Rapid Changes in CB1 Receptor Availability in Cannabis Dependent Males after Abstinence from Cannabis

Supplemental Information

Approvals

This study was approved by the Yale University Institutional Review Boards, the Magnetic Resonance Research Center, and the Yale New Haven Hospital Radiation Drug Research Committee. All participating subjects were informed of the research procedures and provided signed consent in accordance with the Helsinki Declaration of 1975.

Screening Process

Subjects were recruited via local print and Internet advertisements as well as word-of-mouth. After a brief telephone prescreening interview, subjects were invited for a face-to-face screening. All subjects completed a comprehensive screening process that included a psychiatric, medical, and neurological evaluation by a research physician. A Structured Clinical Interview for DSM-IV-TR, Non-Patient Version (SCID-NP) (1) was conducted to verify the primary diagnoses of cannabis dependence (or lack thereof for the comparison group). Detailed history of lifetime exposure to cannabis, other drugs and alcohol was assessed using the Scale Assessing Lifetime Cannabis Use (In Development), the SCID (2), and a locally developed substance use questionnaire (3, 4). Drug and alcohol use over the past six months was quantified using a Time Line Follow Back (TLFB) approach (5). Additionally, an adaptation of the TLFB was used to assess the frequency and amount of cannabis use over the 30 days prior to screening (6, 7). Subjects' status as nonsmokers (tobacco) was ascertained by self-report, the Fagerstrom Test for Nicotine Dependence (8), and breath carbon monoxide (CO) levels. In addition, since one of the goals of the study was to characterize the relationship between cannabis withdrawal and CB_1R availability, CD subjects were required to have a previous history of cannabis withdrawal symptoms which were reported in both the SCID as well as in the collection of substance use history as part of the psychiatric interview (9). A physical examination was conducted. An Allen's test was performed to determine the suitability of the subject for arterial line insertion. Laboratory tests included electrocardiogram, hematology, chemistry, thyroid function tests, PT/PTT, and urinalysis. Urine toxicology for drugs and quantification of urinary THC-COOH (the principle metabolite of THC), as well as spot urine drug tests and tests of blood alcohol content were done

at screening to confirm that subjects were using cannabis but not any other drug or alcohol. Given that smoking cannabis is associated with higher carbon monoxide (CO) levels, if breath CO levels were > 10 ppm, urine cotinine levels (threshold < 50 ng/ml) were also measured to ensure that the elevated CO levels were not related to smoking tobacco. Eligible subjects underwent structural magnetic resonance imaging (MRI) for co-registration of PET data.

Table S1. Schedule of Procedures

Assessment	Screen (~-28 Days)	MRI Visit (~-14 Days)	Check in (~-21, -14, -7)	Day 0 (PET #1)	Day 1	Day 2 (PET #2)	Wk. 1, V. 1	Wk. 1, V. 2	Wk. 2, V.1	Wk. 2, V.2	Wk. 3, V. 1	Wk. 3, V. 2	Wk. 4, V.1	Wk. 4, V.2	Day 28 (+/- 2) (PET #3)
Psychiatric & Medical History	x														
Physical Exam	x														
Clinical Labs	x			x		x									x
Vital Signs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
EKG	x			x											
Quick Dip UTOX	x	x	x				x	x	x	x	x	x	x	x	
TLFB	x	x	x												
Breath CO	x	x	x				x	x	x	x	x	x	x	x	
ETOH Breathalyzer	x	x	x												
Quant. UTOX	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Abstinence Counseling	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Contingency Management	x	x		x		x		x		x		x			x
Structural MRI		x													
Cannabis Withdrawal Assessments				x	x	x	x	x	x	x	x	x	x	x	x
PET Scan				x		x									x

EKG, electrocardiogram; Quick Dip UTOX, instant urine toxicology; TLFB, Time Line Follow Back; Breath CO, breath carbon monoxide; ETOH breathalyzer, alcohol breathalyzer; Quant. UTOX, quantitative urine toxicology; MRI, magnetic resonance imaging; PET, positron emission tomography.

