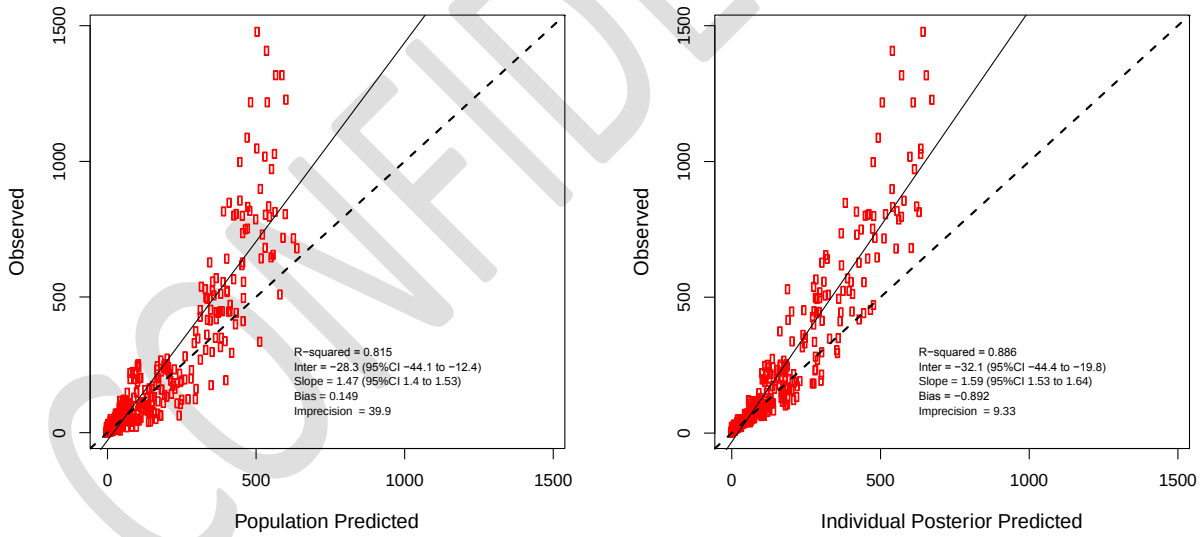
Methodology – Bioanalysis

Plasma samples were assayed for VPA using a liquid chromatography mass spectrometry method (LC-MS). An ABI-3200 Qtrap mass spectrometer with electrospray ionization probe was interfaced with an Agilent 1200 series high performance LC (HPLC) system for sample analysis. The Analyst Software Version 1.4.2 package supplied by Applied Bio-systems (MDS SCIEX) was used to control the LC–MS/MS system, as well as for data acquisition and processing. VPA and benzoic acid (internal standard) were separated on a Xbridge C-18 column, 50 mm × 4.6 mm ID, 3.5 um (waters) under isocratic conditions at a flow rate of 1 mL/min. The mobile phase consisted of H_2O: acetonitrile in ratio of 20:80 (v/v) with 5 mM ammonium formate at pH = 9 adjusted with ammonium hydroxide. The mass spectrometer was operated in the negative mode with selected reaction monitoring for the analytes. The gas temperature was 700 °C with an ion spray voltage of 4500 V, declustering potential (DP) of 33, curtain gas of 45, and collision energy of 11. The mass transitions were monitored at m/z 143.0→143.0 for VPA and m/z 121→77 for internal standard. A plasma sample (200 µL) was pipetted into a microcentrifuge tube and then 20 uL of internal standard working solution (500 µg/mL) and 200 µL of 1%formic acid (v/v) were added. The mixture was vortex-mixed for 30 s and loaded onto a 96-well OASIS HLB SPE cartridge (60 mg, Waters Co., Milford, USA), which had been conditioned by washing with acetonitrile (1 mL) followed by water (2 mL). The OASIS cartridge was rinsed with water (2 mL) followed by 10% methanol (1 mL), and then eluted with 80% acetonitrile (1 mL). Then, 5µL of elutant was transferred into a HPLC vial for injection and injected into the LC/MS system for analysis. The peak area of endogenous components eluted at the retention times of VPA and the internal standard in control plasma was less than 10% of that at the lower limit of

