Evaluation of [^{18}F]2FP3 in pigs and non-human primates

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Abstract

So far, no suitable 5-HT_7R radioligand exists for clinical PET imaging. [^{18}F]2FP3 was first tested *in vivo* in cats and the results were promising for further evaluations. Here, we evaluate the radioligand in pigs and non-human primates (NHP). Furthermore, we investigate species differences in 5-HT_7R binding with [^{3}H]SB-269970 autoradiography in post mortem pig, NHP, and human brain tissue.

Specific binding of [^{18}F]2FP3 was investigated by intravenous administration of the 5-HT_7R specific antagonist SB-269970. [^{3}H]SB-269970 autoradiography was performed as previously described.

[^{18}F]2FP3 was synthesized in an overall yield of 35-45%. High brain uptake of the tracer was found in both pigs and NHPs, however pretreatment with SB-269970 only resulted in decreased binding of 20% in the thalamus, a 5-HT_7R rich region. Autoradiography on post mortem pig, NHP and human tissues revealed that specific binding of [^{3}H]SB-269970 was comparable in the thalamus of pig and NHP.

Despite the high uptake of [^{18}F]2FP3 in both species, the binding could only be blocked to a limited degree with the 5-HT_7R antagonists. We speculate that the affinity of the radioligand is too low for imaging the 5-HT_7Rs *in vivo* and that part of the PET signal arises from targets other than the 5-HT_7R.

1. Introduction

Serotonin (5-hydroxytryptamine, or 5-HT) exerts its many psychophysiological functions through its fourteen different receptors and the latest addition to the family is the serotonin 7 receptor (5-HT_7R). 5-HT_7R is associated with various physiological effects, including serotonin-induced phase-shifting of the circadian rhythm[1]. The 5-HT_7R may also be involved in the pathophysiology of various psychiatric disorders such as anxiety and depression, as has been reviewed by Matthys et al. [2]. The accumulated evidence suggests that the 5-HT_7R can be a relevant drug target for the treatment of these psychiatric disorders. Although *in vivo* studies of the cerebral 5-HT_7R binding with positron emission tomography (PET) would improve the understanding of its role in central nervous system disorders, no suitable PET radioligands for imaging the 5-HT_7R in humans exist.

Several selective 5-HT_7R compounds have been developed[3] and some have been radiolabeled for use in radioligand binding assays and *in vitro* autoradiography studies. For example, [^{3}H]SB-269970 has been used for in vitro experiments in rodent, pig, marmoset, and human brain tissue[4–7]. In recent years, there has been a large effort to identify a radioligand for positron emission tomography (PET) imaging. PET is a very sensitive imaging tool that can be applied to determine receptor availability and occupancy and can be used to inform critically about drug discovery and development decisions[8].

We and others have investigated a number of ligands from different compound classes for the purpose of developing a 5-HT_7R PET radioligand[5,9–15]. Of these, [^{11}C]Cimbi-717 displayed the most promising results for selective 5-HT_7R PET imaging in pigs[5]. A series of ^{18}F-labeled compounds have been developed using SB-269970 as lead compound[16–18] because of its high affinity (K_D = 1.3 nM) and high selectivity for the 5-HT_7R[19]. One promising analogue of SB-269970 is [^{18}F]2FP3 (K_D = 8.4 nM, human recombinant receptors in CHO cells), which displayed promising results when evaluated in cats[16]. The aim of this project was

to evaluate [^{18}F]2FP3 in pigs and non-human primates (NHP) for its potential as PET radioligand for the 5-HT$_7$R. Furthermore, we investigated species differences in 5-HT$_7$R binding in post mortem, pig, NHP and human brain tissue.

2. Methods

2.1 2FP3: Precursor and reference compound syntheses

Precursor (2NP3) and reference compound (2FP3) were synthesized as previously described[16].

2.2 Radiosynthesis of [^{18}F]2FP3

2.2.1 Copenhagen University Hospital: To an aqueous [^{18}F]fluoride solution, Kryptofix®222 (ABX GmbH, Germany), (15 mg), 15 µL potassium carbonate (1 N) and 1-mL acetonitrile were added. The mixture was dried in a stream of nitrogen at 80 °C. The drying procedure was repeated three times until the reaction mixture was absolutely dry. 4 mg (of nitro precursor ((*R*)-4-methyl-1-(2-(1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)ethyl)piperidine (2NP3) dissolved in 0.3 mL DMSO was added, and the reaction mixture heated at 150 °C for 10 min. After dilution with 150 mL of water, the reaction mixture was purified by semi-preparative high-performance liquid chromatography (HPLC) separation (LunaC18(2), 5µm, 250×10 mm; Phenomenex;0.01 borax buffer (pH=9.21)/MeCN (30/70), flowrate: 6 mL; retention time ([^{18}F]2FP3): ~ 600 sec). After diluting the collected HPLC fraction containing [^{18}F]2FP3 with water, the product was trapped on an activated Sep-PakW-C18-cartrige and subsequently eluted with 3 mL of ethanol into 12 mL of phosphate buffered saline (100 mM, pH 7). Quality control was performed to determine radiochemical purity and molar activity (LunaC18(2), 5µm, 150×4.6 mm; Phenomenex;0.01 borax buffer (pH=9.21)/MeCN (30/70), flowrate: 2 mL; retention time [^{18}F]2FP3: 3.01 min).

