Supplementary Information

Supplementary Table S1. Total distribution volume (V_T / f_P, mL·cm^{-3}) of [^{11}C]clozapine ([^{11}C]CLZ) and [^{11}C]deschloroclozapine ([^{11}C]DCZ) in regions of monkey brain

Region	[^{11}C]CLZ				[^{11}C]DCZ			
	BL (n=3)	CNO	CLZ	CTM+CNO	BL (n=3)	CNO	DCZ-Low	DCZ-High
Frontal Cortex	532 ± 68	258	314	390	67 ± 5	35	41	32
Cingulate Cortex	521 ± 38	258	326	402	68 ± 7	32	40	29
Striatum	469 ± 24	288	342	421	55 ± 7	35	44	31
Insula	549 ± 57	258	320	402	71 ± 11	33	41	28
Temporal Cortex	428 ± 39	214	276	343	51 ± 5	26	32	25
Amygdala	688 ± 73	289	365	425	86 ± 10	38	41	30
Hippocampus	464 ± 27	236	309	378	61 ± 9	29	34	27
Thalamus	343 ± 30	248	309	401	46 ± 17	30	35	27
Parietal Cortex	550 ± 90	269	329	438	70 ± 15	32	39	29
Occipital Cortex	410 ± 48	217	278	365	51 ± 9	29	32	26
Cerebellum	250 ± 14	204	224	282	29 ± 2	26	29	24
Right Amygdala (Target)	1440 ± 171	439	361	420	248 ± 40	60	51	27
Left Amygdala (Mirror)	450 ± 53	230	319	375	58 ± 5	32	39	30

V_T / f_P: Total distribution volume corrected for the free fraction in plasma. Values represent mean ± SD from three baseline scans and three blocked scans for each radioligand. The doses of the blocking agents are provided in the legend of Figure 1.

Supplementary Table S2. Definition and explanation of key terms

Term	Definition	Explanation
BP_{ND}	Binding potential	Ratio at equilibrium of specific binding to nondisplaceable compartment. $BP_{ND} = V_S / V_{ND}$ For any brain region, $BP_{ND} \geq BP_{ND}'$, because the numerator of BP_{ND} includes both on-target and off-target binding.
BP_{ND}'	Ratio of on-target binding to nondisplaceable uptake	$BP_{ND}' = V_{S\text{-}on} / V_{ND}$ For this study, $V_{S\text{-}on}$ reflects binding to the transfected receptor. V_{ND} reflects background radioactivity in areas devoid of receptors. Thus, BP_{ND}' is the "signal-to-background ratio" and reflects contrast in the image. BP_{ND}' is the primary outcome measure used to compare [^{11}C]CLZ and [^{11}C]DCZ.
C_{ND}	Concentration of radioligand in the nondisplaceable compartment	C_{ND} is the total concentration of radioligand in the nondisplaceable compartment plus nonspecific binding. $C_{ND} = F + C_{NS}$
C_P	Concentration of radioligand in plasma	C_P is the total concentration of radioligand in plasma and equals Free (F) plus protein binding.
f_{ND}	Free fraction in brain	Ratio at equilibrium of free concentration in brain to free plus nonspecific binding. $f_{ND} = F / C_{ND}$
f_P	Free fraction in plasma	Ratio at equilibrium of free concentration in plasma to free plus nonspecific binding. $f_P = F / C_P$
K_i	Inhibition constant	K_i is measured in vitro and is inversely related to the affinity of the (radio)ligand
V_{ND}	Nondisplaceable distribution volume	Ratio at equilibrium of concentration of radioligand in nondisplaceable compartment to the concentration of radioligand in plasma C_P
V_S	Specific distribution volume	Ratio at equilibrium of the concentration of radioligand that is specifically bound in brain to the concentration of radioligand in plasma C_P. Specific binding refers to displaceable (i.e., high-affinity) binding of radioligand in brain. These radioligands have two types of displaceable binding: on-target (i.e., to the transfected receptor) and off-target (i.e., to endogenous receptors). $V_S = V_{S\text{-}on} + V_{S\text{-}off}$
$V_{S\text{-}off}$	Off-target distribution volume	Off-target binding is displaceable (i.e., high affinity) binding to sites other than the transfected receptor. Such

		off-target binding is to endogenous receptors.
$V_{S\text{-}on}$	On-target distribution volume	For this study, the target is the transfected human receptor. Thus, $V_{S\text{-}on}$ is the ratio at equilibrium of the concentration of radioligand that is bound to the transfected receptor to the concentration of radioligand in plasma C_P.
V_T	Total distribution volume	Ratio at equilibrium of concentration of all radioligand in brain to the concentration of radioligand in plasma C_P. $V_T = V_S + V_{ND} = V_{S\text{-}on} + V_{S\text{-}off} + VND$

Any of the various types of distribution volume V can be corrected (i.e., divided by) the free fraction in plasma of the radioligand, as in V_S / f_P. Correcting for free fraction in plasma reflects the properties of the radioligand binding in the brain and is independent of varying amounts of binding to plasma proteins.

