SUPPORTING INFORMATION

Discrepancies in kappa opioid agonist binding revealed through PET imaging

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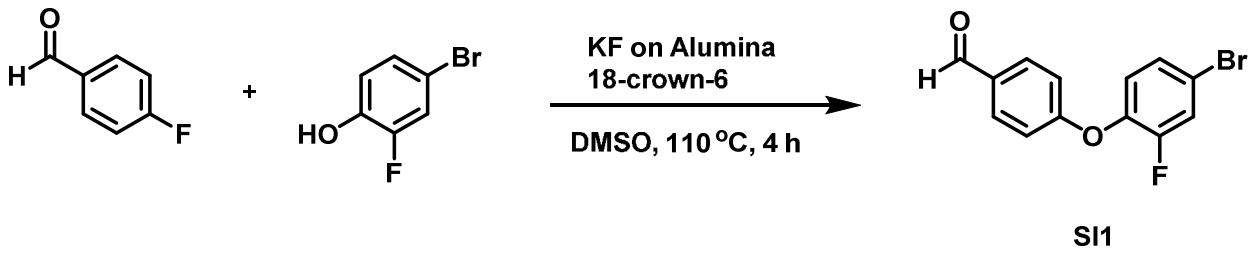
EXPERIMENTAL DATA

Synthesis of LY2459989-precursor

4-(4-bromo-2-fluorophenoxy)benzaldehyde (SI1)



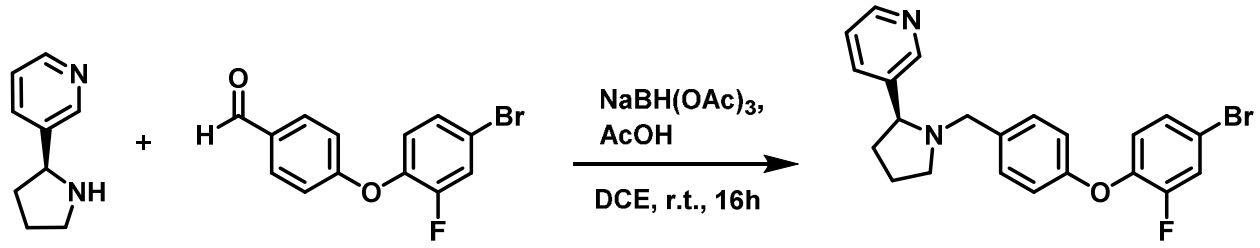
SI1

To a solution of 4-fluorobenzaldehyde (0.248 g, 2.0 mmol) in DMSO (10 mL) was added 4-bromo-2-fluorophenol (0.401 g, 2.1 mmol, 1.05 equiv.) followed by 40 wt% potassium fluoride on alumina (0.407 g, 2.8 mmol, 1.4 equiv.) and 18-crown-6 (0.053 g, 0.2 mmol, 0.1 equiv.). The mixture was heated at 110 ^{o}C for 4 hours before the addition of ethyl acetate (20 mL) and water (100 mL). The phases were separated and the aqueous layer was extracted with ethyl acetate (2 x 10 mL). The combined organic phases were washed with water (25mL), then brine (50 mL), and dried over sodium sulfate. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography on silica (12 g) eluting with 100% hexanes – 98 % hexanes / 2 % ethyl acetate (linear gradient from 0-15 column volumes @ 20 mL/min). The product was concentrated to afford a colorless oil (0.130 g, 22 % yield).

R_f = 0.14 (5 % EtOAc / 95 % hexanes)

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.94 (s, 1H), 7.86 (d, 2H, *J* = 9.0 Hz), 7.40 (d, 1H, *J* = 10.0 Hz), 7.32 (d, 1H, *J* = 10.0 Hz), 7.03 – 7.08 (m, 3H).

(S)-3-(1-(4-(4-bromo-2-fluorophenoxy)benzyl)pyrrolidin-2-yl)pyridine (SI2)



SI2

To a solution of (S)-nornicotine (25 mg, 0.169 mmol) in 1,2-dichloroethane (7 mL) was added 4-(4-bromo-2-fluorophenoxy)benzaldehyde (50 mg, 0.169 mmol) followed by acetic acid (0.254 mmol, 1.5 equiv.) and sodium triacetoxyborohydride (0.254 mmol, 1.5 equiv.). The mixture was stirred overnight at room temperature before the addition of saturated sodium bicarbonate solution (25 mL). The organic was extracted with dichloromethane (3 x 10 mL), combined, washed with brine (25 mL), and dried over sodium sulfate. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography on silica (4 g) eluting with 5% ethyl acetate / 95% hexanes – 60 % ethyl acetate / 40% hexanes (linear gradient from 0-20 column volumes @ 15 mL/min). The product was concentrated to afford a light yellow oil (45 mg, 63 % yield).