Contingency Management

To promote 28 days of abstinence from cannabis and other drug/alcohol use, a contingency management approach with escalating payment was instituted. Subjects were compensated for their time according to the pay schedule outlined below:

Procedure	Amount	Cumulative Payment
Screening Session	$50	50
MRI	$75	150
1st PET Scan: (Arterial Line: $75 + Scan: $375)	$450	$600
Hospitalization	$500	$1100
2nd PET Scan: (Arterial Line: $75 + Scan: $375)	$450	$1550
Week #1 Abstinence	$75	$1625
Week #2 Abstinence	$100	$1725
Week #3 Abstinence	$125	$1850
Week #4 Abstinence	$850	$2700
3nd PET Scan: (Arterial Line: $75 + Scan: $375)	$450	$3150
Grand Total		$3150

Table S2. Schedule of PET Scans

	Scan Day #0	Scan Day #2	Scan Day #28
Cannabis Dependent Subjects	CD state (no more than ~12 hours from last exposure to cannabis)	Confirmed inpatient acute abstinence (~72 hours from last exposure to cannabis)	Confirmed outpatient prolonged abstinence (~28 days from last exposure to cannabis)
Healthy Controls	+	-	+ (in n = 4)

Imaging Methodology

MRI scans (3T) were collected in each subject before the PET scan procedures to 1) exclude individuals with anatomical abnormalities, and 2) to co-register PET and MRI for image analysis. MR imaging was performed on a 3T Trio (Siemens Medical Solutions, Erlangen, Germany) with a circularly polarized head coil. MR acquisition was a Sag 3D magnetization-prepared rapid gradient-echo sequence.

CB1R was measured using the HRRT and [^{11}C]OMAR (aka [^{11}C]JHU75528) (10). This radioligand, an analog of the CB1R antagonist/inverse agonist SR141617A, has high affinity and selectivity for CB1R and has uptake that is consistent with the known distribution of CB1R in the mammalian brain (10). [^{11}C]OMAR has been successfully utilized to study CB1R in healthy human subjects and several neuropsychiatric disorders (11-13). In a human test-retest study [^{11}C]OMAR was shown to possess appropriately fast kinetics for a C-11 labeled ligand, reasonable specific binding signals, and excellent test-retest reproducibility of kinetic parameters (14). To date, over 200 PET scans with [^{11}C]OMAR have been performed at the Yale PET Center including the studies described above.

Prior to PET scanning, intravenous lines and an arterial catheter were placed. The arterial lines permitted measurement of absolute physiological functions by mathematically relating the brain signal (from the PET scanner) to the tracer availability (from the plasma). Immediately prior to each imaging session, [^{11}C]OMAR was prepared with high specific activity by previously described methods adapted to the TRACERlab FXC Pro automated synthesis module (GE Healthcare, Milwaukee, WI; (10)). After a transmission scan with a ^{137}Cs point source, [^{11}C]OMAR (radioactivity dose £ 20 mCi) was injected over one minute and emission data were acquired in list mode for 120 min on the HRRT (spatial resolution of 2.5 to 3 mm). Motion correction was be performed dynamically with measurements from the Vicra (NDI Systems, Waterloo, Ontario) used by a dedicated list-mode reconstruction algorithm (15).

Arterial blood samples were collected during the PET scan for measurement of the plasma input function and high pressure liquid chromatography (HPLC) analysis of radioactive metabolites. Rapid measurements were obtained using an automated blood counter (PBS-101, Veenstra Instruments, Joure, The Netherlands). Radioactive metabolites and unchanged parent compound were assessed by column-switching HPLC analysis method (16) from blood samples drawn at 0, 5, 15, 30, 60, and 90 minutes post-injection.

PET images were reconstructed with a dedicated computer cluster using a list-mode reconstruction algorithm (15) with all corrections (attenuation, normalization, scatter, randoms, dead-time and event-by-event hardware motion correction). Motion-corrected PET data was co-

registered to the subject's T1-weighted 3T MR image using a 6-parameter mutual registration algorithm, which was in turn nonlinearly (BioImage Suite) aligned to a standard MR template in MNI space. Automatic regions of interest delineated on the Anatomical Automatic Labeling template (15) were used to extract time-activity curves from the dynamic PET data. This process permitted direct, automatic determination of volume of distribution (V_T) values using the metabolite corrected arterial plasma input function and the MA1 method with t^* = 30 min (17).

Table S3. Long-Term Test Retest of [^{11}C]OMAR V_T in Healthy Controls (n = 4) Four matched healthy controls were scanned twice at an interval of approximately 4 weeks to replicate the time interval between the 1st and 3rd scan of cannabis dependent individuals. The mean overall change across all brain areas was less than 1% with the greatest change of a 10% reduction in the hypothalamus.

