Role of phenmetrazine as an active metabolite of phendimetrazine: Evidence from studies of drug discrimination and pharmacokinetics in rhesus monkeys

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Supplemental Table 1: Mean values for the pharmacokinetic parameters using non-compartmental analysis: maximum concentration (C_{max}), time to maximum concentration (T_{max}), half-life ($T_{1/2}$), and area under the curve from T_0 min to T_{last} min (AUC_{last}) after treatment with different doses of phenmetrazine or phendimetrazine.

Treatment	**Analyte**	**C_{max} (ng/mL)**	**T_{max} (min)**	**$T_{1/2}$ (min)**	**AUC_{last} (min*ng/mL)**
(+)-Phenmetrazine 0.1 mg/kg	Phenmetrazine	24.3	16.7	178	3973
(+)-Phenmetrazine 0.32 mg/kg	Phenmetrazine	49.6	25	316	10,140
(+)-Phenmetrazine 1.0 mg/kg	Phenmetrazine	156	38.7	281	30,100
(-)-Phenmetrazine 0.32 mg/kg	Phenmetrazine	165	23.2	233	21,670
(-)-Phenmetrazine 1.0 mg/kg	Phenmetrazine	344	28.4	217	53,998
(-)-Phenmetrazine 3.2 mg/kg	Phenmetrazine	1018	36	348	160,361
(-)-Phenmetrazine 10.0 mg/kg	Phenmetrazine	4164	23.2	192	1,480,054
(+)-Phendimetrazine 0.32 mg/kg	Phenmetrazine	15.7	131	714	3706
(+)-Phendimetrazine	Phendimetrazine	137	18	114	15,195

0.32 mg/kg					
(+)-Phendimetrazine 1.0 mg/kg	Phenmetrazine	34.1	163	581	7842
(+)-Phendimetrazine 1.0 mg/kg	Phendimetrazine	268	22	180	35,877
(+)-Phendimetrazine 3.2 mg/kg	Phenmetrazine	141	132	1088	34,868
(+)-Phendimetrazine 3.2 mg/kg	Phendimetrazine	1227	19.2	112	132,945
(-)-Phendimetrazine 1.0 mg/kg	Phenmetrazine	25.3	102	2373	6113
(-)-Phendimetrazine 1.0 mg/kg	Phendimetrazine	391	14	180	49934
(-)-Phendimetrazine 3.2 mg/kg	Phenmetrazine	55	130	1118	12,937
(-)-Phendimetrazine 3.2 mg/kg	Phendimetrazine	853	18	130	109,411
(-)-Phendimetrazine 10.0 mg/kg	Phenmetrazine	308	107	1208	73,372
(-)-Phendimetrazine 10.0 mg/kg	Phendimetrazine	3491	32.4	129	495,589

Supplemental Table 2: Correlations between plasma phenmetrazine levels and percent cocaine-appropriate responding. In the presence of a significant correlation, the effective concentration (nM) that produced 50% cocaine-appropriate responding (EC50) was calculated using linear regression.

	(+)-Phenmetrazine	**(-)-Phenmetrazine**
Overall: Pearson R	0.69*	0.77*
R^2	0.47	0.6
EC50 value	70.14	523.60
10 MIN: Pearson R	0.77*	0.72*
R^2	0.6	0.51
EC50 value	35.24	175.79
30 MIN: Pearson R	0.86*	0.84*
R^2	0.75	0.7
EC50 value	41.21	478.63
56 MIN: Pearson R	0.63*	0.82*
R^2	0.39	0.68
EC50 value	73.11	570.16
100 MIN: Pearson R	0.79*	0.85*
R^2	0.62	0.59
EC50 value	69.02	671.43
180 MIN: Pearson R	0.61*	0.77*
R^2	0.38	0.59
EC50 value	368.13	671.43

300 MIN: Pearson R	0.45	0.58*
R^2	0.2	0.34
EC50 value		2393.32

* Indicates a significant correlation between plasma levels and percent cocaine-appropriate responding ($p < 0.05$).

