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Impact of Isoflurane Anesthesia on D2 Receptor Occupancy by [¹⁸F]fallypride Measured by MicroPET With a Modified Logan Plot

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Abstract

In the previous work, we reported a method that utilized imaging data collected from 60 to 120 min following [¹⁸F]fallypride administration to estimate the distribution volume ratio DVR' (DVR' $\propto$ DVR; $DVR = 1 + BP_{ND}$, where BP_{ND} is a measure of receptor density, DA D2 in this case). In this work, we use this method to assess the effects of isoflurane anesthesia on [¹⁸F]fallypride DVR'.

Methods—Rats were injected with [¹⁸F]fallypride either unconsciously under ~1.5% isoflurane via the tail vein (Group 1) or consciously via a catheter inserted either in the jugular vein (Group 2) or the tail vein (Group 3). After about 1 h of free access to food and water the rats were anesthetized with 1.5% isoflurane and imaged in a microPET for 60 min. The rats that were injected consciously (Groups 2 and 3) were placed in a rat restrainer during [¹⁸F]fallypride injection. They were habituated in that restrainer for 3 days prior to the experiment day to minimize restraint-related stress. For comparison, a control group of rats was imaged for 120 min simultaneously with the administration of [¹⁸F]fallypride i.v. while under 1.5% isoflurane. The DVR' estimates from the 60 min acquisitions were compared with the DVR' from the last 60 min of the 120 min acquisitions (after neglecting the first 60 min). In addition, the striatal time–activity curves were fit with a 2-tissue + plasma compartment model using an arbitrary simulated plasma input function to obtain k_3/k_4 ($\approx BP_{ND}$) for the 60 and 120 min acquisitions.

Results—Isoflurane anesthesia caused a significant reduction, up to 22%, in the DVR' estimates, which were 15.7 ± 0.3 (mean $\pm$ SE) for the controls, 17.7 ± 0.3 for Group 1, 19.2 ± 0.4 for Group 2, and 18.8 ± 0.7 for Group 3. The compartmental model fit produced similar results, ~30% reduction in k_3/k_4 for the 120-min acquisitions compared with the 60-min acquisitions (initial conscious uptake of the radiotracer).

Conclusion—The results of this study demonstrate that isoflurane anesthesia significantly decreases striatal [^{18}F]fallypride BP_{ND} in rats. Of similar importance, this work demonstrates the effectiveness of delayed scans following radiotracer injection and the implication that different types of studies can be conducted simultaneously with this method, including studies of behavioral and environmental impact on brain receptors.

Keywords

[^{18}F]fallypride; dopamine (DA) receptors; isoflurane; graphical analysis; microPET

INTRODUCTION

[^{18}F]Fallypride (Mukherjee et al., 1995) has been shown to be a selective, high affinity DA D2/D3 receptor positron emission tomography (PET) radioligand that is widely used to measure the binding potential (BP_{ND}) with respect to the nondisplaceable radioligand in tissue (Innis et al., 2007). BP_{ND} is an index of receptor density and tracer affinity in the striatum and other D2/3-rich brain regions. Thus, changes in the BP_{ND} are indirect measures of changes in receptor levels. PET imaging has been widely used for studying neurodegenerative diseases and dysfunction such as Parkinson's disease and schizophrenia (Momosaki et al., 2004; Tolwani et al., 1999). In animal studies, anesthesia is usually required, with isoflurane anesthesia being the most commonly used. However, concerns have been raised about the influence of anesthesia on PET receptor binding studies in rodents. Previous studies have reported that isoflurane anesthesia alters PET estimates of the dopamine transporter (DAT) BP_{ND} (Elfving et al., 2003; Harada et al., 2004; Tsukada et al., 1999; Votaw et al., 2003). It has been demonstrated that anesthetics modify neuronal functions and this modification is dependent on the type, amount, and length of application of the anesthetic (Cavazzuti et al., 1987; Crosby et al., 1982; Elfving et al., 2003; Momosaki et al., 2004; Harada et al., 2004; Onoe et al., 1994; Tokunaga et al., 2009; Tsukada et al., 1999, 2002; Votaw et al., 2003).

Whereas the exact mechanisms of the impact of isoflurane anesthesia on the dopaminergic system are not fully understood, it has been shown that inhaled isoflurane reduces the number of membrane externalized DAT sites (Elfving et al., 2003; Votaw et al., 2003). Aldachi et al. (2005) determined that 3% isoflurane anesthesia increased the extracellular concentration of DA and its metabolites in a microdialysis study on rat brains. They suggested that 3% isoflurane anesthesia may increase DA neural activity and DA release.