quantification (5 µg/mL). Ratios of VPA peak areas to the internal standard peak areas were calculated. The calibration curve was constructed using the peak area ratios of the 8 calibration standards (ranging from 5 to 1000 µg/mL) and the best fit was determined by a method of least-squares using a weighting factor of 1/concentration^2. VPA plasma concentration below 5 µg/mL was reported as the low limit of quantification (BLQ) and used as zero in the calculation of mean concentrations and the standard deviations.

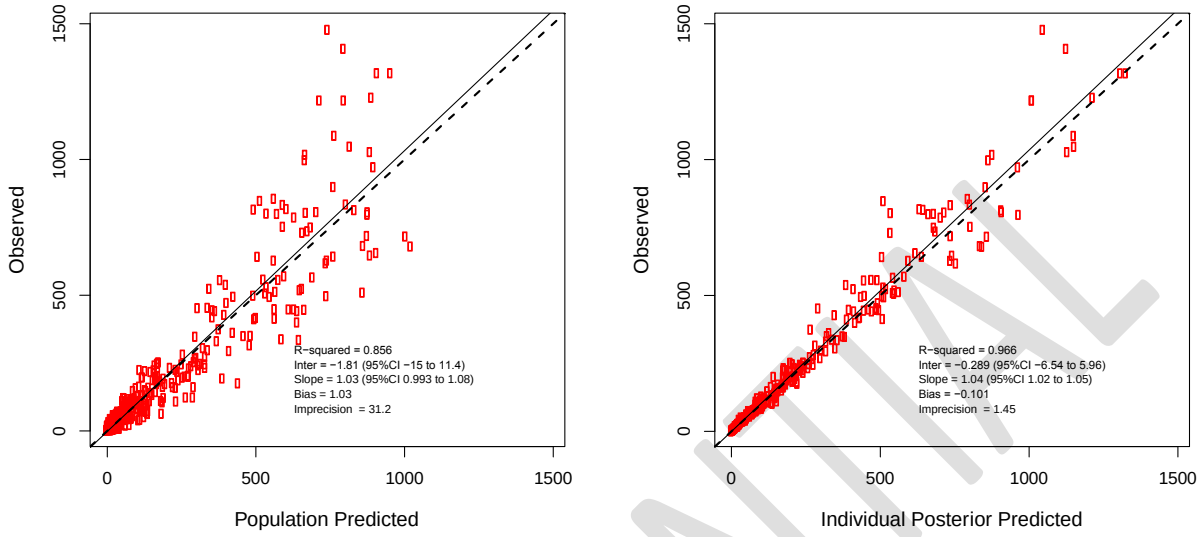
Pharmacokinetic Analyses

Figure S1. Observed versus predicted plots for a 1-compartment (A), 2-compartment (B), 3-compartment (C) linear model

