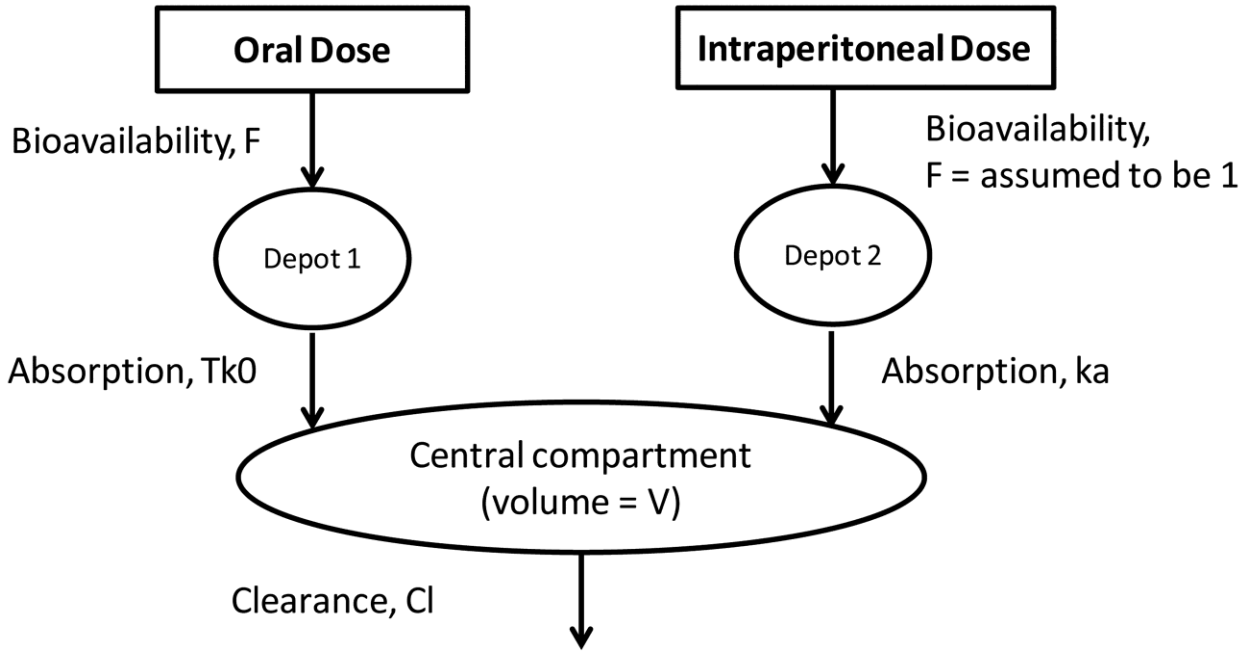
Supplemental Data: Drug Metabolism and Disposition (2018)

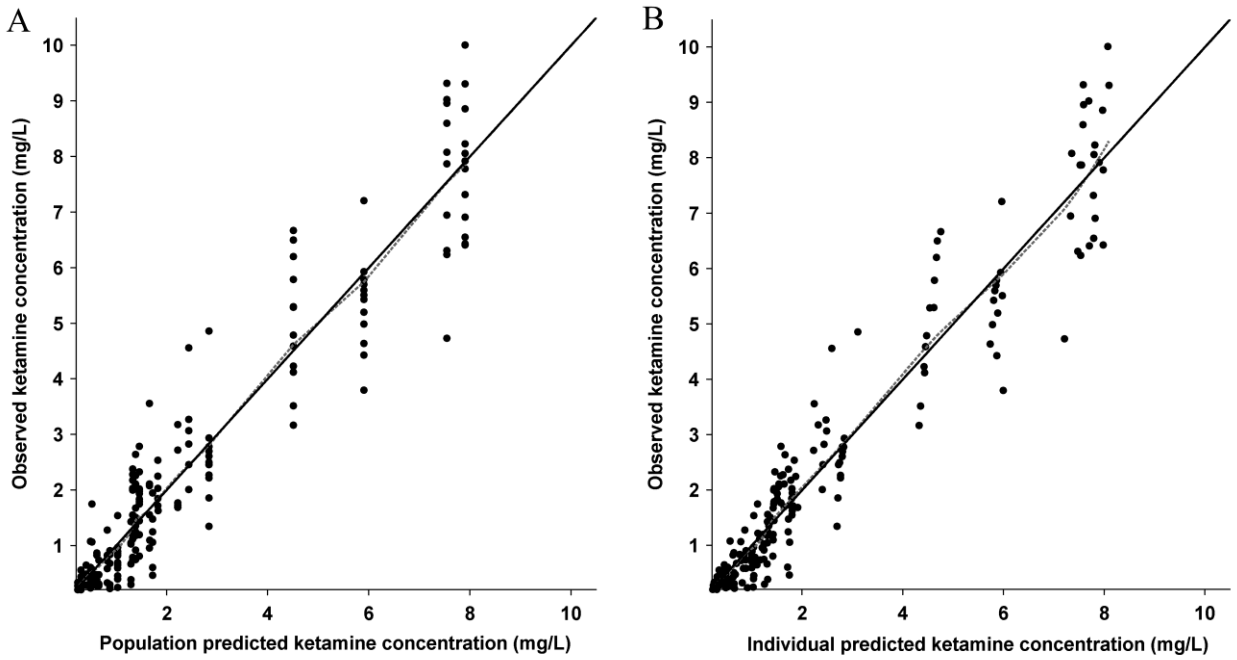
Ketamine *in vivo* exposure and pharmacodynamics is altered by Pgp and Bcrp efflux transporters in mice