Different methods have been developed to minimize or eliminate the impact of anesthesia on neuronal function during PET imaging in animal models. The two most popular methods focus on imaging without the use of anesthesia. One method requires restraining the animal's head, while conscious, during the entire duration of the scan, typically 120 min,

following daily habituation and acclimation for up to 2 weeks (Momosaki et al., 2004; Tokunaga et al., 2009). The other method, applicable to imaging in rats, uses a miniature PET scanner (“RatCAP”) that fits on the rat’s head to enable conscious rat brain imaging without the effects of anesthesia (Schulz et al., 2011). The former method may be stressful to the animals and requires extensive acclimation periods, whereas the latter method is not widely available.

In this work, we use the method of delayed fallypride scans (Tantawy et al., 2009) as a simplified method of assessing and minimizing the effects of isoflurane anesthesia on apparent D2 levels in the rat brain by assessing [^{18}F]fallypride binding to D2 receptors in the striata. This method reduces the duration of time the animal is required to be under anesthesia, while also minimizing stress to the animal.

METHODS

All studies were approved by the Vanderbilt University Animal Care and Use Committee prior to conducting the experiments.

Three groups of male Sprague-Dawley rats (~300 g) were used in four separate sets of experiments. In the first group, three rats were anesthetized with 1.5% isoflurane for 5–10 min during which a tail vein catheter was inserted and the rats were injected with 15 MBq/0.2 ml of [^{18}F]fallypride (Ansari et al., 2006; Kessler et al., 1993) followed by a 0.1 ml saline flush via the catheter. Anesthesia was stopped and the rats were returned to their cages with free access to food and water. Although no formal behavioral studies were made, the rats began to regain consciousness within 2 minutes of isoflurane removal and appeared dizzy and disoriented up to 5 min post isoflurane removal. The rats returned to full activity within 10–20 min post isoflurane removal. At 55 min post [^{18}F]fallypride injection, each rat was anesthetized again with ~1.5% isoflurane anesthesia, placed in a microPET Focus 220 (Siemens Preclinical Solutions, Knoxville, TN), and a 60 min dynamic acquisition was started. Anesthesia began 7–10 min prior to beginning each scan to allow time to position the rat head in the field of view (FOV) of the microPET. Therefore, the microPET dynamic scan was started approximately 62–65 min post [^{18}F]fallypride injection. Five days later, one of the rats was imaged again using the same protocol and an overall n of 4 was used for this group in the statistical analysis described below.

In the second group, 14 rats were anesthetized with 1.5% isoflurane and jugular catheters were inserted into the lateral thoracic vein 5 days prior to the date of the experiment. The rats were habituated in a rat restrainer while flushing the catheters with 0.15 ml of heparinized saline daily beginning 3 days prior to imaging. This method serves not only to keep the catheters patent but also to habituate the animals to a procedure that mimicked the radiotracer injection and minimize stress. On the day of the experiment, the rats were restrained in the same manner and injected with 15 MBq/0.2 ml of [^{18}F]fallypride via the jugular catheters followed by a 0.1 ml saline flush. The restraining and injection lasted less than 3 min, after which the rats were returned to their home cages and allowed free access to food and water for 55 min. The rats were then anesthetized with 1.5% isoflurane and imaged for 60 min in the microPET. To compare the kinetics of conscious uptake of [^{18}F]fallypride

to uptake of the radiotracer under anesthesia, six of the rats were imaged again one week later following the protocol previously described (Tantawy et al., 2009). Briefly, the rats were anesthetized with 1.5% isoflurane and placed in the microPET FOV. The rats were injected with 15 MBq/0.2 ml of [^{18}F]fallypride via the jugular catheters followed by a 0.1 ml saline flush simultaneously with the start of a 120 min dynamic acquisition.

In the third group, 10 rats were habituated in a rat restrainer for 5 min each day beginning 3 days prior to the start of the experiment. On the day of the experiment, each rat was positioned in the rat restrainer and a tail vein catheter was inserted while they were conscious. Prior to catheter insertion, the tail was sprayed with lidocaine, an analgesic, to minimize the pain. Approximately 15 MBq/0.2 ml of [^{18}F]fallypride was administered via the catheter followed by a 0.1 ml saline flush. After injection, each rat was allowed free access to food and water for 55 min. Subsequently, the rats were anesthetized with 1.5% isoflurane and imaged for 60 min in the micro- PET in a dynamic acquisition mode.

The 60 min dynamic acquisition was divided into six frames of 600 s duration each. The 120 min dynamic acquisition was divided into five frames of 60 s duration, one frame of 300 s and 11 frames of 600 s (Tantawy et al., 2009). All datasets were reconstructed using the OSEM-2D algorithm into $128 \times 128 \times 95$ slices with a voxel size of $0.095 \times 0.095 \times 0.08$ cm³ after correcting for scatter and attenuation. The resulting images were coregistered to an MR template (Rubins et al., 2003; Schweinhardt et al., 2003) using the medical imaging analysis tool AMIDE (Loening and Gambhir, 2003). Anatomical volumetric regions-of-interest (ROIs) were drawn around the striatum and cerebellum and the resulting time–activity curves (TACs) were corrected for partial volume effects using recovery coefficients derived from a cone-shaped phantom (Tantawy et al., 2009).