Supplemental Table 3: Correlations between plasma phenmetrazine or phendimetrazine levels and percent cocaine-appropriate responding after phendimetrazine administration. In the presence of a significant correlation, the effective concentration (nM) that produced 50% cocaine-appropriate responding (EC50) was calculated using linear regression.

	(+)-Phendimetrazine		**(-)-Phendimetrazine**	
	Phenmetrazine	Phendimetrazine	Phenmetrazine	Phendimetrazine
Overall: Pearson R	0.35*	0.62*	0.16	0.45*
R^2	0.12	0.39	0.00	0.2
EC50 value	91.62	302.69		5834.45
10 MIN: Pearson R	0.46	0.46	-0.16	0.05
R^2	0.21	0.21	0.03	0.00
EC50 value				
30 MIN: Pearson R	0.60*	0.67*	0.31	0.46
R^2	0.36	0.45	0.09	0.21
EC50 value	35.08	401.79		
56 MIN: Pearson R	0.64*	0.75*	0.58*	0.67*
R^2	0.41	0.56	0.33	0.44
EC50 value	32.43	219.79	317.69	2917.43
100 MIN: Pearson R	0.62*	0.59*	0.71*	0.68*
R^2	0.38	0.35	0.5	0.46
EC50 value	74.13	311.17	274.79	2243.88
180 MIN: Pearson R	0.67*	0.65*	0.5	0.42
R^2	0.45	0.43	0.25	0.17

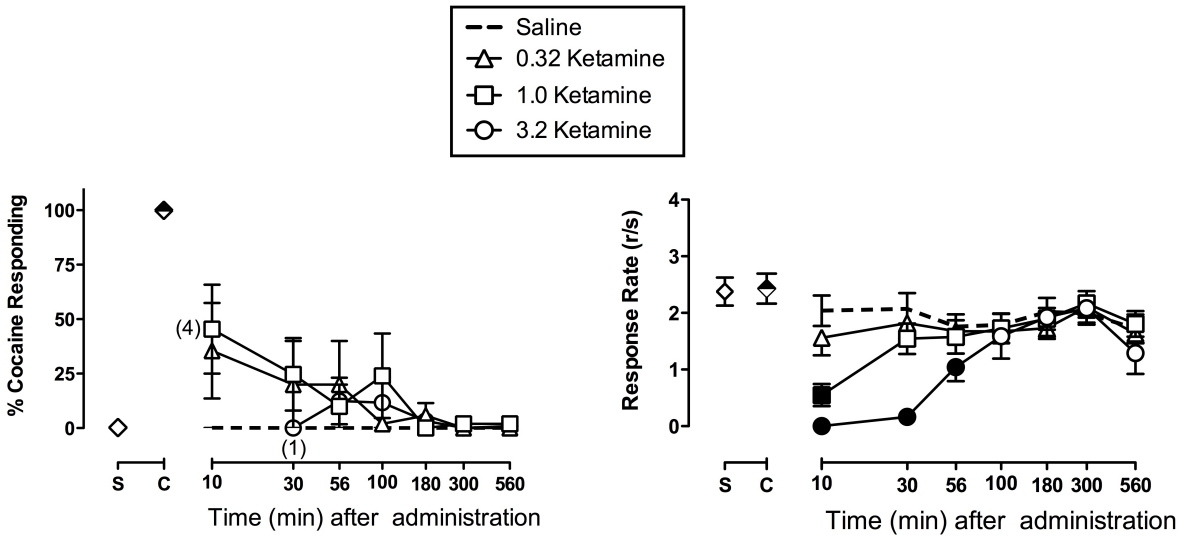
EC50 value	108.89	244.34		
300 MIN: Pearson R	0.56*	0.6*	0.57*	0.38
R^2	0.34	0.36	0.32	0.15
EC50 value	241.55	314.77	1142.88	

* Indicates a significant correlation between plasma levels and percent cocaine-appropriate responding ($p < 0.05$).