Samit Ganguly, John C. Panetta, Jessica K. Roberts and Erin G. Schuetz

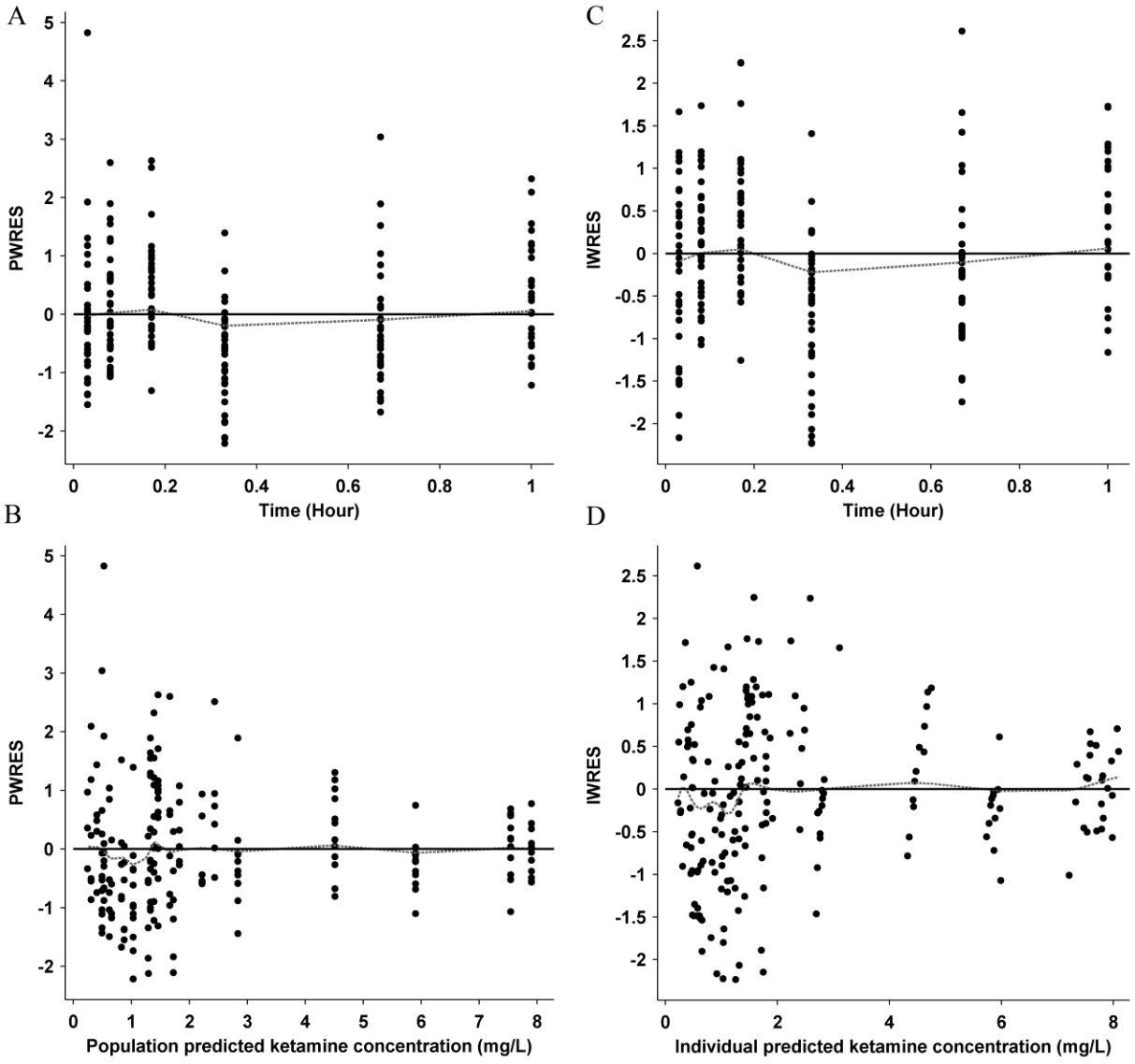
Supplemental Figure S1. Ketamine one-compartmental distribution with zero order absorption for PO and first-order absorption for the IP dosing and first-order elimination from the central compartment