R_f = 0.25 (50 % EtOAc / 50 % hexanes)

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.63 (s, 1H), 8.50 (d, 1H, *J* = 3.5 Hz), 7.78 (d, 1H, *J* = 8.0 Hz), 7.33 (d, 1H, *J* = 10.0 Hz), 7.24 – 7.28 (m, 1H), 7.19 – 7.22 (m, 3H), 6.85 – 6.89 (m, 3H), 3.73 (d, 1H, *J* = 13.5 Hz), 3.41 (t, 1H, *J* = 8.5 Hz), 3.10 – 3.14 (m, 2H), 2.20 – 2.28 (m, 2H), 1.40 – 1.92 (m, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 155.7, 154.1 (d, *J* = 251.9 Hz), 149.7, 148.7, 143.8 (d, *J* = 11.5 Hz), 139.5, 135.1, 134.9, 130.1, 127.8 (d, *J* = 3.9 Hz), 123,7, 122.5, 120.7 (d, *J* = 20.4 Hz), 117.5, 115.8 (d, *J* = 8.8 Hz), 67.1, 57,6. 53.7, 35.4, 22.7.

MS ESI+ (m/z) calc'd for $C_{22}H_{21}BrFN_2O$ $[M+H]^+$, 427.1; observed, 427.1

IMAGING : TIME-ACTIVITY CURVES

Baseline time-activity curves for each radiotracer from 60 min dynamic PET-CT in Sprague-Dawley rat. Only select regions shown for clarity (high and low binding regions). Data shown for each radiotracer was averaged from 3 scans. Mean reported without error bars for enhanced clarity. High binding ROI = average of nucleus accumbens, amygdala, caudate putamen, hypothalamus, thalamus, periaqueductal gray, ventral tegmental area, midbrain, and olfactory.

[^{11}C]GR103545 PET: baseline time-activity curves from Sprague-Dawley rat.