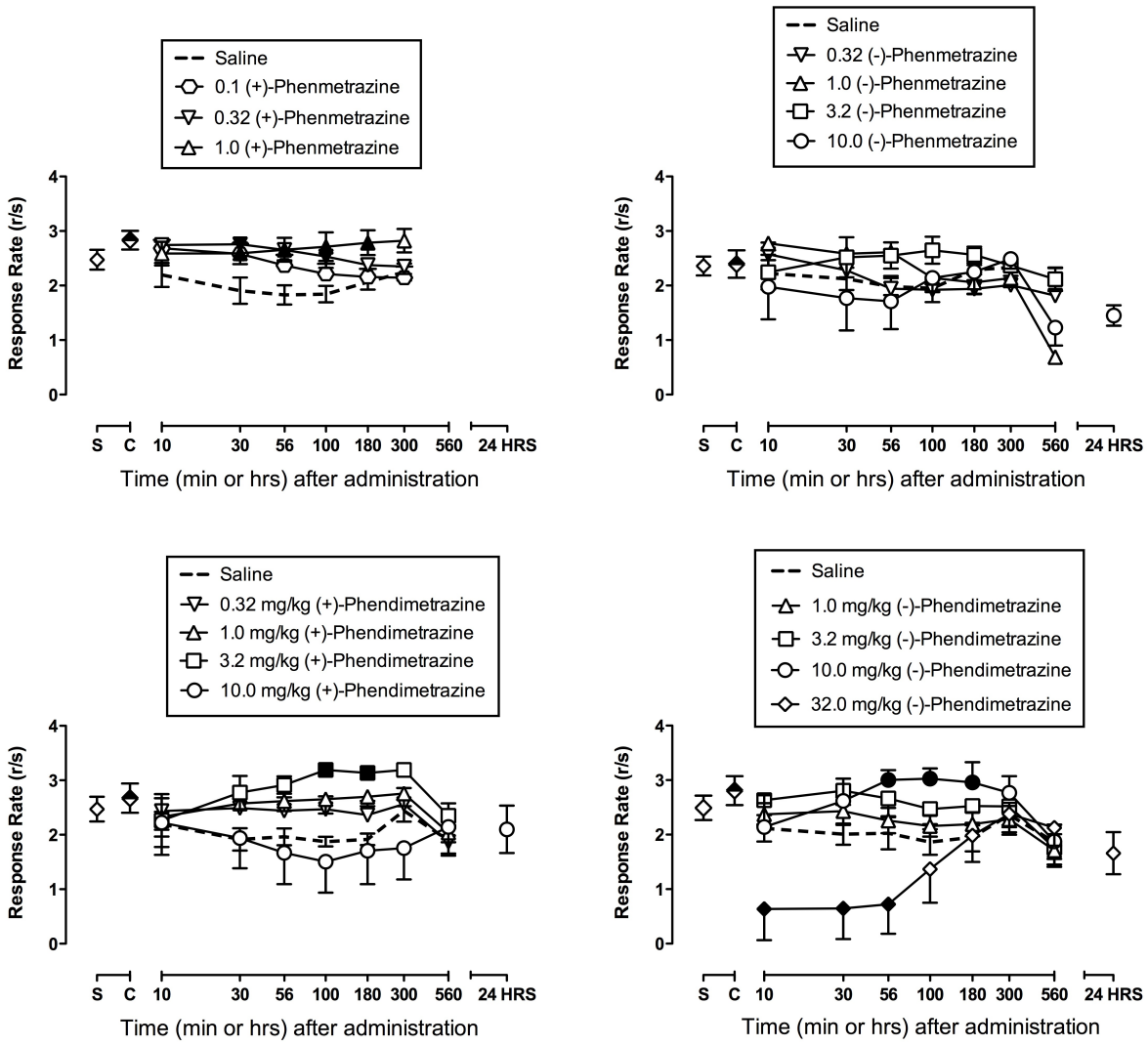
Supplemental Table 4: Mean EC50 values and corresponding 95% confidence limits as a function of time after (+)-phenmetrazine or (-)-phenmetrazine administration. EC50 values (nM) were calculated using linear regression and only in the presence of a significant correlation between plasma phenmetrazine (nM) levels and percent cocaine-appropriate responding.