2.2.2 Molecular NeuroImaging LLC: [^{18}F]fluoride solution (Cardinal Health, CT, USA) was trapped onto an ion exchange column (Waters QMA light) before being eluted in the reactor with a solution of Kryptofix®222 (ABX GmbH, Germany), (10 mg) and potassium carbonate (1.2 mg) in aqueous acetonitrile (1 mL, acetonitrile/water 4/1 v/v). The mixture was dried in a stream of helium and under vacuum at 95 °C for 4 min. Acetonitrile (1 mL) was added to the reactor and the drying continued for 3 min. A second portion of acetonitrile (1 mL) was added, and the drying was continued for 3 min before the reactor was cooled to 70 °C. A solution of the nitro precursor (2NP3, 2 mg) dissolved in DMSO (1 mL) was added, and the reaction mixture heated at 150 °C for 10 min. After dilution with water (2 mL) and HPLC eluent (2 mL), the reaction mixture was purified by semi-preparative HPLC (Agilent Eclipse XDB, 250x10 mm) using a mixture of methanol/water/triethylamine (70/30/0.1 v/v/v) at a flow rate of 4 mL/min. The retention time of [^{18}F]2FP3 was 750 sec. After diluting the collected HPLC fraction containing [^{18}F]2FP3 with water (15 mL), the product was trapped on a Waters tC18 light Sep-Pak cartridge and eluted with ethanol (1 mL) into saline (10 mL). The cartridge was rinsed with saline (4 mL), adding the rinse to the product. Quality control was performed to determine radiochemical purity and molar activity (Waters XBridge C18, 5µm, 150×4.6 mm, eluting with methanol/water/triethylamine (70/30/0.1 v/v/v) at a flow rate of 1 mL/min). The retention time of [^{18}F]2FP3 was 10 min.

2.3 In vivo imaging in pigs

Three female domestic pigs (crossbreed of Landrace x Yorkshire x Duroc, mean weight ± S.D., 23 ± 0.6 kg) were used for *in vivo* PET imaging. All animal procedures were approved by the Danish Council for Animal Ethics (journal no. 2012-15-2934-00156).

[^{18}F]2FP3 was synthesized according to the method described in section 2.2.1. The tracer was given as intravenous (i.v.) bolus and the injected dose was 365 ± 88 MBq (mean ± SD, n = 3).

The pigs were scanned for 150 min in list mode. Ninety minutes after the injection of the radioligand, a challenge of 1 mg/kg SB-269970 (Tocris Bioscience) was given i.v. In one pig, a constant infusion of 1 mg/kg/h was started 30 min prior to the injection of [^{18}F]2FP3 resulting in a total infusion time of 3 hours.

See supplementary information for more details on animal procedure, blood sampling, metabolite analysis, reconstruction and pre-processing of PET data.

2.4 In vivo imaging in NHP

Two male rhesus monkeys (Macaca mulatta), one male and one female, were used as research subjects. These animals were housed and imaged following identical procedures at the Yale University School of Medicine in New Haven, Connecticut. PET imaging was carried out under institutional animal care protocols complying with Federal regulations. Animal care approval and oversight for this study was provided by the Yale University Institutional Animal Care and Use Committee.

[^{18}F]2FP3 was synthesized according to section 2.2.2 and was administered via iv bolus over 3 minutes and the injected doses were 182.7 ± 65 MBq (mean ± SD, n = 3). Following radiotracer administration, list mode emission data was collected over 180 min on the microPET FOCUS 220 camera (Siemens Medical Solutions, Inc.). A transmission scan with a Ge-68 source prior to injection of radiotracer was used for attenuation correction. In one animal SB-269970 (3 mg/kg) was administrated i.v. over 15 min starting 70 min before the injection of the radioligand. In a different experiment, WAY100635 (1 mg/kg) was administrated i.v. over 5 min starting 35 min before the injection of the radioligand.

See supplementary information for more details on animal procedure, MR imaging, reconstruction and pre-processing of PET data.

