stimulation.

case, the intraterminal store of the amine is not affected. We can thus hypothesize that GLU neurotransmission mediates the effects of sensory stimulation on [¹⁸F]FDOPA accumulation we report here. Its direct action on the tonic DA release, can reduce the amine stores and the resulting DA overflow can activate DA autoreceptors leading to a decrease of DDC activity [5,26].

The extrasynaptic nature (tonic DA release) of the amine overflow after sensory stimulation that we propose is also confirmed by previous similar experiments but using [¹¹C]raclopride [25]. In that case no change in binding was detected after sensory stimulations of the forelimb in both humans and cats, suggesting that a too small amount of synaptic DA was released to displace the radioligand. However, amphetamine, known to activate the extrasynaptic reverse transport of DA [15,18,23], and to increase DA extracellular levels by several hundred fold, has only a modest effect on [¹¹C]raclopride binding [10,16]. This observation suggests that [¹¹C]raclopride binding is likely much more sensitive to changes in intrasynaptic DA levels or occurring near the synapses than to alterations occurring in the extracellular space distant from the synapses. The absence of effect of the sensory stimulations on the [¹¹C]raclopride binding is thus well in line with the proposal of a major effect on the tonic DA release.

In summary, the present study using [¹⁸F]FDOPA, together with a previous study using [¹¹C]raclopride and the same experimental paradigm, suggests that the mechanism of DA release triggered by sensory stimulations is extrasynaptic (tonic) and likely mediated by GLU neurotransmission. The present study also shows that PET gives the opportunity to characterize in human, in vivo, the ability for nigrostriatal DA system to respond to sensory stimulations and to precise the mechanism of DA release.

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