Data analysis

The distribution volume ratio DVR' (Tantawy et al., 2009) for each 60 min scan was estimated using the Logan plot (Logan, 2000) tool in version 2.6 of the commercial PMOD software (PMOD Technologies, Zürich, Switzerland, www.pmod.com) where the cerebellum, which has few or no D2 receptors, was taken as the reference tissue. These DVR' estimates were compared with the DVR' values estimated using only the last 60 min of the 120 min scans obtained in this work ($n = 6$) combined with the 120 min scans obtained from our previous work ($n = 14$) (Tantawy et al., 2009) after neglecting the first 60 min. The subjects that were imaged while anesthetized for 120 min during and after radiotracer injection will be labeled as control rats in the text to follow.

To verify our results obtained using graphical analysis, we used the 120-min scans and the 60-min scans of the six rats in Group 2 to estimate k_3/k_4 ($\approx \text{BP}_{\text{ND}}$ (Innis et al., 2007)) using a 2-tissue + plasma compartmental model in the following way: the simulated arbitrary 120 min plasma input function used in our previous work (Tantawy et al., 2009) was used to first fit the 120 min striatal TACs with a 2-tissue compartment model using PMOD. Then K_1 and k_2 obtained from this fit were fixed and used in conjunction with the same 120-min plasma input function in a 2-tissue + plasma compartmental model on the striatal TACs obtained from the 60 min acquisitions. This was carried out on a subject specific manner, i.e., each of

the six rats had a separate estimate of K_1/k_2 . The ratio of k_3/k_4 from the fit of the 60 min acquisitions was compared to k_3/k_4 from the fit of the 120 min acquisitions, i.e.

$$\text{Ratio} = \frac{\left(k_3/k_4\right)_{60\text{min-cons.uptake}}}{\left(k_3/k_4\right)_{120\text{min}}}.$$

This ratio provides an approximate estimate of the comparison of the BP_{ND} between the rats that were not subjected to anesthesia in the first 60 min post [^{18}F]fallypride injection and those that were subjected to anesthesia from the time of the injection to the end of the scan. This ratio will be compared to the ratio of DVR' in the conscious uptake phase to DVR' in the nonconscious uptake phase ($\text{DVR}'_{\text{conscious uptake}}/\text{DVR}'_{\text{unconscious uptake}}$).

A one-way ANOVA statistical test followed by a Tukey multiple comparison test was carried out using GraphPad Prism software, version 4.03 (GraphPad Software Inc.) comparing the DVR' estimates among the different groups including the DVR' estimates of the controls. An overall probability of $P < 0.05$ was selected as the significance threshold.

RESULTS

While the TACs of the 60 min acquisitions and the TACs of the last 60 min of the 120 min acquisitions appear to be similar in shape (Fig. 1), DVR' estimates measured via graphical analysis (Fig. 2) for the rats that were conscious during the initial 60 min after [^{18}F]fallypride injection were up to 22% higher than the controls that were anesthetized for a 120 min period after injection, $\text{DVR}'_{\text{(conscious uptake)}}/\text{DVR}'_{\text{(unconscious uptake)}} = 19.2/15.7 = 1.22$, $P < 0.001$ (Table I), where $\text{DVR}'_{\text{(unconscious uptake)}}$ are DVR' estimates for the last 60 min of the 120 min data after neglecting the first 60 min (Tantawy et al., 2009). The compartmental modeling revealed a 34% increase in $\text{D}_2 \text{BP}_{\text{ND}}$ in the absence of isoflurane anesthesia during the initial uptake period of [^{18}F]fallypride, $[(k_3/k_4)_{\text{(conscious uptake)}}]/[(k_3/k_4)_{\text{(unconscious uptake)}}] = 1.34$. This is in good agreement with the ratio found using graphical analysis, i.e., when comparing DVR' of the 60 min acquisitions ($\text{DVR}' = 19.2 \pm 0.4$, see Table I) to DVR of the 120 min acquisitions [$\text{DVR} = 13.5 \pm 0.3$, from (Tantawy et al., 2009) plus the six additional rats studied in this work].

The rat that was imaged twice in Group 1 (unconscious injection via the tail vein + initial conscious uptake) demonstrated excellent reproducibility: $\text{DVR}'_{\text{(day 1)}} = 17.4 \pm 0.3$ and $\text{DVR}'_{\text{(day 2)}} = 17.3 \pm 0.5$.

There was no statistically significant difference ($P > 0.05$) in DVR' when comparing all conscious uptake groups to one another.