2.5 Kinetic modelling

The PET imaging data for both pig and NHP were analysed with the Logan graphical analysis (LGA), using the metabolite corrected arterial plasma concentration to calculate the total distribution volume (V_T).

2.6 In vitro autoradiography

For autoradiography, smaller pieces of one non-human primate brain were sliced on a HM500OM cryostat (Microm Intl GmbH) in 20 µm coronal sections except for cerebellum which was sliced in the sagittal plane. Human temporal cortical tissue was obtained from anterior temporal lobectomies in patients with medically intractable temporal lobe epilepsy with hippocampal onset, aged 19 and 55 years. Written informed consent was obtained from both patients before surgery. The study was approved by the Ethical Committee in the Capital Region of Denmark (H-2-2011-104) and performed in accordance with the Declaration of Helsinki. The tissue was dissected and immediately frozen on dry ice and stored at -80°C until use. Human brain tissue was sliced in 12 µm coronal sections. Autoradiography was performed on 3 to 6 adjacent sections for both NHP and human brain tissue. See supplementary information for details of the autoradiographic protocol.

3. Results

3.1 Radiosynthesis of [^{18}F]2FP3

Injection-ready [^{18}F]2FP3 for pig studies was prepared according to section 2.2.1 in 40-65 min from the end of bombardment with a radiochemical yield of 0.4-2 GBq (Fig. 1). The radiochemical yield was 35.2% (n = 3). The molar activity at the time of injection (TOI) was 24 ± 23 GBq/µmol (n = 3) resulting in injected masses of 12.5 ± 12.4 µg. For NHP studies the radiosynthesis of [^{18}F]2FP3 (see section 2.2.2) was automated using a commercial

radiofluorination device (GE Tracerlab FXFN) to afford high radiochemical yields (43.8%, *n* = 3) and specific activities of 270.4 ± 44.2 GBq/μmol (*n* = 3) resulting injected masses of 0.116 ± 0.110 μg. Both radiochemical labs confirmed the identity of the product by co-injection with a sample of reference compound, which under the same elution conditions showed an identical retention time. The compound was stable over 4 h and the radiochemical purity > 98 %.

3.2 In vivo studies of [^{18}F]2FP3 in pigs

[^{18}F]2FP3 readily entered the pig brain and displayed reversible tracer kinetics (Fig. 2A and 2D) but with relatively slow wash-out of the tracer. The distribution of the radioligand was to some degree in accordance with the 5-HT$_7$R distribution within pigs[5] with high uptake in the thalamus and lowest uptake in the cerebellum. However, an unexpected higher uptake was found in the striatum, a region that has a low density of 5-HT$_7$Rs. In pigs the binding of [^{18}F]2FP3 was challenged by administrating the 5-HT$_7$R specific antagonist SB-269970 (1 mg/kg) 90 minutes after the injection of the radioligand (Fig. 2A). Administration of SB-269970 resulted in an increase in plasma radioactivity, suggesting that SB-269970 displaces [^{18}F]2FP3 from peripheral receptor sites but no significant change was observed in any of the brain TACs. The specificity of radioligand binding was also investigated by starting a constant infusion of SB-269970 (1 mg/kg/h) 30 min prior to the injection of [^{18}F]2FP3 (Fig. 2B) and lasting throughout the scan time (total infusion time 3h). This resulted in a slightly higher uptake of radioligand across all brain regions, but with visually similar kinetics compared to the baseline experiment. Although a higher uptake was seen after pretreatment with SB-269970, changes in radioactivity in the blood and metabolism of the radioligand should also be considered. Therefore, we quantified the radioligand uptake in the brain using the Logan graphical model (LGA). Kinetic modelling revealed a decrease in binding in all investigated regions of the brain (see Supplementary Table 1) and the occupancy of SB-269970 in the pig

brain was determined to 35 % by the occupancy plot (Fig 2C). Additionally, the occupancy plot determined the non-displaceable distribution volume to 6.29 ml/cm^3.

3.3 In vivo studies of [^{18}F]2FP3 in NHP

After intravenous injection, [^{18}F]2FP3 rapidly entered the NHP brain (Fig. 3A). The radioligand accumulation was fairly uniform in the NHP brain but higher initial uptake of radioligand was found in cerebellum and occipital cortex and lower accumulation was found in the thalamus (Fig. 3B). The tracer kinetics of [^{18}F]2FP3 appeared fast with peak uptake after approximately 10 min followed by fast wash-out from all investigated brain regions. The specificity of binding of [^{18}F]2FP3 was also investigated in NHPs by administrating SB-269970 (3 mg/kg) and the 5-HT$_{1A}$R selective WAY100635 (1 mg/kg). In both blocking experiments, the TACs had slightly faster washout rates consistent with a modest blocking effect of the drugs (Fig. 3C and 3D). Quantification of binding after administration of SB-269970 revealed a decrease in V_T of 13 % in the thalamus and of 20 % in the temporal cortex (Supplementary Table 2). Administration of WAY100635 resulted in similar blocking effects, with decreases in V_T values: 20 % in the thalamus and 19 % in the temporal cortex. It was not possible to reliably estimate occupancy of the two drugs and non-displaceable binding of [^{18}F]2FP3 in the NHP due to the low blocking effects of drugs.