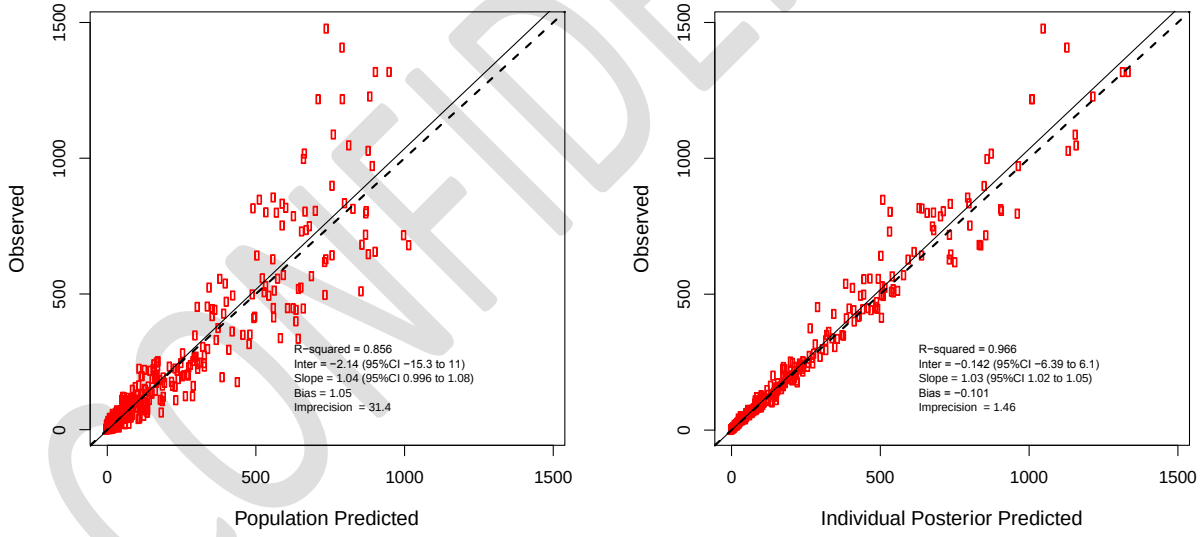


A

Supplementary Data: Pharmacokinetics and Safety of High-Dose Intravenous Valproic Acid in Healthy Subjects: A Dose Escalation Trial To Support Clinical Translational Studies, Georgoff et al