DISCUSSION

In this work, we show that the delayed scan method can be a useful tool to indirectly probe pharmacological changes in tracer binding. The method of delayed scans allowed us to measure brain activity using PET, while reducing exposure to and minimizing the impact of

anesthesia. We were able to verify in this work that DVR' and subsequently BP_{ND} are reduced in the presence of isoflurane anesthesia using graphical analysis (Logan, 2000; Tantawy et al., 2009) and then again using a 2-tissue + plasma compartmental model. The difference in DVR' estimates between conscious fallypride uptake and the unconscious uptake depicted in these studies have implications for the impact of isoflurane anesthesia on the dopaminergic system. A decrease in DVR' and subsequently BP_{ND} indicates an increase in extracellular concentrations of DA. The exact mechanism of how isoflurane anesthesia impacts the dopaminergic system is still an issue of debate. Elfving et al. (2003) and Votaw et al. (2003) showed that 1% inhalation of isoflurane (light anesthesia) reduces the number of available DA reuptake binding sites in rhesus monkey brains to less than 50%, as measured with [^{18}F]FECNT, a highly selective DAT ligand (Goodman et al., 2000). It was shown that increasing isoflurane from 1% to 2% (deep sleep) further decreased DAT BP_{ND} to about 63% less than the awake rhesus monkeys. On the other hand, it was shown that the BP_{ND} of [^{11}C]β-CFT to the DAT in the rhesus monkey striatum under 1.5% isoflurane conditions is increased by 50% compared to the awake condition (Harada et al., 2004; Tsukada et al., 1999), although CFT is not as selective at the DAT as FECNT. Adachi et al. (2005) studied the effects of isoflurane anesthesia on extracellular concentrations of DA and its metabolites in rats using in vivo microdialysis techniques. They measured a ~20% increase in extracellular concentrations of DA when the rats inhaled 3% isoflurane. On the other hand, 1% isoflurane inhalation caused a reduction in the extracellular concentrations of DA to ~80% of the awake value. The above examples from the literature as well as many other reports available demonstrate that the impact of isoflurane anesthesia on the dopaminergic system depends on the dosage of isoflurane used, the affinity of the receptor against the radiotracer used, the length of time of isoflurane exposure, as well as the experimental method. For example, in Votaw et al. (2003), the comparison studies between the awake and the 1% and the 2% isoflurane anesthetized rhesus monkeys were carried out on the same day, which may not have allowed enough recovery time from the first isoflurane anesthesia session. On the other hand, Tsukada et al. (Tsukada et al., 1999) carried out the awake and the anesthetized studies on two separate days.

In the current study, the lower estimates of the [^{18}F]fallypride DVR' (and subsequently BP_{ND}) in anesthetized rats is consistent with an increase in DA levels and a decrease in DAT. Our results are consistent with those of Votaw and Elfving, who reported that isoflurane causes a reduction in membrane externalized DAT during anesthesia, leading to a decreased DA reuptake and increased occupancy of the D2 receptor by extracellular DA.

Despite the habituation and acclimation of the rats, a tail vein injection in conscious rats could have induced stress-related DA release. It has been shown in a microdialysis study on the brains of male Sprague-Dawley rats that tail-shock stress produced up to a 25% increase in striatal extracellular DA relative to baseline which was marginally significant ($P = 0.05$) during the first 15 min after stress termination (Abercrombie et al., 1989). This increase in DA release in the striatum due to tail-shock stress appeared to have a prolonged time course compared to the nucleus accumbens and the medial frontal cortex though the stress-induced increase in DA release in the striatum was small relative to baseline compared to the nucleus accumbens (39% increase) and the medial frontal cortex (95% increase). Pie et al. (1990)

determined in a microdialysis study that a 2-min tail pinch on male Sprague Dawley rats increased striatal DA release by up to 17%. On the other hand, the DA levels from striatal dialysates within 10 min following the application of a 5-min tail pinch varied considerably between the rats and ranged from 5 to 483% with an average of 238%. In addition, DA levels in the striatal dialysates appeared to be 20%–30% above baseline 30 min after the 5-min tail pinch application and maintained these elevated levels for nearly 80 min before returning to baseline levels. Antelman et al. (1975) carried out behavioral studies on rats using tail pinch as a source of stress and found their behavior to be similar to that of amphetamine induced behavior. Amphetamine causes a drop in the binding of fallypride to D2/3 (Morris and Yoder, 2007; Mukherjee et al., 2005; Narendran et al., 2009; Riccardi et al., 2005; Slifstein et al., 2004). Although the rats were habituated for a few days prior to injection, some degree of stress may have been induced by restraining the animals during insertion of the tail vein catheter. In addition, we predict that inserting a catheter in a rat's tail while the rat is conscious induces stress similar to that of a tail pinch. Although statistical significance was not achieved, this could explain why the mean DVR' estimates of Group 3 (conscious injection via the tail vein) appear slightly lower than the mean DVR' estimates of Group 2 (conscious injection via the jugular catheter). In addition, differences in the amount of stress experienced among animals in Group 3 may have contributed to the dispersion and high degree of variability in the results of the DVR' estimates for Group 3 compared to any other group (Fig. 3).