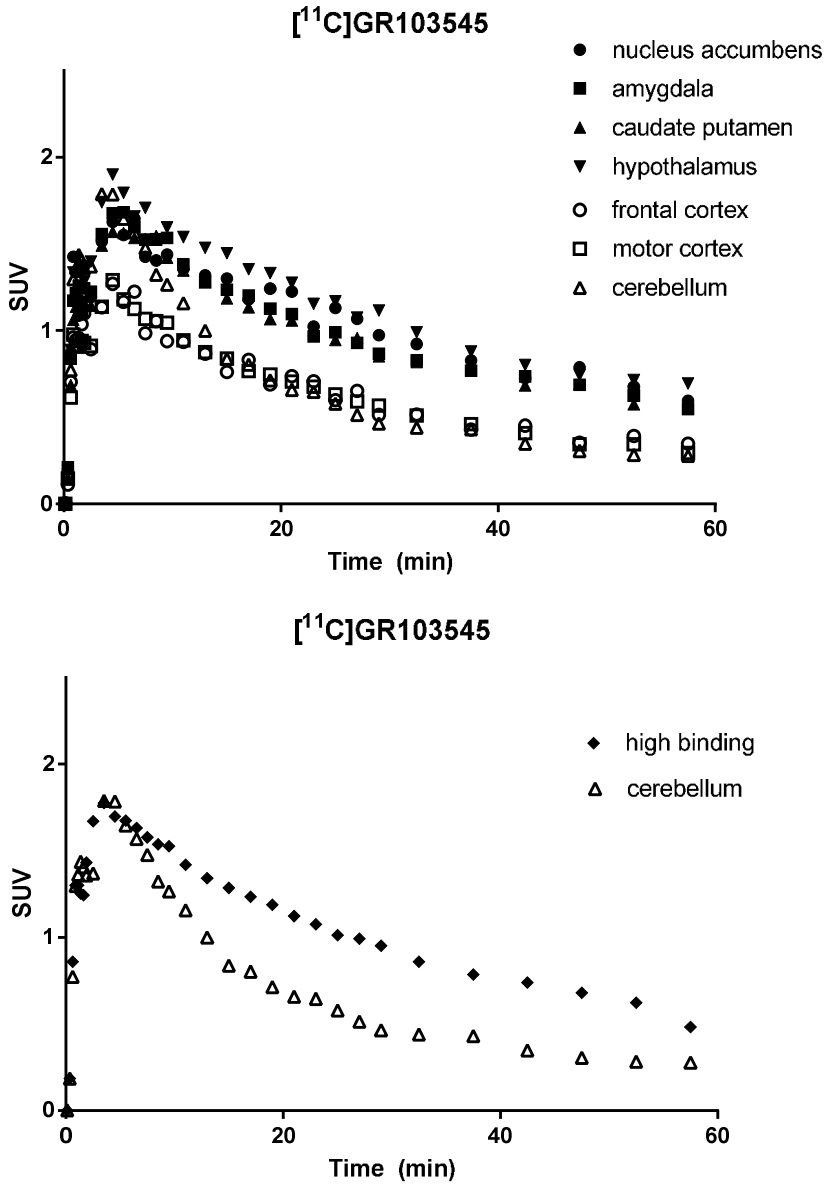


Figure S1. Time-activity curve for [^{11}C]GR103545 at baseline.

[^{11}C]LY2459989 PET: baseline time-activity curves from Sprague-Dawley rat.

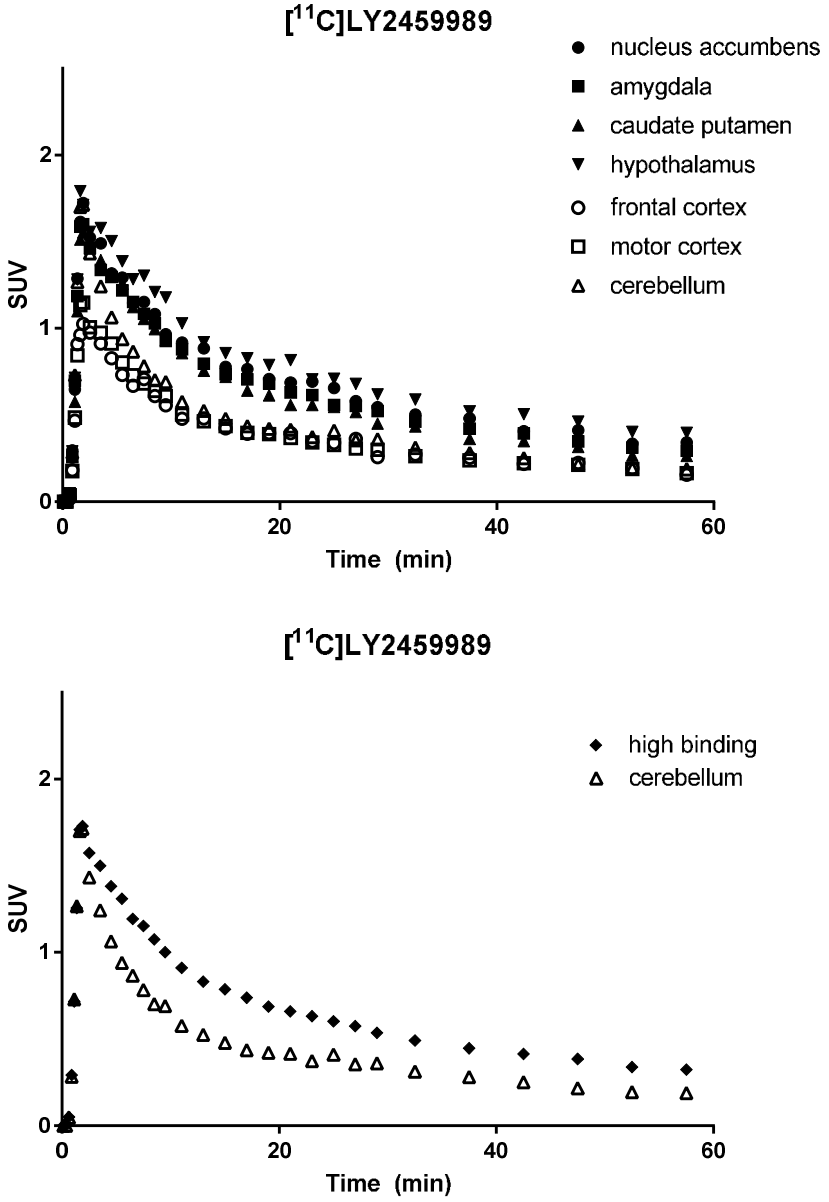


Figure S2. Time-activity curve for [^{11}C]LY2795050.