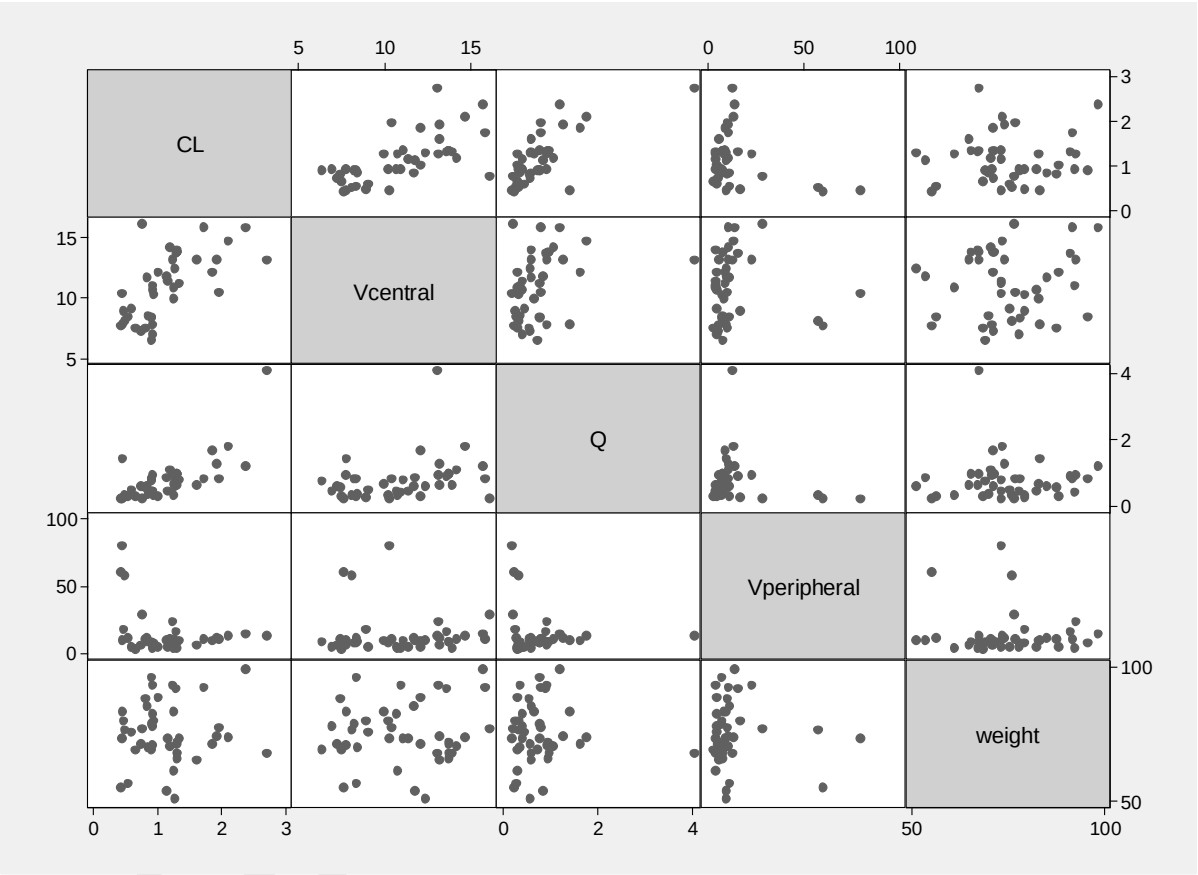
B



C

Supplementary Data: Pharmacokinetics and Safety of High-Dose Intravenous Valproic Acid in Healthy Subjects: A Dose Escalation Trial To Support Clinical Translational Studies, Georgoff et al

Figure S2. Scatter matrix plot of 2-compartment model system parameters against weight with the associated correlation matrix tabulated below



R-values	CL	Vc	Q1	Vp_1	Weight
CL	1				
Vc	0.646	1			
Q_1	0.7242	0.3278	1		
Vp_1	-0.2971	-0.0757	-0.1633	1	
Weight	0.0703	0.1029	-0.0091	-0.0821	1

Supplementary Data: Pharmacokinetics and Safety of High-Dose Intravenous Valproic Acid in Healthy Subjects: A Dose Escalation Trial To Support Clinical Translational Studies, Georgoff et al

Pharmacodynamic Safety Analyses

Figure S3. Heart rate over time by valproic acid weight-based dose group (mg/kg)