In our work, we followed the interpretations of the impact of isoflurane anesthesia on the dopaminergic system outlined in the cited literature which they point to changes in the dopaminergic system itself. However, one cannot neglect the fact that isoflurane anesthesia may have a physiological impact and therefore alter blood delivery of the radiotracer, i.e., change K_1 and/or k_2 . This may have some contribution to the increase in DVR' as compared with DVR. However, since we are using a reference tissue method of analysis, we assume that the underlying K_1 and k_2 between the cerebellum and the striatum are the same minimizing contribution of physiological or blood metabolite changes to DVR'. In the same manner, in our compartmental modeling simulations, since we are not aware of any changes in the [^{18}F]fallypride metabolism in the blood because of isoflurane anesthesia, we assumed the plasma input function is the same between the fully anesthetized state and the awake state during the initial 60 min uptake. Therefore, we kept K_1 and k_2 obtained from the 120 min scans fixed to be able to estimate k_3/k_4 in the conscious uptake phase. However, this would not be the case if the plasma input function did change between the conscious uptake and unconscious uptake phases, and in this case we would have no way of knowing what K_1 and k_2 would be in the conscious uptake phase and therefore we would not be able to use a compartmental modeling to estimate k_3/k_4 .

In a recent study, Schulz et al. (2011) used the Rat- CAP to image rat brains using [^{11}C]raclopride in conscious rats and determined a 10.6% increase in D2 BP_{ND} as compared to D2 BP_{ND} of the same rats imaged (1 h later) under ketamine–xylazine anesthesia (Schulz et al.). Although they used ketamine–xylazine anesthesia during the imaging session in the unconscious uptake phase, their results agree with our results in displaying that anesthesia results in lower D2 BP_{ND} as compared with conscious uptake of the radiotracer. An

attachment bracket for the Rat- CAP was surgically secured to the outside of the rats' skulls before the scans while under anesthesia. The stress from the surgery added with the anesthesia exposure during surgery may have caused the difference in BP_{ND} between conscious imaging and unconscious imaging to be as low as 10.6% and without those two factors, BP_{ND} may have been greater.

In a pharmaceutical drug challenge, if it is anticipated that the drug will cause a small change in the BP_{ND} estimates, then to obtain the best results, it may be desirable to use the conscious injection plus initial conscious uptake method presented in this work and inject [^{18}F]fallypride via a jugular catheter. This can be beneficial in several ways: (i) the possibility of [^{18}F]fallypride competing with anesthesia-induced DA release is removed, (ii) without anesthesia impacting the dopaminergic system, a more accurate assessment of the ability of the pharmaceutical drug to displace [^{18}F]fallypride is obtained, (iii) data will be more representative of those seen in human studies since human radiotracer uptake is almost always executed in a conscious state, (iv) rat behavioral studies can be performed during the conscious initial uptake phase, i.e., during the first 50–60 min post [^{18}F]fallypride injection, and therefore behavioral study results can be obtained in conjunction with PET results, (v) from the practical standpoint of cost and time, this method will allow for double the throughput compared with the conventional 120 min scans commonly used (Christian et al., 2000; Dalley et al., 2007; Tantawy et al., 2009; Tokunaga et al., 2009). Similarly, single prolonged stress (SPS), e.g., tail pinching, (Yamamoto et al., 2007) has an impact on the dopaminergic system and also can be studied by the method of delayed fallypride scans. SPS can be carried out during the first 60 min post [^{18}F]fallypride injection to investigate the immediate effect of SPS on the dopaminergic system, which until now has been done via behavioral observations or microdialysis only.

Further studies are needed to determine the length of time anesthesia affects the dopaminergic system. For that, we suggest an experiment in which each rat is placed under isoflurane anesthesia for approximately 1 h, and then they are divided into groups in which a conscious [^{18}F]fallypride injection is initiated at different times following anesthesia, e.g., 2, 4, 8, 24, 48, and 36 h post isoflurane inhalation followed by ~60 min conscious uptake of the radiotracer and then imaged for 60 min while under anesthesia.

The method of delayed scans following [^{18}F]fallypride injection should be applicable across all species studies: rats, nonhuman primates, and humans. In addition to the reasons mentioned above, initial conscious uptake of [^{18}F]fallypride can help minimize the risk of death to animals, including nonhuman primates, during a study caused by prolonged exposure to anesthesia.

In our lab, we have begun using this method in all of our [^{18}F]fallypride studies. In addition, we plan to use a dual rat bed developed in our institute (Tantawy et al., 2007) to further increase the throughput, compared with the conventional 120 min single-bed scans.