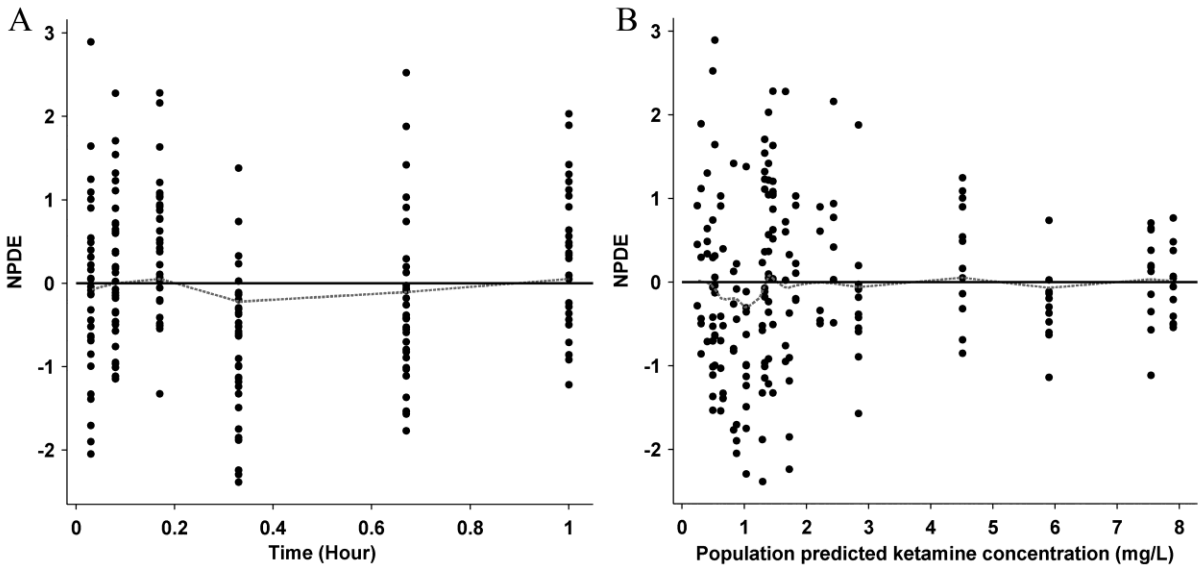
Supplemental Figure S2. Diagnostic plots – observed vs. population and individual predicted ketamine concentrations. Observed ketamine concentration versus population predicted (A) and (B) individual predicted ketamine concentration for the final ketamine population PK model. The solid line represents the line of identity and the gray line represent the spline of the model. The plots show that the spline is very close to the line of identity, which implies that the final model was able to successfully predict the observed ketamine concentrations in serum.