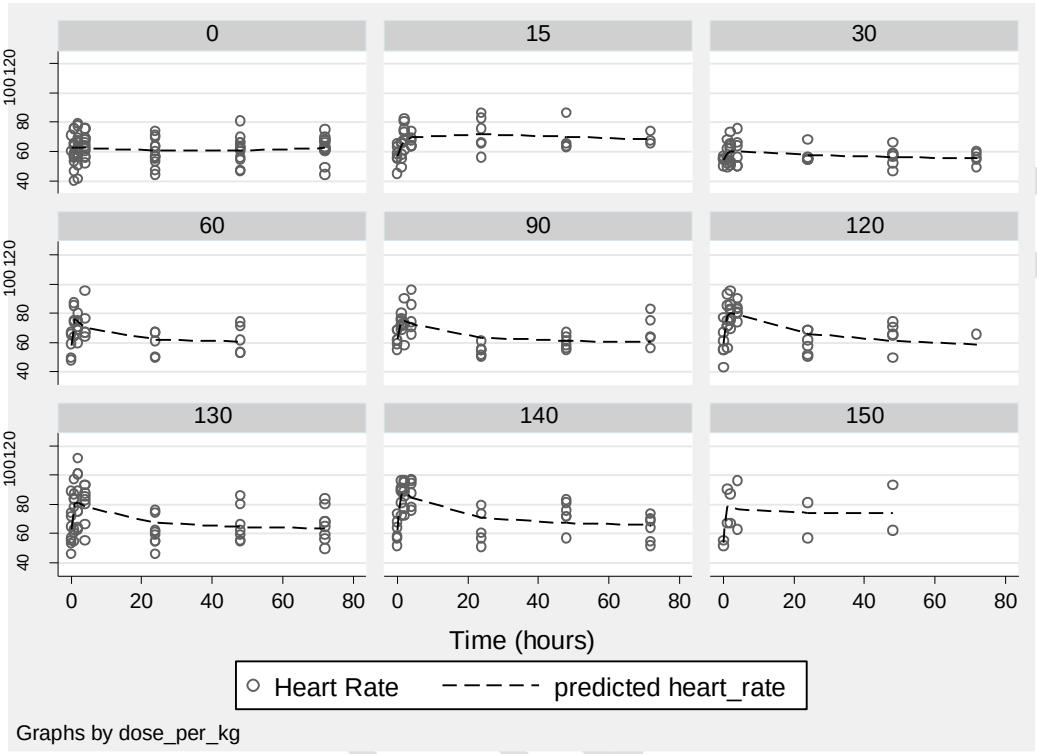
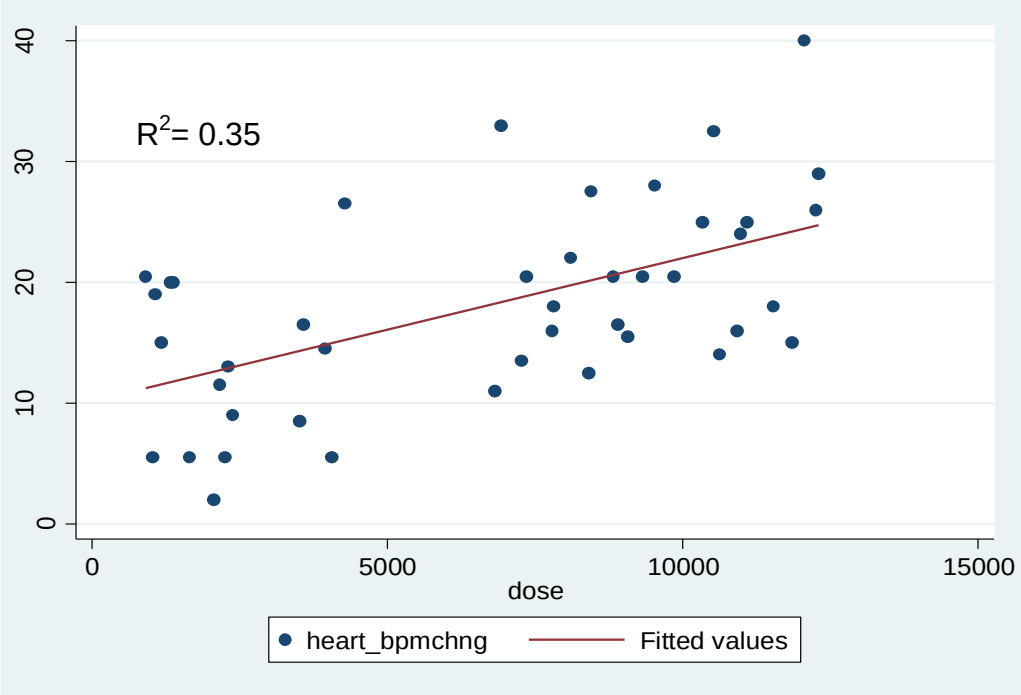


Figure S4 . Scatter and linear regression fit plot of the change in heart rate (beats per minute) over valproic acid dose (mg)



Source	SS	df	MS	Number of obs	=	429
				F(1, 427)	=	229.54
Model	10301.97	1	10301.9695	Prob > F	=	0
Residual	19164.18	427	44.8809719	R-squared	=	0.3496
				Adj R-squared	=	0.3481
Total	29466.14	428	68.8461321	Root MSE	=	6.6993

heart_bpmchng	Coef.	Std. Err.	t	P>t	[95% Conf.	Interval]
dose	0.001265	8.35E-05	15.15	0.000	.0011005	0.0014286
_cons	9.543962	0.655827	14.55	0.000	8.254911	10.83301