3.4 Assessment of metabolic profile

Finally, the systemic metabolism of [^{18}F]2FP3 was determined *in vivo*. The metabolism of [^{18}F]2FP3 in pigs was fast, with only 50 % of parent compound remaining after 10 min (Fig. 4A). When a challenge of 1 mg/kg SB-269970 was given after 90 minutes of scan time, the plasma parent compound fraction as well as activity in whole blood and plasma increased, suggesting that [^{18}F]2FP3 was displaced by SB-269970 at peripheral receptor sites. Pretreatment with SB-269970 did not influenced the rate of metabolism of the parent compound significantly.

In NHPs, the rate of metabolism of [^{18}F]2FP3 was slower than in pigs, with 50 % parent compound remaining after 60 min (Fig. 4B). Contrary to what was observed in pigs, the pretreatment with SB-269970 lead to an increased metabolic breakdown of [^{18}F]2FP3 in NHPs. This effect was not observed with pre-administration of WAY100635.

In both species, the metabolites detected were more polar than the parent compound and thus unlikely to cross the blood-brain barrier (Supplementary Figure 1).

3.6 In vitro autoradiography

The specific binding (SB) was determined with [^{3}H]SB-269970 autoradiography in the human, NHP, and pig temporal cortex: 18.9 ± 4.99 (human), 21.5 ± 4.32 (NHP), and 41.0 ± 3.03 (pig) fmol/mg tissue equivalent (TE). SB was also determined in the thalamus and cerebellum of NHP and pig tissue: 32.3 ± 3.61 (NHP, thalamus), and 45.6 ± 4.99 0.19 (pig, thalamus), 14.3 ± 0.93 (NHP, cerebellum) and 13.4 ± 2.22 (pig, cerebellum) fmol/mg TE. The results from pig brain tissue are taken from Hansen et al. (2014)[5] Comparing the specific binding of [^{3}H]SB-269970 and the V_T of [^{18}F]2FP3 revealed no apparent relationship between the in vitro and in vivo measures for the pig (Fig. 5C). No relationship was found for the NHP data either, although only three different regions could be compared.

4. Discussion

In pigs, we observed a high uptake of the radioligand and a relative slow wash-out from the brain, which could indicate specific binding to the target. However, pretreatment with SB-269970 (1 mg/kg/h for 3h) resulted in a [^{18}F]2FP3 occupancy of 35% whereas the same dose of SB-269970 resulted in 60% occupancy of the 5-HT_7R with [^{11}C]Cimbi-717 in pigs[5], suggesting that [^{18}F]2FP3 has either off-target binding or high non-specific binding. The NHPs also had high brain uptake of [^{18}F]2FP3 but with faster tracer kinetics than in the pig. Further,

the regional separation between the TACs was minimal and pretreatment with SB-269970 and with WAY100635 resulted in 13-20% decrease in V_T values, respectively. This supports that in the NHP, [^{18}F]2FP3 has limited but measurable specific binding to the 5-HT$_7$R and 5-HT$_{1A}$R *in vivo*. The binding to the 5-HT$_{1A}$R was unexpected, given that 2FP3 does not have any appreciable affinity for the 5-HT$_{1A}$R *in vitro*[16]. Although WAY100635 is considered highly selective for the 5-HT$_{1A}$R, it does retain some affinity for the 5-HT$_7$R (248 nM)[20]. With the administered dose (1 mg/kg), it is therefore possible that WAY100635 also occupied a fraction of the 5-HT$_7$Rs. The mismatch between regional distribution volumes of [^{18}F]2FP3 and the *in vitro* autoradiography outcomes support that [^{18}F]2FP3 has off-target binding. We can only speculate in the nature of the potential *in vivo* off-target binding as affinity was only tested for the 5-HT$_7$, 5-HT$_{1A}$ and 5-HT$_6$ receptors and with apparent affinity for the two latter targets[16]. Similarly, low affinity for the D_2 (790 nM), D_3 (631 nM) and 5-HT$_6$ (3.21 µM) receptors was identified of the closely related compound 12 in the publication by Lovell et al.[19]. The blocking effect obtained in these studies contrasts the results obtained in cats, where the TACs strongly indicated a blocking effect after pre-administration of SB-269970 (5 mg/kg). However, since the arterial input function was not determined in these experiments, the binding before and after pretreatment with SB-269970 was not quantified[16].