[^{11}C]LY2795050 PET: baseline time-activity curves from Sprague-Dawley rat.

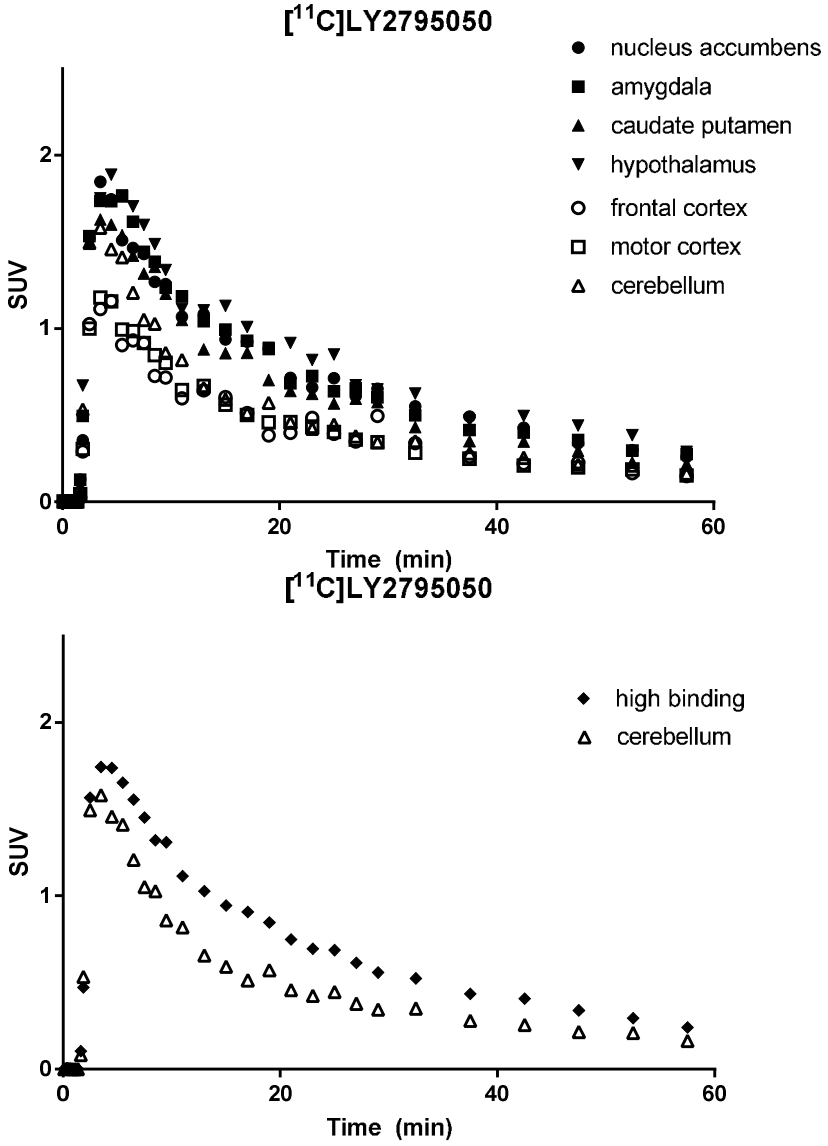
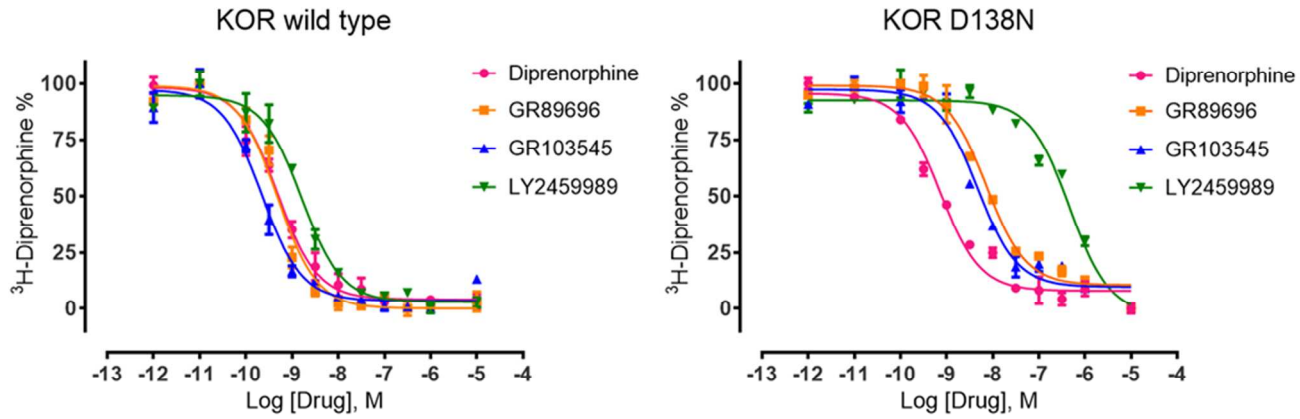