Supplementary Table S3. Off-target binding of [^{11}C]clozapine ([^{11}C]CLZ) and [^{11}C]deschloroclozapine ([^{11}C]DCZ) determined with two-tissue compartment model in regions of monkey brain

Region	$V_{S\text{-}off}$ / f_P (mL · cm^{-3})	
	[^{11}C]CLZ	[^{11}C]DCZ
Frontal Cortex	274 ± 29	41 ± 5
Cingulate Cortex	263 ± 37	41 ± 4
Striatum	210 ± 66	28 ± 7
Temporal Cortex	169 ± 35	24 ± 4
Occipital Cortex	151 ± 27	24 ± 6
Cerebellum	-9 ± 65	3 ± 4
Left Amygdala (mirror)	192 ± 70	32 ± 7

$V_{S\text{-}off}$ / f_P: off-target distribution volume corrected for the free fraction in plasma

Values represent mean ± SD from three baseline scans and three blocked scans for each radioligand. The doses of the blocking agents are provided in the legend of Figure 1.

Supplementary Table S4. Effect of lipophilicity and affinity on radioligand binding in plasma and brain.

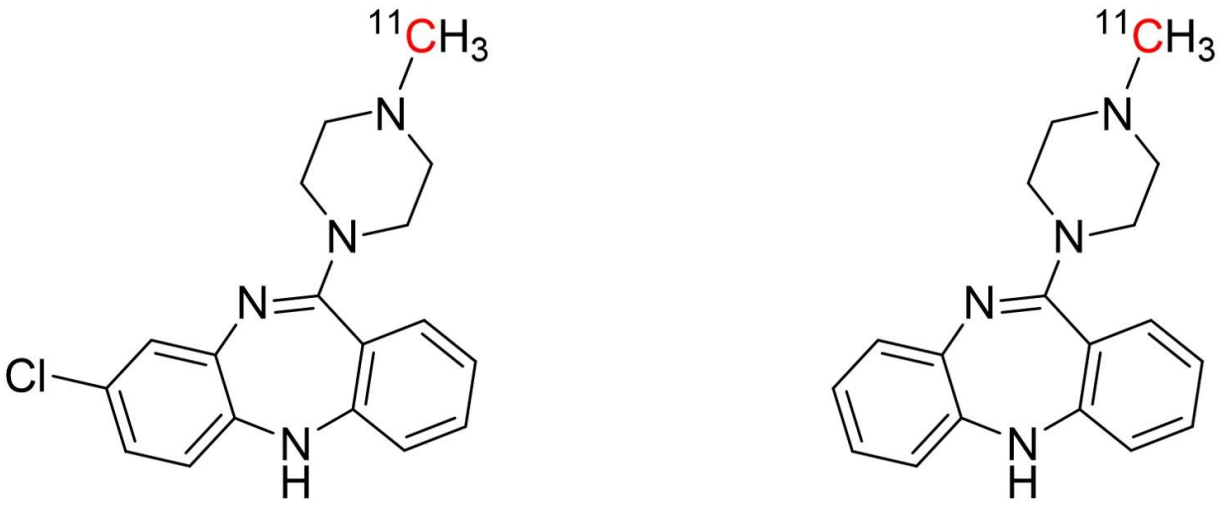
Radioligand	Lipophilicity (clog*D*)[a]	Affinity K_i [b](nM)	Plasma f_p (%)	Brain		
				f_{ND} (%)	$V_{S\text{-}on} / f_p$ (mL·cm^{-3})	V_{ND} / f_p (mL·cm^{-3})
[^{11}C]CLZ	3.4	0.9	6	0.4	990	258
[^{11}C]DCZ	2.6	4.2	22	3.8	190	27

cLog*D*: calculated log*D*; K_i: inhibition constant; f_p: free fraction in plasma; f_{ND}; free fraction in brain; $V_{S\text{-}on} / f_p$: on-target displaceable binding corrected for free fraction in plasma (i.e., to the transfected receptor); V_{ND} / f_p: nondisplaceable uptake corrected for free fraction in plasma.

[a]Values were calculated using Pallas 3.70 software and ACD software (version 9.04; Advanced Chemistry Development, Inc., Toronto, Canada). Note: affinity is inversely related to K_i.