CONCLUSION

The results of this work support the idea that isoflurane anesthesia influences the release of D2 DA, reducing the number of unoccupied D2 receptors. Using the delayed scans approach (Tantawy et al., 2009) the effects of anesthesia on [^{18}F]fallypride estimates of available DA D2 receptors at both baseline and following pharmacological challenges can be minimized. In addition, the method of delayed scans can be used as a useful tool to probe the impact of stress and other environmental conditions on neurotransmission via a well designed experiment in which stress, such as tail pinching or SPS, is introduced to the rat during the first 60 min of conscious uptake of [^{18}F]fallypride. The delayed scan method also may allow evaluation of the effects of behavioral challenges on available DA D2 receptor levels without the need for conscious scanning in animals. This method can be used in conjunction with behavioral studies and is especially applicable for studies involving a pharmaceutical drug challenge, which will eliminate the competition between the drug and the anesthesia-induced DA release. The method of delayed scans also has merit when applied to human studies. Although anesthesia is not used in these human studies, this method offers the advantage of reduced scan time, which should reduce motion artifacts in addition to increasing throughput. In our lab, we have successfully begun using this method in all our [^{18}F]fallypride studies.

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REFERENCES

- Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ. Differential effect of stress on in vivo dopamine release in striatum. Nucleus accumbens, and medial frontal cortex. *J Neurochem.* 1989; 52:1655–1658. [PubMed: 2709017]
- Adachi YU, Yamada S, Satomoto M, Higuchi H, Watanabe K, Kazama T. Isoflurane anesthesia induces biphasic effect on dopamine release in the rat striatum. *Brain Res Bull.* 2005; 67:176–181. [PubMed: 16144652]
- Ansari MS, Kessler RM, Clanton JA, de Paulis T, Baldwin RM. Comparison of three [^{18}F]fallypride methods intended for automated remote chemistry modules. *J Nucl Med.* 2006; 47:159P.
- Antelman SE, Szechtman H, Chin P, Fisher AE. Tail pinch-induced eating, gnawing and licking behaviour in rats: Dependence on the nigrostriatal dopamine system. *Brain Res.* 1975; 99:319–337. [PubMed: 1182545]
- Cavazzuti M, Porro CA, Biral GP, Benassi C, Barbieri GC. Ketamine effects on local cerebral blood-flow and metabolism in the rat. *J Cereb Blood Flow Metab.* 1987; 7:806–811. [PubMed: 3121648]
- Christian BT, Narayanan TK, Shi B, Mukherjee J. Quantitation of striatal and extrastriatal D-2 dopamine receptors using PET imaging of [^{18}F]fallypride in nonhuman primates. *Synapse.* 2000; 38:71–79. [PubMed: 10941142]
- Crosby G, Crane AM, Sokoloff L. Local changes in cerebral glucose-utilization during ketamine anesthesia. *Anesthesiology.* 1982; 56:437–443. [PubMed: 7081728]
- Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Lääne K, Peña Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science.* 2007; 317:1033–1035. [PubMed: 17717168]