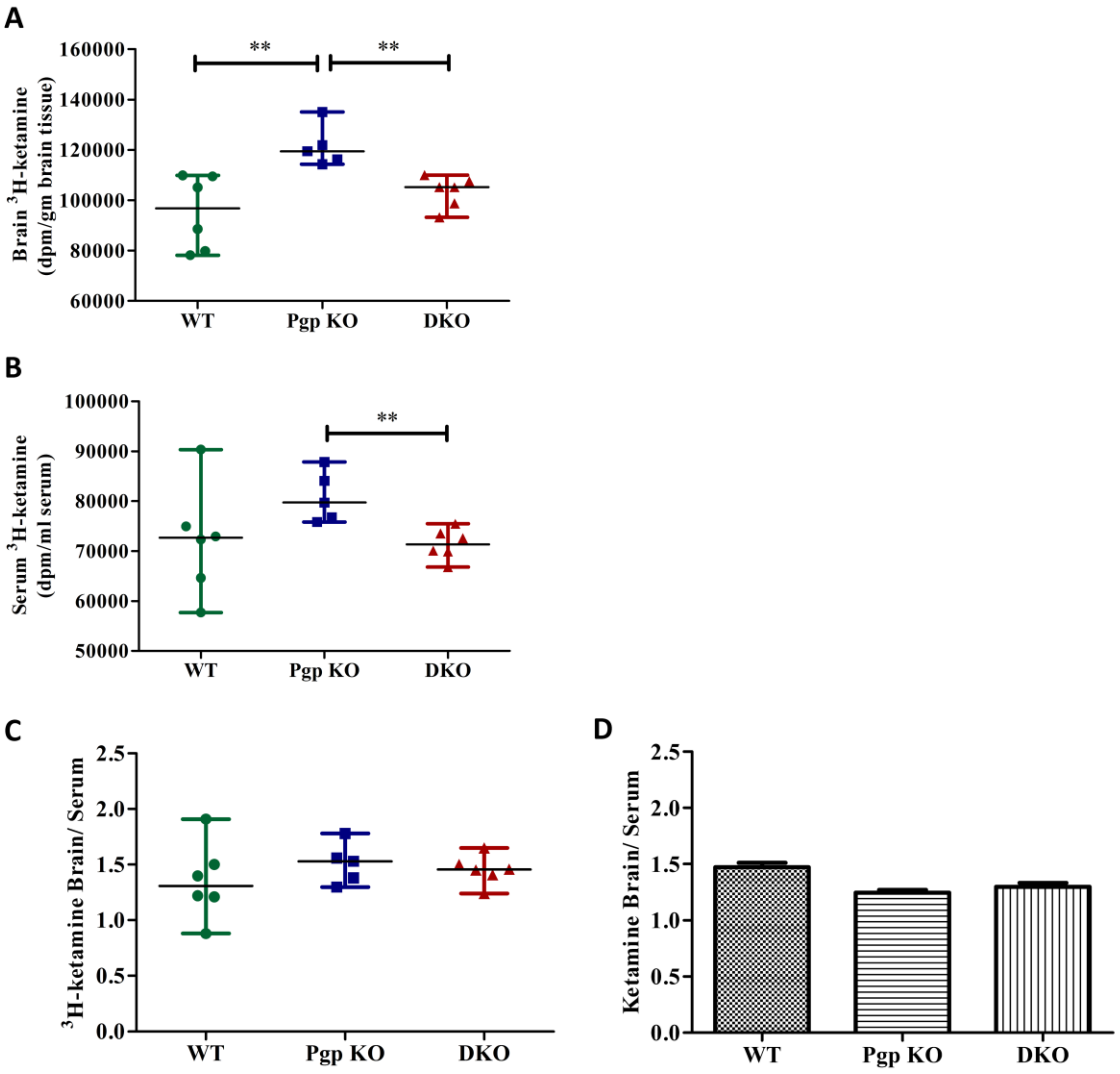
Supplemental Figure S3. Diagnostic residual plots. (A) & (B) – population weighted residual (PWRES) vs. time and predicted ketamine concentration, respectively. (C) & (D) – Individual weighted residual (IWRES) vs. time and predicted concentration, respectively. The solid line represents the reference line at zero and the gray line represents the spline of the model. The plots show there was no systematic bias in the model over time or concentration range. The majority of the population residuals were equally distributed around zero and within ± 2, which indicates that the predicted data is within an acceptable range to the observed data.



Supplemental Figure S4. Normalized prediction distribution error (NPDE) plots.
(A)– NPDE vs. time and predicted ketamine concentration, respectively. (B) – NPDE vs. time and predicted concentration, respectively. The solid line represents the reference line at zero and the gray line represents the spline of the model. The plots shows a lack of model misspecification for the final model.