	Mean Difference	% Change
Amygdala	-0.03	-1.62
Caudate	0.02	1.56
Cerebellum	0.05	3.30
Cingulum_ant	0.04	2.20
Cingulum_post	0.01	0.78
Frontal	0.04	3.05
Hippocampus	0.02	1.15
Hypothalamus	-0.14	-10.44
Insula	0.03	1.52
Occipital	0.04	2.60
Pallidum	-0.03	-1.22
Parietal	0.06	4.09
Putamen	0.02	1.11
Temporal	0.01	0.80
Thalamus	0.03	2.11
Overall	0.01	0.73

Table S4. Comparison of CB1 Availability in Cannabis Dependence vs. Healthy Controls at Baseline Across Studies

	Hirvonen *et al.* (2012) (18)	Current Study
Timing of Scan	Next day from last exposure to cannabis	Within 12 hours of last exposure to cannabis
Amygdala	No difference	Lower (sig.)
Caudate	No difference	Lower (sig.)
Cerebellum	No difference	No difference
Anterior Cingulate	Lower (sig.)	Lower (sig.)
Posterior Cingulate	Lower (sig.)	Lower (sig.)
Frontal Cortex	Lower (sig.)	Lower (sig.)
Hippocampus	Lower (sig.)	Lower (sig.)
Parahippocampus	Lower (sig.)	Not done
Pons	No difference	Not done
Hypothalamus	Not done or reported	Lower (sig.)
Insula	Lower (sig.)	Lower (sig.)
Occipital	Lower (sig.)	Lower (sig.)
Pallidum	Not done or reported	Lower (trend)
Parietal Cortex	Lower (sig.)	Lower (sig.)
Putamen	No difference	Lower (sig.)
Temporal Cortex	Lower (sig.)	Lower (sig.)
Thalamus	No difference	No difference
Midbrain	No difference	Not done

Table S5. Comparison of [^{11}C]OMAR V_T in Cannabis Dependence After 2-4 Days of Abstinence vs. Healthy Controls Across Studies

	Ceccarini *et al.* 2013 (19)	Current Study
Timing of Scan	4 ± 1.7 days of abstinence	2 days of abstinence
Amygdala	Lower (n.s)	Lower (n.s)
Caudate	Lower (n.s)	Lower (n.s)
Cerebellum	Lower (n.s)	Lower (n.s)
Anterior Cingulate	Lower (sig.)	Lower (n.s)
Posterior Cingulate	Lower (sig.)	Lower (n.s)
Frontal Cortex	Lower (n.s)	Lower (n.s)
Hippocampus	Lower (n.s)	Lower (n.s)
Parahippocampus	Lower (n.s)	Not done
Pons	Lower (n.s)	Not done
Hypothalamus	Not done or reported	Lower (n.s)
Insula	Lower (sig.)	Lower (n.s)
Occipital	Lower (n.s)	Lower (n.s)
Pallidum	Lower (n.s)	Lower (n.s)
Parietal Cortex	Lower (n.s)	Lower (n.s)
Putamen	Lower (n.s)	Lower (n.s)
Temporal Cortex	Lower (sig.)	Lower (n.s)
Thalamus	Lower (n.s)	Lower (n.s)

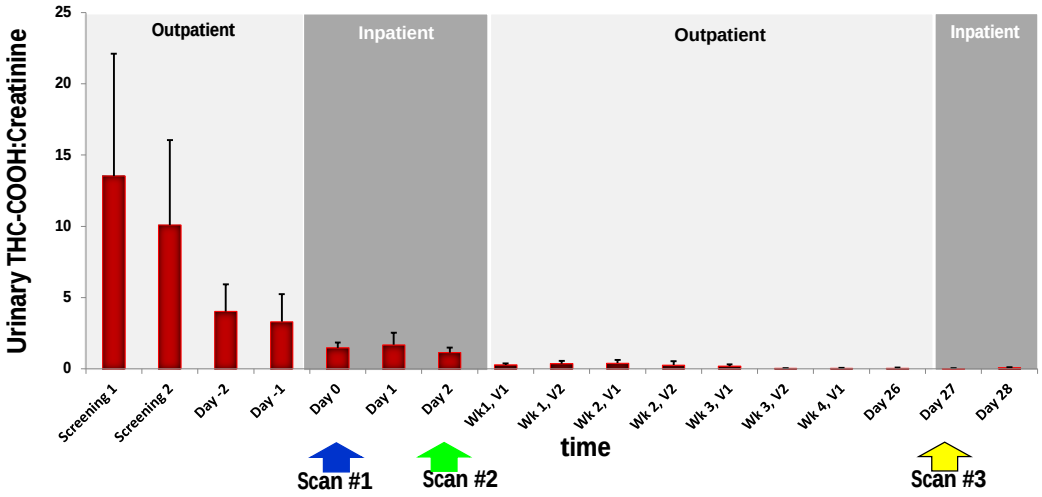


Figure S1. Urinary THC-COOH: Creatinine Ratio. Urinary THC:COOH to creatinine ratio (x axis) plotted over time (y axis). The time period is divided into epochs based on whether subjects were inpatient or outpatient. The timings of the 3 scans are shown below the y axis.