Time (min)	**(+)-Phenmetrazine EC50 (95% CL)**	**(-)-Phenmetrazine EC50 (95% CL)**
10	198.6 (94.2 – 371.5)	990.8 (252.9 – 2084.5)
30	232.3 (151.4 – 331.1)	2697.7 (1633.0 – 4415.7)
56	413.0 (185.8 – 2483.1)	3221.1 (1918.7 – 5794.3)
100	389.0 (245.5 – 835.6)	3176.9 (2079.7 – 5260.2)
180	2074.9 (609.5 – 8,994,976)*	3784.4 (2037.0 – 10,641.4)
300	Could not calculate	13,520.7 (3698.3 – 4,920,395)*
	(+)-Phenmetrazine EC50 (95% CL) after (+)-Phendimetrazine	**(-)-Phenmetrazine EC50 (95% CL) after (-)-Phendimetrazine**
10	Could not calculate	Could not calculate
30	198.2 (77.1 – 2766.9)	Could not calculate
56	183.2 (65.8 – 547.0)	1794.7 (550.8 – 33,496,544)
100	418.8 (184.9 – 8590.1)	1548.8 (666.8 – 18,663.8)
180	615.2 (276.7 – 9057.3)	Could not calculate
300	1361.5 (399.9 – 9.55x10^10)	6456.5 (1238.8 – 6.31x10^16)

* Indicates that 95% confidence limits do not overlap compared to the 10 min time point.