Supplemental Figure S5. Ketamine brain/serum ratios in wild-type, PgpKO and Pgp/Bcrp-dKO mice. Brain ketamine in mice after IP dosing with ketamine (100 mg/kg) + 1 µCi ketamine tracer. Results are expressed as median radioactivity (A,B) or brain/serum radioactivity ratio ± range (C). Brain/serum concentration ratio (D) was measured by HPLC-UV based method after single intraperitoneal dose of ketamine (100 mg/kg). Significance is calculated using Mann-Whitney test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$



Supplemental Table S1. Noncompartmental analysis parameters for Ketamine IP and PO PK at 100 mg/kg

		IPPK at 100 mg/kg (Mean ± SD)			
Parameters	Unit	WT	Bcrp KO	Pgp KO	Double KO
$\mathbf{t_{1/2}}$	min	22.93 ± 2.33	27.75 ± 6.45	22.72 ± 3.78	21.89 ± 3.50
$\mathbf{T_{max}}$	min	10.00	5.00	10.00	10.00
$\mathbf{C_{max}}$	µg/ml	7.88 ± 1.38	8.60 ± 1.03	7.90 ± 0.98	8.77 ± 1.08
$\mathbf{AUC_{inf}}$	µg*min /ml	296.61 ± 17.07	298.4 ± 38.43	278.33 ± 29.1	315.15 ± 25.38

		POPK at 100 mg/kg (Mean ± SD)			
Parameters	Unit	WT	Bcrp KO	Pgp KO	Double KO
$\mathbf{t_{1/2}}$	min	17.86 ± 4.23	22.26 ± 4.07	30.85 ± 9.54	26.56 ± 6.25
$\mathbf{T_{max}}$	min	10.00	10.00	10.00	10.00
$\mathbf{C_{max}}$	µg/ml	1.83 ± 0.52	1.82 ± 0.54	2.0 ± 0.34	**3.03 ± 0.87** *
$\mathbf{AUC_{inf}}$	µg*min /ml	43.91 ± 6.9	**52.82 ± 3.92** *	**66.91 ± 7.86** ***	**85.93 ± 8.2** ***

All pharmacokinetic parameters were calculated by noncompartmental analysis using PK package in R, with variability calculated using bootstrap t method.
AUCs are compared by one way ANOVA with Newman-Keuls Multiple Comparison Test to compare all the groups. The results in the table show the comparison between each transporter genotype vs. WT. * $p< 0.05$, ** $p<0.01$, *** $p<0.001$.
C_{max} of the dKO and WT is compared using Mann Whitney Test, * $p < 0.0.5$.

Supplemental Table S2. Descriptive Statistics of Ketamine induced dLORR in male FVB mice (WT, Bcrp $^{-/-}$, Pgp $^{-/-}$, Bcrp-Pgp $^{-/-}$)

	WT	**Bcrp KO**	**Pgp KO**	**dKO**
dLORR Time (minutes)	n = 12	n = 12	n = 12	n = 11
Minimum	0.0	0.0	0.0	13.0
25% Percentile	0.75	3.5	8.0	14.0
Median	6.0	7.5	**12.0***	**16.0*****
75% Percentile	11.5	12.75	17.25	19.0
Maximum	15.0	17.0	20.0	22.0
Mean	6.5	8.0	11.58	**16.73**
Std. Deviation	5.45	5.69	6.20	3.13
Std. Error	1.57	1.64	1.79	0.94
Lower 95% CI of mean	3.04	4.38	7.64	14.62
Upper 95% CI of mean	9.96	11.61	15.52	18.83

Significance is calculated using Mann-Whitney test by comparing the median dLORR in each genotype with the median dLORR in the WT mice. * $p < 0.05$, ** $p< 0.01$, *** $p< 0.001$.

Supplemental Table S3. Descriptive statistics of Ketamine induced Loss of righting reflex (LORR) in male WT FVB mice with or without 100 mg/kg oral Elacridar 1.5 hours before intraperitoneal ketamine dosing.

	50 mg/kg	**50 mg/kg + ECD**	**100 mg/kg**	**100 mg/kg + ECD**	**200 mg/kg**
dLORR Time (minutes)	n = 6	n = 6	n = 5	n = 5	n = 5
Minimum	0.0	1.00	6.00	11.00	33.00
25% Percentile	0.0	2.50	6.00	12.50	33.00
Median	0.00	**3.50***	6.00	**14.00***	34.00
75% Percentile	2.00	4.50	7.50	17.00	42.50
Maximum	2.00	6.00	8.00	18.00	44.00
Mean	0.67	3.5	6.60	14.60	37.00
Std. Deviation	1.03	1.64	0.89	2.61	5.15
Std. Error	0.42	0.67	0.40	1.17	2.30
Lower 95% CI of mean	-0.42	1.78	5.49	11.36	30.61
Upper 95% CI of mean	1.75	5.22	7.71	17.84	43.39

Significance is calculated using the Mann-Whitney test by comparing each ECD treated ketamine dose group by their corresponding no ECD pretreatment group. * $p < 0.05$.