- Elfving B, Bornholm B, Knudsen GM. Interference of anaesthetics with radioligand binding in neuroreceptor studies. *Eur J Nucl Med Mol Imaging*. 2003; 30:912–915. [PubMed: 12715241]
- Goodman MM, Kilts CD, Keil R, Shi B, Martarello L, Xing D, Votaw JR, Ely TD, Lambert P, Owens MJ, Camp VM, Malveaux E, Hoffman JM. 18F-labeled FECNT: A selective radioligand for PET imaging of brain dopamine transporters. *Nucl Med Biol*. 2000; 27:1–12. [PubMed: 10755640]
- Harada N, Ohba H, Fukumoto D, Kakiuchi T, Tsukada H. Potential of [18F]β-CFT-FE(2β-Carbomethoxy-3β-(4-fluorophenyl)-8-(2-[18F]fluoroethyl)nortropine) as a dopamine transporter ligand: A PET study in the conscious monkey brain. *Synapse*. 2004; 54:37–45. [PubMed: 15300883]
- Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang SC, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab*. 2007; 27:1533–1539. [PubMed: 17519979]
- Kessler RM, Votaw JR, de Paulis T, Bingham DR, Ansari MS, Mason NS, Holburn G, Schmidt DE, Votaw DB, Manning RG, Ebert MH. Evaluation of 5-[18F]fluoropropylepidepride as a potential PET radioligand for imaging dopamine D₂ receptors. *Synapse*. 1993; 15:169–176. [PubMed: 8278895]
- Loening AM, Gambhir SS. AMIDE: A free software tool for multimodality medical image analysis. *Mol Imaging*. 2003; 2:131–137. [PubMed: 14649056]
- Logan J. Graphical analysis of PET data applied to reversible and irreversible tracers. *Nucl Med Biol*. 2000; 27:661–670. [PubMed: 11091109]
- Momosaki S, Hatano K, Kawasumi Y, Kato T, Hosoi R, Kobayashi K, Inoue O, Ito K. Rat-PET study without anesthesia: Anesthetics modify the dopamine D1 receptor binding in rat brain. *Synapse*. 2004; 54:207–213. [PubMed: 15476291]
- Morris ED, Yoder KK. Positron emission tomography displacement sensitivity: predicting binding potential change for positron emission tomography tracers based on their kinetic characteristics. *J Cereb Blood Flow Metab*. 2007; 27:606–617. [PubMed: 16788713]
- Mukherjee J, Yang Z-Y, Das MK, Brown T. Fluorinated benzamide neuroleptics3. Development of (S)-N-[(1-allyl-2-pyrrolidinyl) methyl]-5-(3-[18F]fluoropropyl)-2,3-dimethoxybenzamide as an improved dopamine D-2 receptor tracer. *Nucl Med Biol*. 1995; 22:283–296. [PubMed: 7627142]
- Mukherjee J, Christian BT, Narayanan TK, Shi B, Collins D. Measurement of d-amphetamine-induced effects on the binding of dopamine D-2/D-3 receptor radioligand, 18F-fallypride in extra-striatal brain regions in non-human primates using PET. *Brain Res*. 2005; 1032:77–84. [PubMed: 15680944]
- Narendran R, Frankle WG, Mason NS, Rabiner EA, Gunn RN, Searle GE, Vora S, Litschge M, Kendro S, Cooper TB, Mathis CA, Laruelle M. Positron emission tomography imaging of amphetamine-induced dopamine release in the human cortex: A comparative evaluation of the high affinity dopamine D_{2/3} radiotracers [11C]FLB 457 and [11C]fallypride. *Synapse*. 2009; 63:447–461. [PubMed: 19217025]
- Onoe H, Inoue O, Suzuki K, Tsukada H, Itoh T, Mataga N, Watanabe Y. Ketamine increases the striatal N-[C-11]methylpiperone binding in vivo—Positron emission tomography study using conscious rhesus-monkey. *Brain Res*. 1994; 663(2):191–198. [PubMed: 7874501]
- Pei Q, Zetterstrom T, Fillenz M. Tail pinch-induced changes in the turnover and release of dopamine and 5-hydroxytryptamine in different brain regions of the rat. *Neuroscience*. 1990; 35:133–138. [PubMed: 1694282]
- Riccardi P, Li R, Ansari MS, Zald D, Park S, Dawant B, Anderson S, Doop M, Woodward N, Schoenberg E, Schmidt D, Baldwin R, Kessler R. Amphetamine-induced displacement of [18F]fallypride in striatum and extrastriatal regions in humans. *Neuropsychopharmacology*. 2005; 31:1016–1026. [PubMed: 16237395]
- Rubins DJ, Melega WP, Lacan G, Way B, Plenevaux A, Luxen A, Cherry SR. Development and evaluation of an automated atlas-based image analysis method for microPET studies of the rat brain. *Neuroimage*. 2003; 20:2100–2118. [PubMed: 14683714]