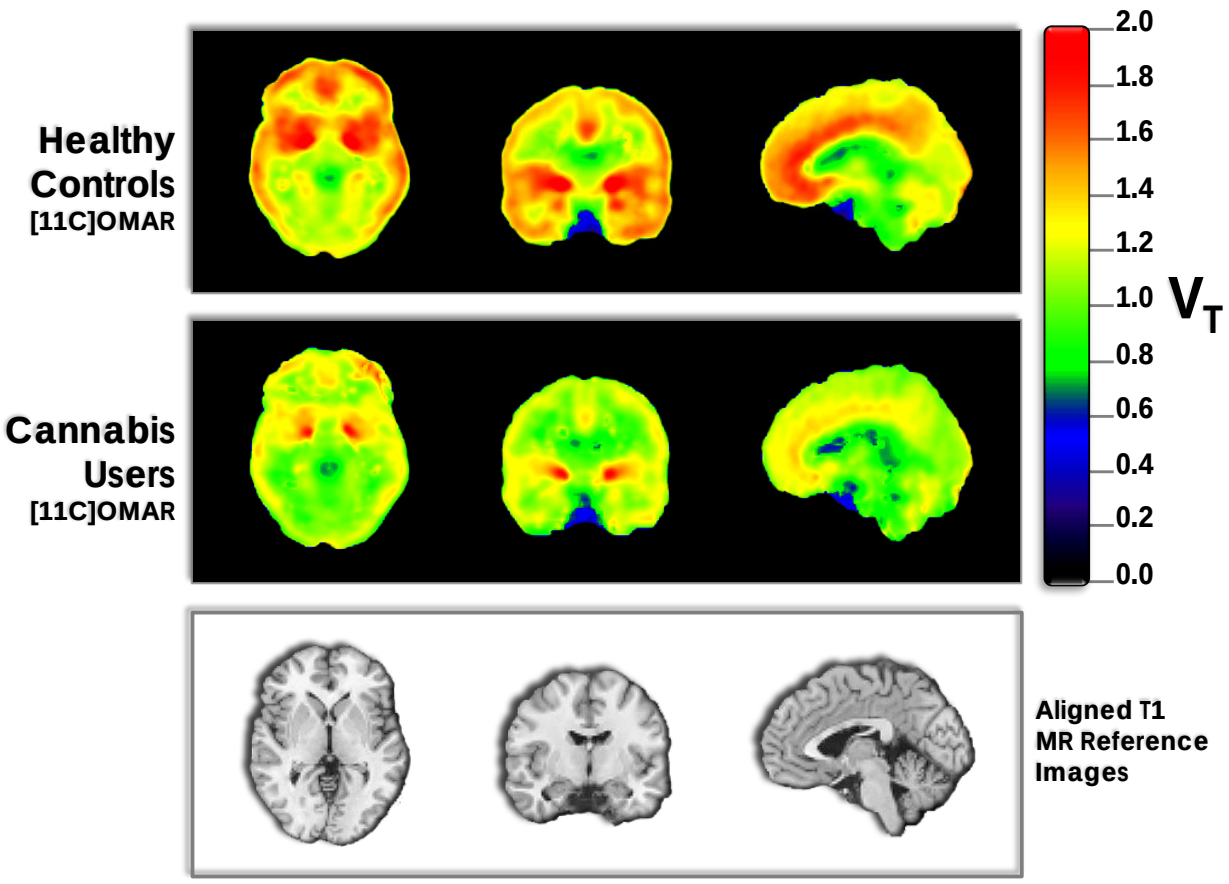
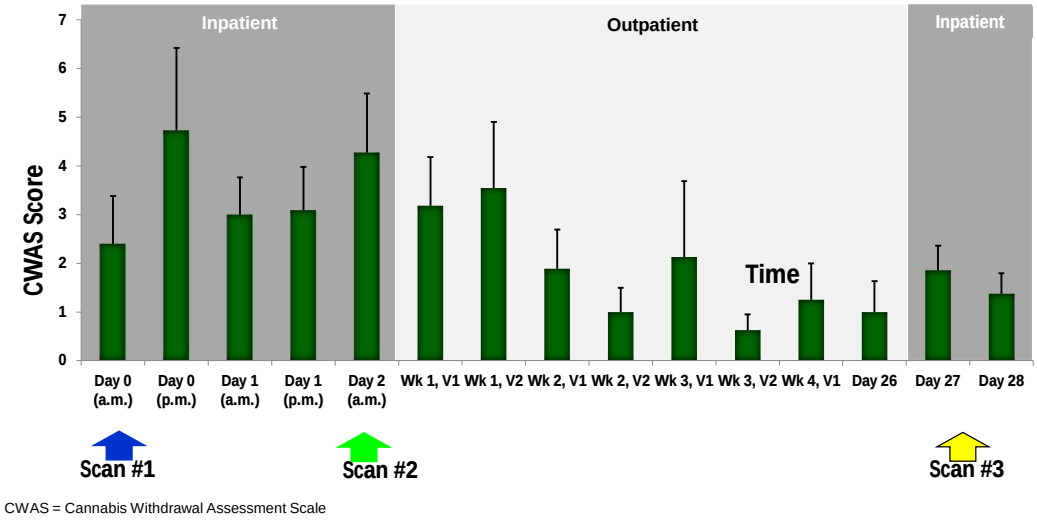