There could be species differences to the amino acid sequence in the 5-HT$_7$R, however comparing the amino acid sequence using NCBI BLAST of the predicted (partial) sequence of the cat 5-HT$_7$R (XP_006938099) and the pig 5-HT$_7$R (XP_005671333) resulted in 99 % sequence identity. Comparing the pig and the NHP (XP_001143930) sequences, however, resulted in 96 % sequence identity. The exact function of these amino acids in relation to loops, helices, or more importantly the binding pocket is not known. If these amino acids interact with ligands, it could potentially result in a large difference in binding between species.

The binding potential is the product of receptor density (B_{max}) and the *in vivo* affinity of the radioligand ($1/K_D$). In humans, the 5-HT_7R is, along with the 5-HT_4R, one of the least abundant 5-HT receptors[7,21]. Thus, to determine the amount of 5-HT_7Rs in the NHP and compare this directly to amounts in human brain, *in vitro* autoradiography was performed on post mortem NHP and human tissue and directly compared to that of post mortem pig. Generally, the level of binding of the 5-HT_7R radioligand, [^{3}H]SB-269970, was higher in the thalamus and temporal cortex of the pig compared to NHP and human. In the cerebellum, the levels of specific binding were comparable between pig and NHP. The specific binding in the human temporal cortex were similar to the NHP temporal cortex. However, the amount of specific binding found in all regions in the present study was 10-fold higher than those reported by Varnäs et al.[7] using the same radioligand, but with small differences in the experimental protocol. Varnäs et al. report 5-7-fold difference in binding between the temporal cortex and thalamus, and if we extrapolate this ratio to our own data, the predicted specific binding in the human thalamus would be in the range of 116-162 fmol/mg TE. Unfortunately, we did not have access to thalamic tissue from a post mortem human brain to confirm this hypothesis. PET imaging with [^{11}C]Cimbi-717 in pigs demonstrated that specific binding *in vivo* could be detected at 5-HT_7R levels as low as 20 fmol/mg[5]. To obtain a high binding potential, a high affinity of the radioligand for the target is necessary, especially if the target in question has low receptor density (B_{max}). The affinity of [^{18}F]2FP3 (8.4 nM) was determined by competition binding experiments in over-expressing h5-HT_7R CHO cells[16]. Unfortunately, the K_D has not been determined in rats and cats[16,18] and neither did we determine the affinity in the current study. We speculate that the affinity of [^{18}F]2FP3 may be too low for the 5-HT_7R and this could be one reason why we saw relatively low occupancies with SB-269970, both in pigs and in NHP. Lastly, selectivity of the radioligand is also an important factor, especially in the case of developing ligands for the 5-HT_7R, which has a relatively low B_{max} in the brain compared to

other targets. As described by Herth et al., a 100-200-fold difference in K_D is needed to ensure less than 10 % of the signal from e.g. the α1 adrenergic receptor in the thalamus[13]. The full selectivity profile of 2FP3 has not been established, and thus we cannot rule out that some of the PET signal arises from other targets in the brain. The high uptake in the striatum (which has low abundance in 5-HT_7s and 5-HT_{1A}Rs) of both pigs and NHP may be another indication of off-target binding.

A limitation to the studies with [^{18}F]2FP3 in pigs was varying specific activities resulting in injected masses ranging from 2.6 - 26 µg. However, we found that baseline V_T values in two different pigs were very similar despite the large difference in injected mass, it is unlikely that the injected radioligand has caused a self-blockade. Nevertheless, the occupancy plot (Fig 2C) was made with the baseline experiment with the highest molar activity. Another limitation of the studies in pigs is that the baseline and blocking experiments was not performed in the same animal, due to the long half-life of the radioligand. The baseline and blocking experiment were, however, carried out in the same animal.

Recently, 2FP3 served as a lead compound for further development of radioligands for the 5-HT_7R with higher affinity for the receptor[17]. Unfortunately, these chemical modifications rendered the radioligands substrates for the P-glycoprotein and thus the brain uptake in rats of the two of the radiolabelled compounds was rather low. Due to species differences of the P-glycoprotein it is possible that the evaluation of the most promising of these new compounds, 2F3P3, will have brain uptake in the pig and NHP brain and that the increased affinity will increase the chances of imaging the 5-HT_7Rs *in vivo*.

5. Conclusion

Although initial [^{18}F]2FP3 experiments in cats showed some promise for the radioligand being a good 5-HT_7R PET radioligand we here show in pigs and in NHP that the brain distribution

of the radioligand does not match the 5-HT_7R distribution determined *in vitro*. Furthermore, we could only detect limited effects of blocking the 5-HT_7R in these two species. We speculate that the affinity of the radioligand is too low for imaging the 5-HT_7Rs *in vivo* and that part of the PET signal arises from targets other than the 5-HT_7R and 5-HT_{1A}R.