- Schulz D, Southeikal S, Junnarkar SS, Pratte J-F, Purschke ML, Stoll SP, Ravindranath B, Maramraju SH, Krishnamoorthy S, Henn FA, O'Connor P, Woody CL, Schlyer DJ, Vaska P. Simultaneous assessment of rodent behavior and neurochemistry using a miniature positron emission tomograph. *Nat Methods*. 2011; 8:347–352. [PubMed: 21399637]
- Schweinhardt P, Fransson P, Olson L, Spenger C, Andersson JL. A template for spatial normalisation of MR images of the rat brain. *J Neurosci Methods*. 2003; 129:105–113. [PubMed: 14511814]
- Slifstein M, Narendran R, Hwang D-R, Sudo Y, Talbot PS, Huang Y, Laruelle M. Effect of amphetamine on [^{18}F]fallypride in vivo binding to D_2 receptors in striatal and extrastriatal regions of the primate brain: Single bolus and bolus plus constant infusion studies. *Synapse*. 2004; 54:46–63. [PubMed: 15300884]
- Tantawy MN, Peterson TE, Kessler RM. Rat brain PET imaging using a dual rat bed. *J Nucl Med*. 2007; 48(Suppl 2):1441P.
- Tantawy MN, Jones CK, Baldwin RM, Ansari MS, Conn PJ, Kessler RM, Peterson TE. [^{18}F]fallypride dopamine D_2 receptor studies using delayed microPET scans and a modified Logan plot. *Nucl Med Biol*. 2009; 36:931–940. [PubMed: 19875049]
- Tokunaga M, Seneca N, Shin R-M, Maeda J, Obayashi S, Okauchi T, Nagai Y, Zhang M-R, Nakao R, Ito H, Innis RB, Halldin C, Suzuki K, Higuchi M, Suhara T. Neuroimaging and physiological evidence for involvement of glutamatergic transmission in regulation of the striatal dopaminergic system. *J Neurosci*. 2009; 29:1887–1896. [PubMed: 19211895]
- Tolwani RJ, Jakowec MW, Petzinger GM, Green S, Waggle K. Experimental models of Parkinson's disease: Insights from many models. *Lab Anim Sci*. 1999; 49:363–371. [PubMed: 10480640]
- Tsukada H, Nishiyama S, Kakiuchi T, Ohba H, Sato K, Harada N, Nakanishi S. Isoflurane anesthesia enhances the inhibitory effects of cocaine and GBR12909 on dopamine transporter: PET studies in combination with microdialysis in the monkey brain. *Brain Res*. 1999; 849:85–96. [PubMed: 10592290]
- Tsukada H, Miyasato K, Kakiuchi T, Nishiyama S, Harada N, Domino EF. Comparative effects of methamphetamine and nicotine on the striatal [(11) C]raclopride binding in unanesthetized monkeys. *Synapse*. 2002; 45(4):207–212. [PubMed: 12125041]
- Votaw JR, Smith MB, Hua J, Voll R, Martarello L, Levey AI, Bowman FD, Goodman M. Interaction of isoflurane with the dopamine transporter. *Anesthesiology*. 2003; 98:404–411. [PubMed: 12552200]
- Yamamoto S, Morinobu S, Fuchikami M, Kurata A, Kozuru T, Yamawaki S. Effects of single prolonged stress and D-cycloserine on contextual fear extinction and hippocampal NMDA receptor expression in a rat model of PTSD. *Neuropsychopharmacology*. 2008; 33:2108–2116. [PubMed: 17957211]

Figure 1.

Time-activity curves (TACs) of the cerebellum and striatum (average of left and right striatum) for rats in Group 2 ($n = 6$) comparing a 55-min initial conscious uptake period of the radiotracer followed by a 60-min PET scan while under ~1.5% anesthesia to the full 120-min PET scans in which the rats were fully anesthetized with ~1.5% isoflurane from the start of the radiotracer administration through the end of the scan. The TACs are displayed as mean $\pm$ SE. SUV: standard uptake value. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 2.

Sample Logan plots of a rat injected with [^{18}F]fallypride via a jugular catheter: (a) 60 min prior to the start of a 60-min dynamic acquisition on the microPET (conscious uptake during the first ~60 min) ($\text{DVR}' = 19.1 \pm 0.6$); (b) 30 s after the start of a 120 min dynamic acquisition (unconscious uptake) ($\text{DVR} = 15.2 \pm 0.8$). (c) Logan plot of the last 60 min of the full 120 min data after neglecting the first 60 min ($\text{DVR}' = 16.8 \pm 1.3$). [Color figure can be viewed in the online issue, which is available at www.wileyonlinelibrary.com.]

Figure 3.

Statistical display of DVR' estimates obtained from controls ($n = 20$): rats imaged for 120 min while under ~1.5% isoflurane anesthesia and DVR' was measured for the last 60 min of the 120 min data after neglecting the first 60 min. Group 1 ($n = 4$): Rats were anesthetized and then injected with [^{18}F]fallypride via the tail vein (TV) and then were allowed to wake up and set free in their cages. Group 2 ($n = 14$): Rats were restrained and injected with [^{18}F]fallypride consciously via a jugular catheter (JC) and then set free in their cages. Group 3 ($n = 10$): Rats were restrained, injected with [^{18}F]fallypride via the TV consciously and then set free in their cages. Groups 1–3 were fed ad libitum while free in their cages for 55 min and then were anesthetized with ~1.5% isoflurane and imaged in a microPET Focus 220

for 60 min (conscious fattypride uptake for ~60 min + imaging while anesthetized for 60 min).

TABLE I

Mean DVR' estimates of the different groups of rats studied in this work and previous work (Tantawy et al., 2009)

Study	DVR' (mean $\pm$ SE)	P
Controls: last 60 min of 120 min data after neglecting the first 60 min (unconscious injection + unconscious uptake)	15.7 $\pm$ 0.3, (n = 20)	–
Group 1: unconscious injection via the tail vein (TV) + initial conscious uptake	17.7 $\pm$ 0.3, (n = 4)	>0.05
Group 2: Conscious injection via a jugular catheter (JC) + initial conscious uptake	19.2 $\pm$ 0.4, (n = 14)	<0.01
Group 3: Conscious TV injection + initial conscious uptake	18.8 $\pm$ 0.7 (n = 10)	<0.01

DVR' was obtained via graphical analysis. A one way ANOVA with a Dunnett's multiple comparison test was carried out where the animals imaged for a full 120 min in the microPET under ~1.5% isoflurane anesthesia were taken as the controls.