Figure S2. Grand Averaged CB1R Availability in CDs vs. HCs at Baseline. Grand averaged [^{11}C]OMAR V_T in HCs (top row) and CDs (middle panel) at baseline in the horizontal (left column), coronal (middle column) and sagittal (right column). Bottom row shows structural MRI.



CWAS = Cannabis Withdrawal Assessment Scale

Figure S3. Cannabis Withdrawal Symptoms Over Time. Cannabis withdrawal symptoms measured by the Cannabis Withdrawal Assessment Scale (x axis) plotted over time (y axis). The time period is divided into epochs based on whether subjects were inpatient or outpatient. The timings of the 3 scans are shown below the y axis.

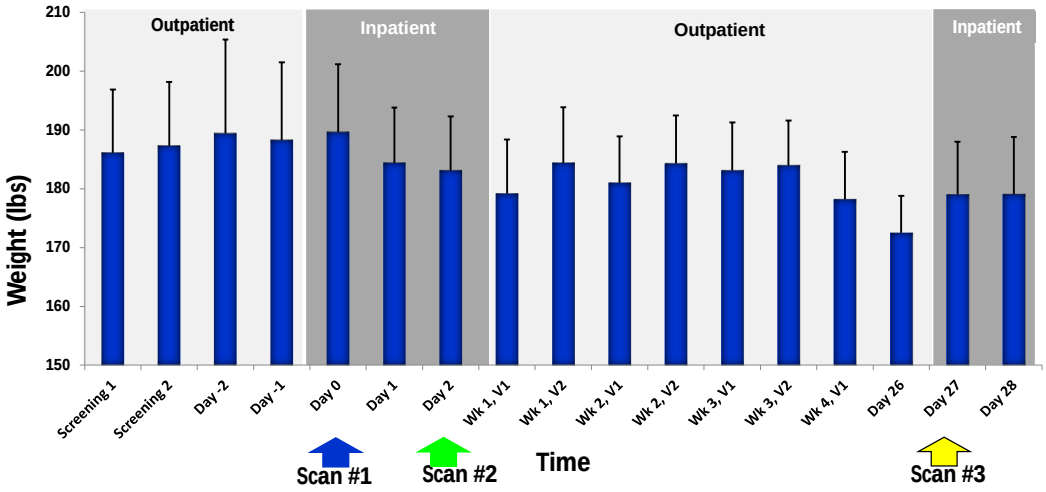


Figure S4. Body Weight Over Time. Body weight in pounds (lbs) (x axis) plotted over time (y axis). The time period is divided into epochs based on whether subjects were inpatient or outpatient. The timings of the 3 scans are shown below the y axis.

Supplemental References

1. First MB, Spitzer RL, Gibbon M, Williams JBW (2002): *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition. (SCID-I/NP)*. New York: Biometrics Research, New York State Psychiatric Institute, .