Figure S3. Time-activity curve for [^{11}C]LY2795050.

SODIUM CONCENTRATION EFFECTS ON *IN VITRO* BINDING

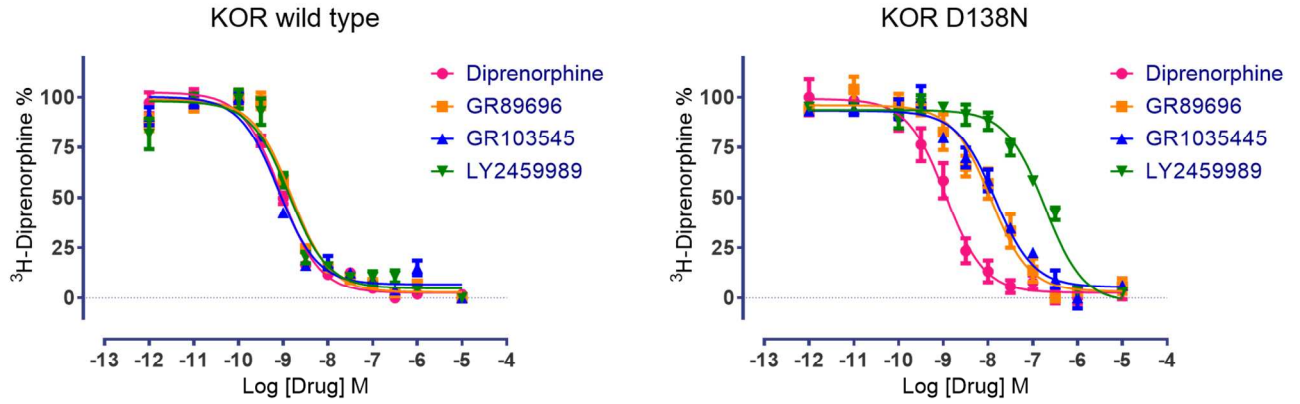
Standard binding buffer



	Diprenorphine	GR89696	GR103545	LY2459989
Ki (KOR WT)	1.603e-010	1.601e-010	7.405e-011	5.412e-010

	Diprenorphine	GR89696	GR103545	LY2459989
Ki (KOR D138N)	4.474e-010	4.433e-009	2.901e-009	2.871e-007

HEPES buffer + 100 mM NaCl



	Diprenorphine	GR89696	GR1035445	LY2459989
Ki (KOR WT)	2.977e-010	5.112e-010	2.657e-010	4.530e-010

	Diprenorphine	GR89696	GR1035445	LY2459989
Ki (KOR D138N)	6.312e-010	6.328e-009	8.003e-009	1.024e-007

Figure S4. *In vitro* radioligand binding studies to compare sodium effects on agonist binding. With 25 mM HEPES (100 mM NaCl; pH 7.4) compound affinity trends similarly to standard binding buffer (50 mM Tris, 0.1mM EDTA, 10mM $MgCl_2$, 0.1% BSA, pH 7.4). Although a slight change in affinity was observed, there was no significant sodium effect on agonist binding.