Chemistry, stock solution preparation and SA adjustment and measurement

MP was synthesized using a published method (2*1*) and provided greater than 95% radiochemically pure *d-threo*-[^{11}C]methylphenidate with SA of greater than 55.5kBq/pmol at end of synthesis. Quantities produced ranged from 444MBq to 900MBq in approximately 1 to 2mL isotonic saline at end of synthesis.

MP stock solution was prepared by dissolving *d-threo*-Methylphenidate hydrochloride (JML Biopharm Inc. www.jmlbiopharm.com) in isotonic saline and filtered through a 0.22μm filter to provide a 0.9mM (214μg/mL) stock solution. The concentration of the solution was determined by HPLC from standard calibration curves using a Waters Sunfire C18 3.5μm (3.0x150mm) column. (Mobile phase: 30:70 acetonitrile/0.17M ammonium formate; flow rate: 0.6mL/min; λ 225nm; retention time: 3.5min). A known amount of MP, ranging from 12.5μg to 125μg, was added to the high specific activity MP sample using the stock solution. The sample was mixed and two identical 200μL samples were removed and weighed. One syringe was used for injection and the mass of MP in the second sample was analyzed by HPLC and a SA at time of injection was calculated.

Scanning

The rats were anesthetized and maintained with a 2% isoflurane/O_2 gas mixture. All studies were performed on a SIEMENS/ Concorde microPET Focus 120 (*24*), which has an approximately $(1.6\ mm)^3$ resolution and a plane-to-plane separation of 0.79 mm. Each rat was positioned in a stereotaxic headholder similar to that used in the surgical

procedures. This headholder was securely mounted to the scanner bed and allowed accurate repositioning of the animal within the ear bars and the mouthpiece. An anesthetic cone fit over the nose and allowed smooth delivery of the anesthetic mixture isoflurane/O_2 without movement of the animal. After a 6-min transmission scan with a ^{57}Co source, a 61min long emission scan was performed starting at tracer injection. 3.7MBq/100g of body weight of tracer was administered as a bolus with SA ranging from 0.009kBq/pmol to 191kBq/pmol corresponding to a tracer mass ranging from 93.8mg/100g of body weight to 0.0041mg/100g as detailed in table 1.

Data were acquired in list mode, histogrammed into 6x30s, 2x60s, 5x300s, 2x450s, and 2x480s, and reconstructed using Fourier rebinning and filtered backprojection after applying normalization, scatter, attenuation, and sensitivity corrections. Attenuation correction factors were calculated after segmentation and energy scaling of the measured attenuation coefficients. To limit the amount of time the rats were kept under anesthesia, each rat underwent at most two scans per day, where the two tracer injections were administered 120 min apart. The higher SA scan was always performed first to minimize potential residual tracer occupancy effects. Since the animals were not moved between these two scans, the low SA scan was always performed following either a medium or high SA to be able to re-use the same region of interest (ROI) placement as defined on the corresponding higher SA scan since on the low SA scan the striata were not as clearly visible. The standard scanning protocol thus involved either a high and a low SA, or a medium-high and a low SA on the same rat on the same day followed by a medium-high or a high and a medium-low SA on another day. Care was

taken to reposition the animals as accurately as possible between separate scanning sessions. All procedures were approved by the UBC Animal Care Committee.

Data Analysis

The high SA tracer scan was used as a reference image for analysis of the medium and low SA tracer scans. To facilitate ROI placement and image co-registration an average image of all dynamic frames was created for each scan. First a brain atlas (*25*) which contains the delineation of the structures of interest, in particular the striata and cerebellum, was manually co-registered to the brain area in the high SA tracer scan. All image co-registration was completed using the MINC software package (McConnell Brain Imaging Centre, Montreal Neurological Institute, McGill University), which requires the user to select like points in both images in order to calculate a rigid body transformation. Using the co-registered atlas for guidance, a rectangular (2.6 x 3.5) mm^2 ROI was placed on each striatum on three consecutive transaxial planes (plane thickness 0.796mm) and a (6.9 x 2.6) mm^2 ROI was placed on the cerebellum. Since the image-atlas registration was not always perfect, it was found that a manual adjustment of the ROIs on the PET images using the realigned atlas as guidance was more accurate than directly transporting atlas defined ROIs onto the PET images. The ROIs were then replicated on each frame of the dynamic sequence for the extraction of a time activity curve (TAC). Each of the medium and low SA tracer scans were co-registered to the high SA scan using the same method as for the atlas-image co-registration. The ROIs defined

on the high SA tracer scan were carried over to the co-registered medium and low SA scans for TAC extraction. In the cases in which a low SA tracer scan was completed on the same day as the high SA tracer scan no co-registration was required and the ROIs were used directly on the low SA tracer scan, since the animals were not moved between the two scans.

				NS	R1	R2	R3	R4	R5	R6	R7
B/F*				N/A	0.85	0.32	0.63	0.12	0.13	0.08	0.27
DD(eq 6)				N	*0.36*	*0.76*	*0.58*	*0.91*	*0.87*	*0.94*	*0.81*
DD(eq 7)				Y	0.35	0.86	0.64	1	0.98	1.05	0.9
	Fit	**Variables**	**# points**								
B_{max}	Linear	B = S-C (eq3)	3	N	658	285	508	-53	30	-42	363
		B = S-C(eq3)	4	N	662	826	1462	-3500	-143	-259	603
		B =$(B/F)_e$ x F(eq4)	3	N	462	221	340	-82	43	-31	296
		B =$(B/F)_e$ x F(eq5)	**3**	**Y**	***290***	***47***	***182***	***0***	***1***	***-15***	***82***
		B =$(B/F)_e$ x F(eq4)	4	N	430	542	1009	-7150	-83	-148	383
		B =$(B/F)_e$ x F(eq5)	4	Y	234	147	533	1	4	24	15
	Non-linear	F (eq 1)	4	N	437	435	879	-8526	-81	-149	409
		F (eq 2)	4	Y	351	505	982	-3227	-314	NA	221
		F (eq 1)	5	N						NA	1365
		F(eq 2)	5	Y						9	110
K_d^{app}	Linear	B = S-C(eq3)	4	N	776	2662	2531	-24427	-1155	-3199	2033
		B =$(B/F)_e$ x F(eq4)	3	N	545	666	562	-619	320	-398	990
		B =$(B/F)_e$ x F(eq5)	**3**	**Y**	***409***	***242***	***392***	***-28***	***-71***	***245***	***522***
		B =$(B/F)_e$ x F(eq4)	4	N	503	1731	1748	-49693	-671	-1832	1382
		B =$(B/F)_e$ x F(eq5)	4	Y	322	880	1223	-128	-316	-419	86
	Non-linear	F (eq 1)	4	N	514	1353	1507	-59219	-666	-1834	1376
		F (eq 2)	4	Y	351	505	982	-3227	-314	NA	221
		F (eq 1)	5	N						4758	NA
		F(eq 2)	5	Y						709	-1765

TABLE 1. B_{max} (pmol/mL) and K_d^{app} (pmol/mL) values for the lesion side for each animal (R1-R7). Negative values are found when the data are dominated by noise (very low specific uptake in the striatum) and consequently have no biological meaning (see figure 3). NA indicates absolute values of the parameter > 10^8. The bolded row show the model that yielded the highest number of plausible fits. NS N indicates no NS subtraction and NS Y indicates NS subtraction.
*$(B/F)_e$ was estimated from the high SA scan.