2. Spitzer RL, Williams JBW, Gibbon M, First MB (1990): *Structured Clinical Interview for DSM-III-R-Patient Edition (SCID-P, Version 1.0).* Washington, DC: American Psychiatric Press.

3. Skosnik PD, Krishnan GP, D'Souza DC, Hetrick WP, O'Donnell BF (2014): Disrupted gamma-band neural oscillations during coherent motion perception in heavy cannabis users. *Neuropsychopharmacology*.

4. Skosnik PD, D'Souza DC, Steinmetz AB, Edwards CR, Vollmer JM, Hetrick WP, *et al.* (2012): The effect of chronic cannabinoids on broadband EEG neural oscillations in humans. *Neuropsychopharmacology*. 37:2184-2193.

5. Sobell LC, Sobell MB (1992): Timeline follow-back: A technique for assessing selfreported alcohol consumption. In: Allen RZLJ, editor. *Measuring alcohol consumption: Psychosocial and biological methods.* New Jersey: Humana Press. pp 41-72.

6. Hjorthoj CR, Fohlmann A, Larsen AM, Arendt M, Nordentoft M (2012): Correlations and agreement between delta-9-tetrahydrocannabinol (THC) in blood plasma and timeline follow-back (TLFB)-assisted self-reported use of cannabis of patients with cannabis use disorder and psychotic illness attending the CapOpus randomized clinical trial. *Addiction*. 107:1123-1131.

7. Hjorthoj CR, Hjorthoj AR, Nordentoft M (2012): Validity of Timeline Follow-Back for self-reported use of cannabis and other illicit substances--systematic review and meta-analysis. *Addict Behav*. 37:225-233.

8. Fagerstrom KO (1978): Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. *Addict Behav*. 3:235-241.

9. Budney AJ, Novy PL, Hughes JR (1999): Marijuana withdrawal among adults seeking treatment for marijuana dependence. *Addiction*. 94:1311-1322.

10. Horti AG, Fan H, Kuwabara H, Hilton J, Ravert HT, Holt DP, *et al.* (2006): 11C-JHU75528: a radiotracer for PET imaging of CB1 cannabinoid receptors. *J Nucl Med*. 47:1689-1696.

11. Wong DF, Kuwabara H, Horti AG, Raymont V, Brasic J, Guevara M, *et al.* (2010): Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C]OMAR. *Neuroimage*. 52:1505-1513.

12. Neumeister A, Normandin MD, Murrough JW, Henry S, Bailey CR, Luckenbaugh DA, *et al.* (2012): Positron emission tomography shows elevated cannabinoid CB1 receptor binding in men with alcohol dependence. *Alcohol Clin Exp Res*. 36:2104-2109.

13. Neumeister A, Normandin MD, Pietrzak RH, Piomelli D, Zheng MQ, Gujarro-Anton A, *et al.* (2013): Elevated brain cannabinoid CB1 receptor availability in post-traumatic stress disorder: a positron emission tomography study. *Mol Psychiatry*. 18:1034-1040.

14. Normandin MD, Zheng MQ, Lin KS, Mason NS, Narendran R, Lin SF, *et al.* (2015 in press): Imaging the cannabinoid CB1 receptor in human with [11C]OMAR: Assessment of kinetic analysis methods, test-retest reproducibility, and gender differences. *Journal of cerebral blood flow and metabolism*.

15. Carson RE, Barker WC, Liow J-S, Adler S, Johnson CA (2003): Design of a motion-compensation OSEM List-mode Algorithm for Resolution-Recovery Reconstruction of the HRRT. *Conf Record IEEE Nuclear Science Symposium and Medical Imaging*. Portland, OR.

16. Hilton J, Yokoi F, Dannals RF, Ravert HT, Szabo Z, Wong DF (2000): Column-switching HPLC for the analysis of plasma in PET imaging studies. *Nucl Med Biol*. 27:627-630.

17. Ichise M, Toyama H, Innis RB, Carson RE (2002): Strategies to improve neuroreceptor parameter estimation by linear regression analysis. *J Cereb Blood Flow Metab*. 22:1271-1281.

18. Hirvonen J, Goodwin RS, Li CT, Terry GE, Zoghbi SS, Morse C, *et al.* (2012): Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. *Mol Psychiatry*. 17:642-649.

19. Ceccarini J, Kuepper R, Kemels D, van Os J, Henquet C, Van Laere K (2013): [F]MK-9470 PET measurement of cannabinoid CB receptor availability in chronic cannabis users. *Addict Biol*.