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7. Compliance with ethical standards

7.1 Conflict of interest

GMK has been an invited lecturer at Pfizer A/S, worked as a consultant and received grants from H. Lundbeck A/S and is a stock holder of Novo Nordisk/Novozymes.

7.2 Author contribution

Study conception and design: HDH, MMH, GMK, CCC, OB, GT. Acquisition of data: HDH, MMH, AD, JHM, OB, CCC, SL. Analysis and interpretation of data: HDH, MMH, OB, CCC, GMK, GT. Writing the manuscript: HDH, MMH, GMK, CCC, OB. All authors revised the manuscript.

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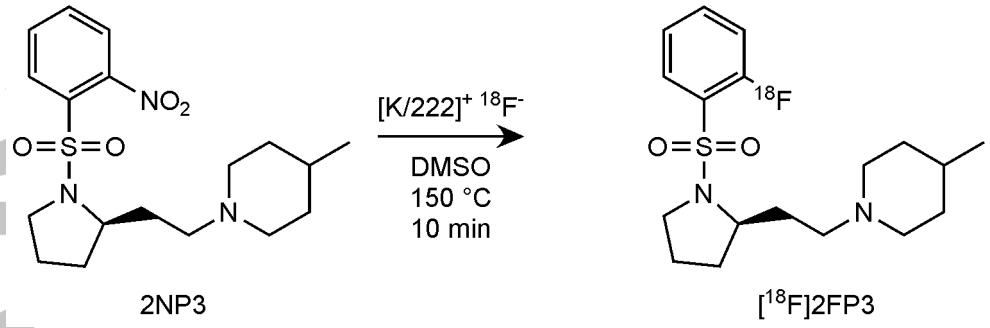

Fig. 1 Radiosynthesis of [^{18}F]2FP3.

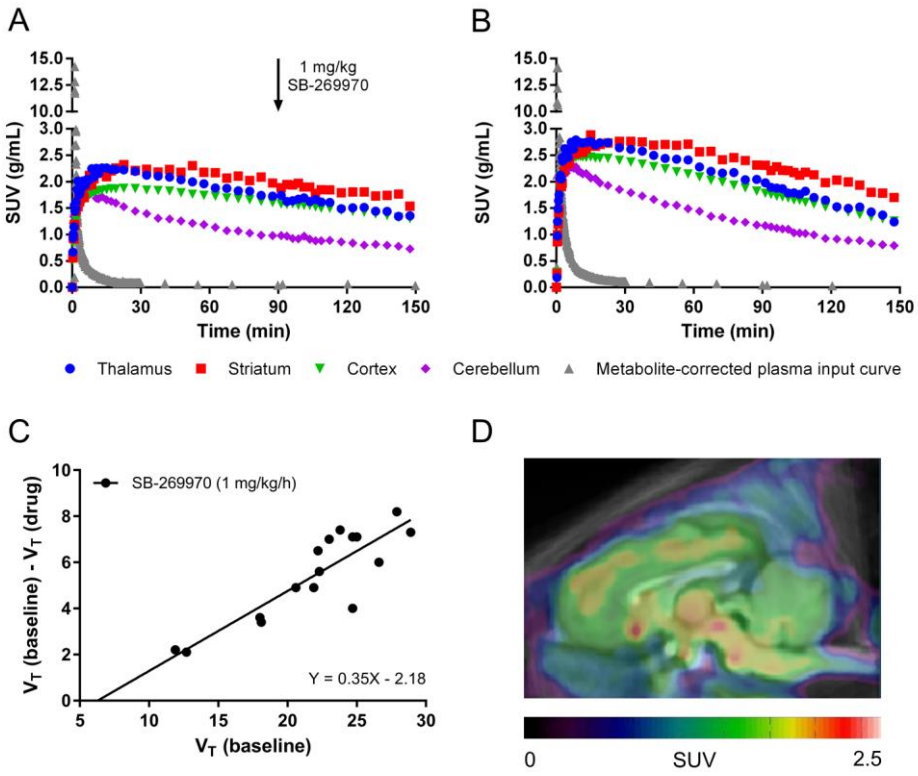

Fig. 2 A) Representative TACs from an experiment with a within-scan challenge of SB-269970 at 90 min. **B)** Representative TACs from an experiment with pre-administration of SB-269970 (1 mg/kg/h for 3h). **C**) Occupancy plot for [^{18}F]2FP3 determined by two scans in two different pigs: one pig with a baseline scan (V_T (baseline)) and another pig scanned after pre-administration of SB-269970 (1 mg/kg/h, V_T (drug)). **D)** Summed PET image (0-90 min) of the baseline biodistribution of [^{18}F]2FP3 in the pig brain.