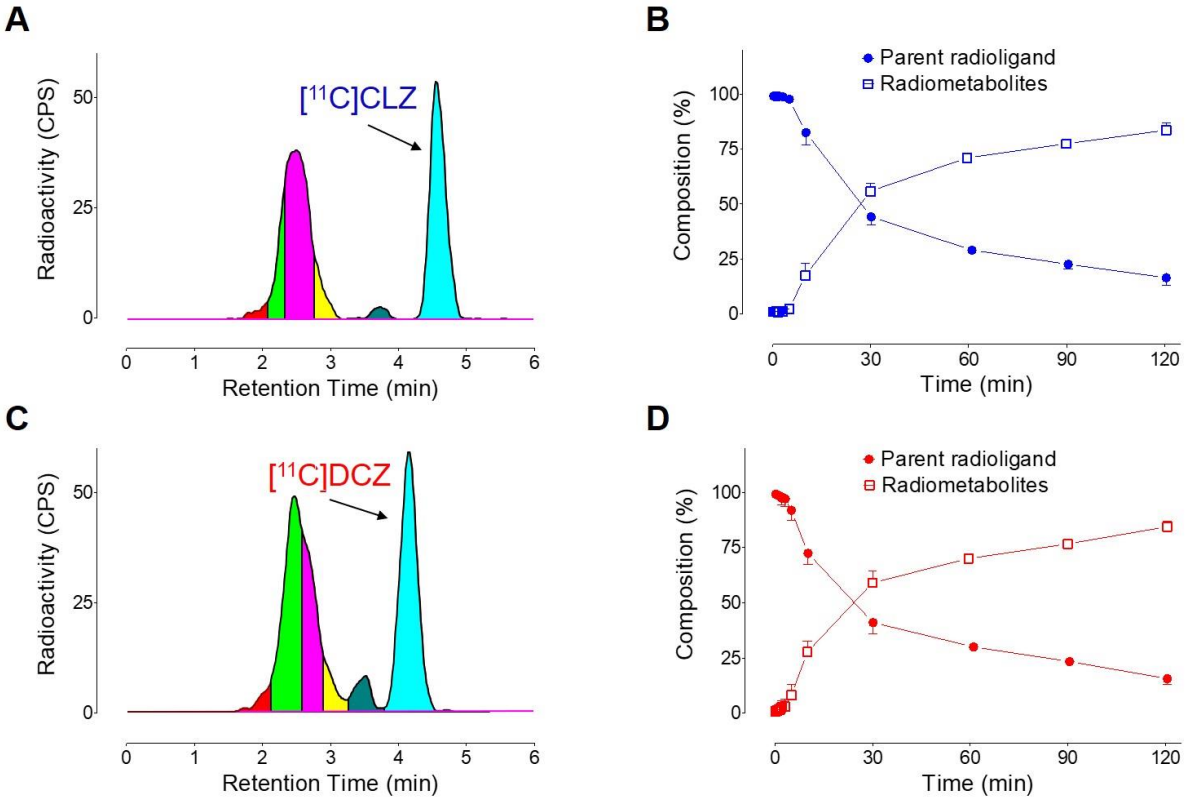
[b]Values were reported by Nagai and colleagues[1].

Supplementary Figure S1



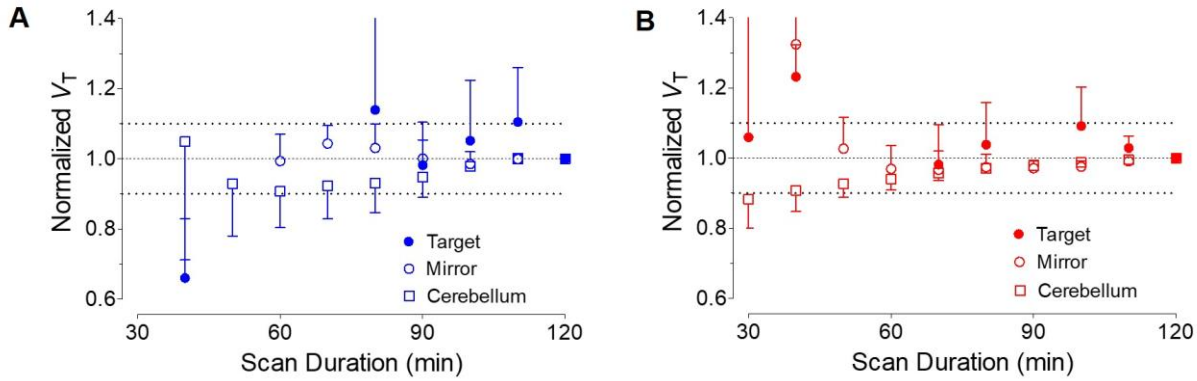
Supplementary Figure S1. Structures of DREADDs Ligands [^{11}C]CLZ and [^{11}C]DCZ.

Supplementary Figure S2



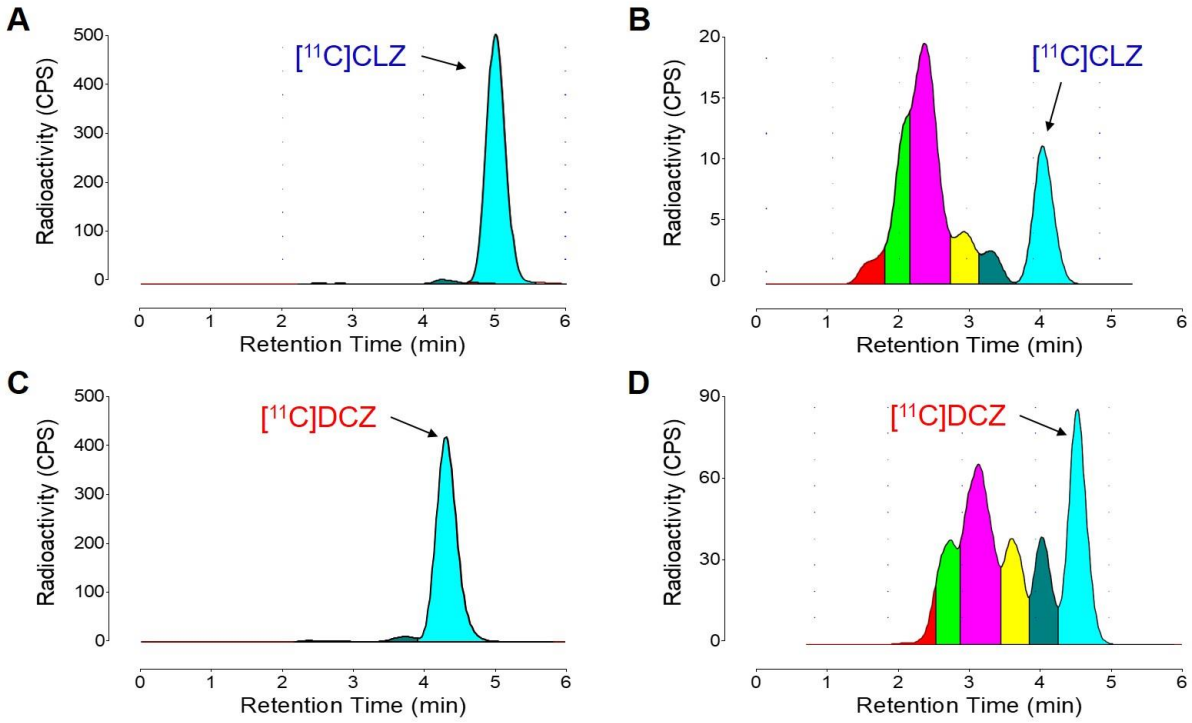
Supplementary Figure S2. Radiochromatograms and percentage composition of monkey plasma after injection of [^{11}C]clozapine ([^{11}C]CLZ) **(A, B)** or [^{11}C]deschloroclozapine ([^{11}C]DCZ) **(C, D)**. The radiochromatograms **(A** and **C)** of plasma acquired at 30 minutes post-administration showed that radiometabolites, eluting before the parent tracer, constituted the majority of radioactivity: 41% for [^{11}C]CLZ and 38% for [^{11}C]DCZ. The percentage composition over time **(B** and **D)** for both radioligands was also similar: 50% composition occurred slightly before 30 minutes for both. Error bars represent the standard deviation (SD) of three scans.

Supplementary Figure S3



Supplementary Figure S3. Total distribution volume (V_T) time stability of [^{11}C]clozapine ([^{11}C]CLZ) **(A)** and [^{11}C]deschloroclozapine ([^{11}C]DCZ) **(B)** for three regions: target (right amygdala), mirror (left amygdala), and cerebellum. V_T was calculated using an unconstrained two-tissue compartment model with increasingly truncated acquisition times, from 0-120 minutes to 0-30 minutes. All V_T values were normalized as a percentage of the terminal value attained from 120 minutes of imaging. Data represent mean ± SD of three scans.

Supplementary Figure S4



Supplementary Figure S4. Radiochromatograms of activity extracted from rat brain 30 minutes after injection of [^{11}C]clozapine ([^{11}C]CLZ) **(A)** or [^{11}C]deschloroclozapine ([^{11}C]DCZ) **(B)**. Most of the radioactivity for both radioligands represented parent radioligand: 98% for [^{11}C]CLZ and 97% for [^{11}C]DCZ.

Supplementary References

1. Nagai Y, Miyakawa N, Takuwa H, Hori Y, Oyama K, Ji B *et al.* Deschloroclozapine, a potent and selective chemogenetic actuator enables rapid neuronal and behavioral modulations in mice and monkeys. *Nat Neurosci* 2020; 23**:** 1157-1167.