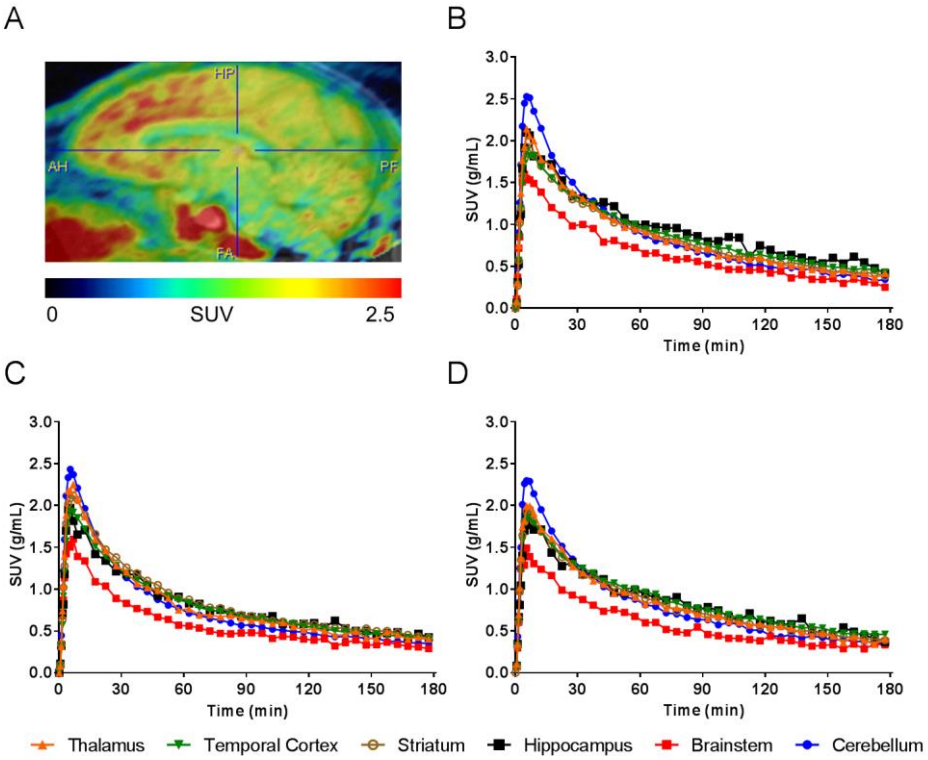

Fig. 3 A) Summed PET image of the baseline biodistribution of [^{18}F]2FP3 in the NHP brain. **B)** Representative baseline TACs for [^{18}F]2FP3. **C)** Representative TACs after pre-administration of SB-266970 (3 mg/kg). **D)** TACs after pre-administration of WAY100635 (1 mg/kg).

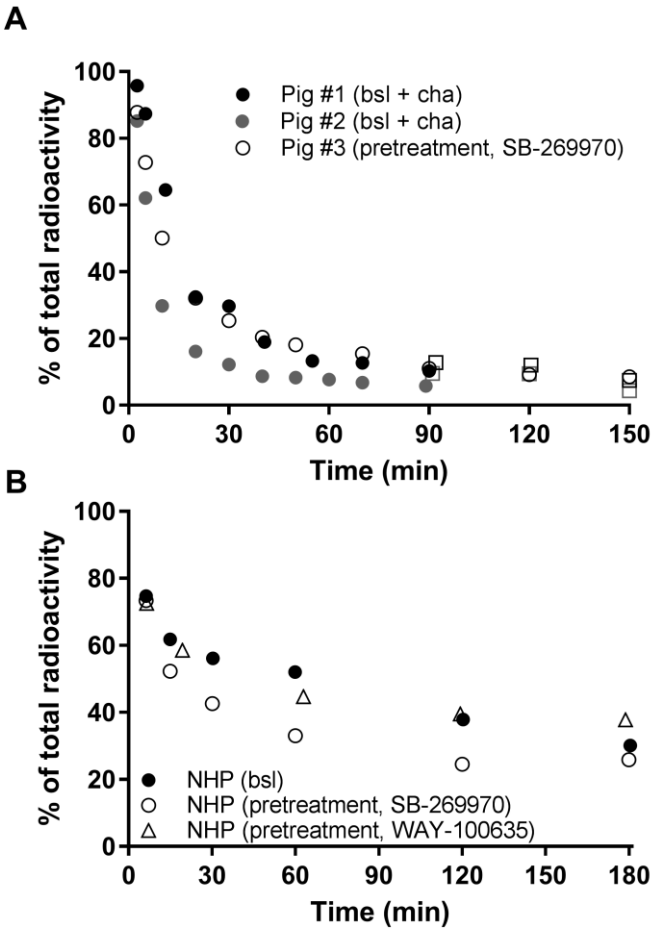

Fig. 4 HPLC analysis of parent compound ([^{18}F]2FP3) in plasma in pigs (**A**) and NHP (**B**) as a function of time after intravenous injection. Filled circles represent baseline (bsl) conditions, open circles represent an experiment where the animal has been pretreated with SB-269970, and open triangles represent an animal pretreated with WAY-100635. In pigs, open squares represent the parent compound in plasma after a challenge (cha) with SB-269970 ninety min after the injection of the radioligand. In pigs, experiments with 0-90 min baseline and 90-120 min challenge phase, were repeated and values are presented for each experiment, as indicated with different colors. All other experiments were performed once.

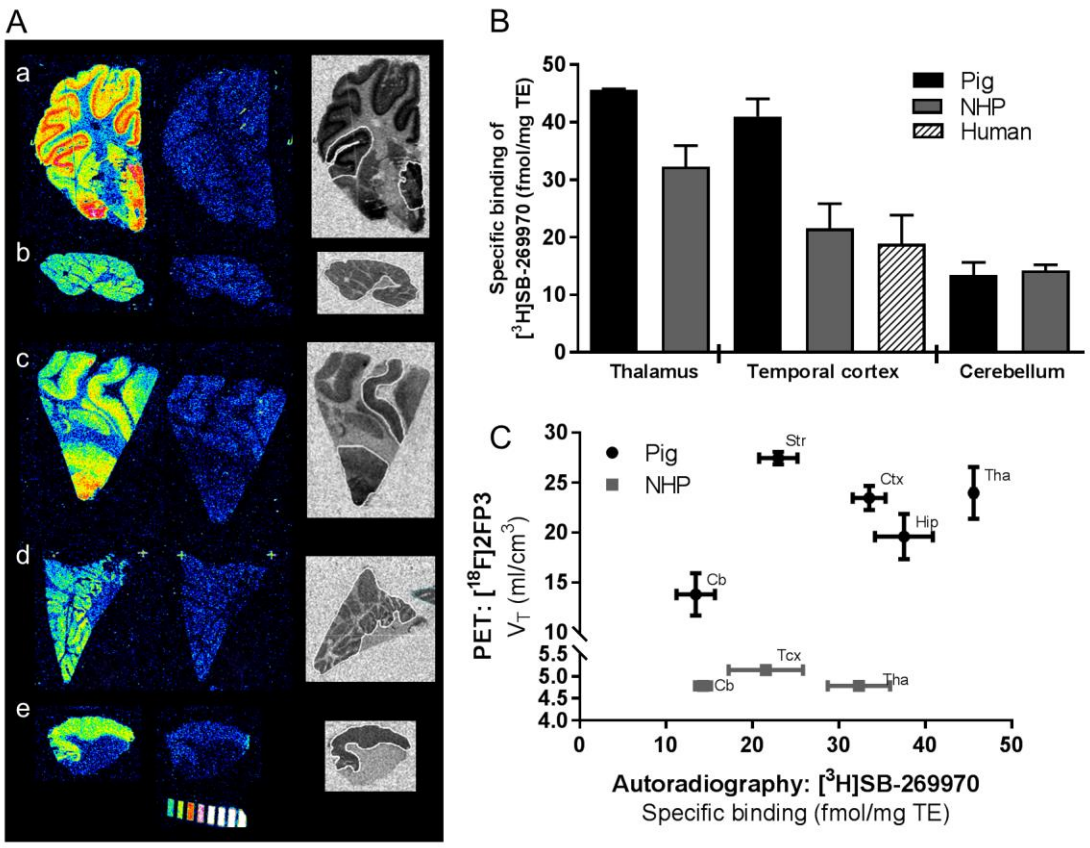

Fig. 5 A) Representative sections of 5-HT$_7$R autoradiography in pigs (a+b), non-human primates (c+d), and human (e). Total binding in left panel, non-specific binding determined with 10 µM SB-258719 in middle panel, and indication of ROI in right panel. **B)** Bar graph showing the amount of specific binding found in human, non-human primate (NHP) and pig post mortem brain tissue. **C)** Comparison of the binding of [^{3}H]SB-269970 and [^{18}F]2FP3 in pig and NHP tissue. All [^{3}H]SB-269970 data for pig tissue is taken from Hansen et al.[5] Cb: cerebellum, Ctx: cortex, Hip: Hippocampus, Str: striatum, Tha: Thalamus, Tcx: temporal cortex.