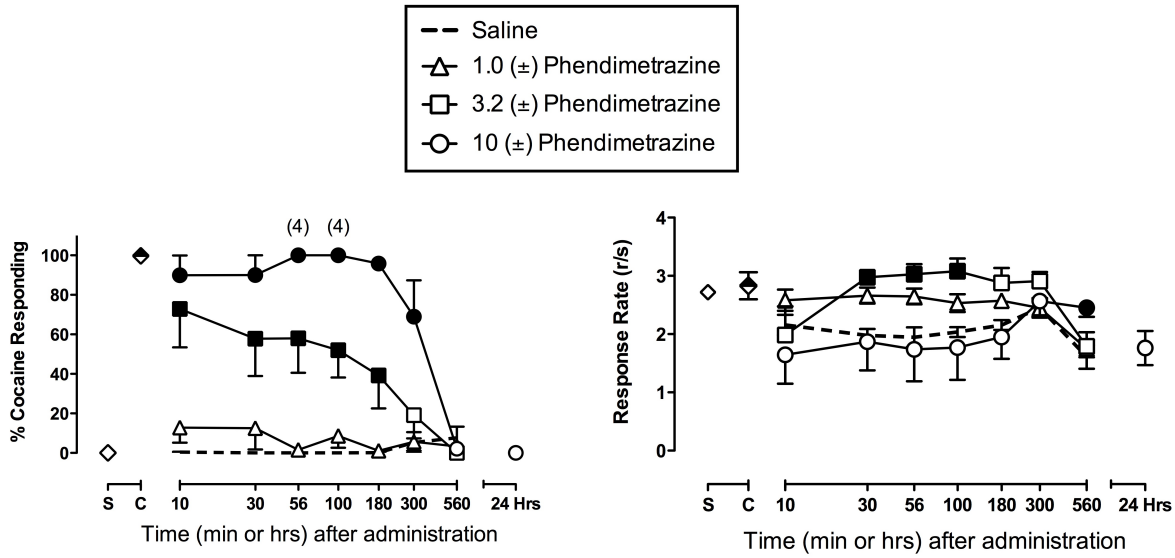


Supplemental Figure 1: Magnitude and time course of cocaine-like discriminative stimulus effects produced by the *N*-methyl *D*-aspartate antagonist ketamine in rhesus monkeys (n=5). Effects of separate saline tests are also shown for comparison. Ordinates: percent cocaine-appropriate responding. Abscissae: time in min after administration (log scale). Symbols above "S" and "C" represent the group averages ± SEM for all training sessions preceding test sessions when the saline- and cocaine- associated keys were correct, respectively. Filled symbols represent statistical significance ($p < 0.05$) within a given time point compared to experimental sessions when saline was administered (dotted line). All points represent the mean ± SEM of 5 monkeys except where indicated by numbers in parentheses in the top panels. In these cases, responding was eliminated in some monkeys, and the parenthetic number indicates the number of subjects responding at levels sufficient to contribute to the data point. Two-way repeated-measures ANOVA indicated no significant main effect of ketamine dose, time, or a significant interaction for percent cocaine responding. For rates of responding, there was a significant main effect of ketamine dose ($F_{3,12}$=14.6, $p<0.05$), time ($F_{6,24}$=29.0, $p<0.05$) and a significant interaction ($F_{18,72}$=6.3, $p<0.05$). Post-hoc analysis indicated that 1.0 mg/kg ketamine significantly decreased rates of responding at 10 min only, and 3.2 mg/kg ketamine significantly decreased rates of responding from 10 to 56 min compared to saline.

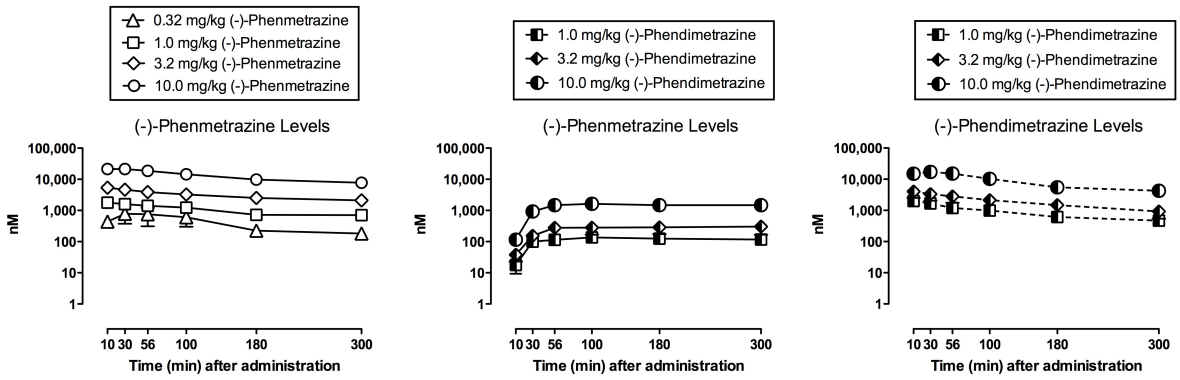


Supplemental Figure 2: Effects of (+)-phenmetrazine, (-)-phenmetrazine, (+)-phendimetrazine, and (-)-phendimetrazine on rates of operant behavior in rhesus monkeys (n=5). Ordinates: Rates of responding in responses per second. Abscissae: time in min after administration (log scale). Symbols above "S" and "C" represent the group averages ± SEM for all training sessions preceding test sessions when the saline- and cocaine- associated keys were correct, respectively. Filled symbols represent statistical significance ($p < 0.05$) within a given time point compared to experimental sessions when saline was administered (dotted line). All points represent the mean ± SEM of 5 monkeys. For (+)-phenmetrazine, there was a significant main effect of (+)-phenmetrazine dose ($F_{3,12}$=3.8, p<0.05), time ($F_{5,20}$=2.4, p<0.05) and a significant interaction ($F_{15,60}$=2.5, p<0.05) for rates of responding. Post-hoc analysis indicated that 0.32 mg/kg significantly increased rates of responding compared to saline from 30 to 100 min, and 1.0

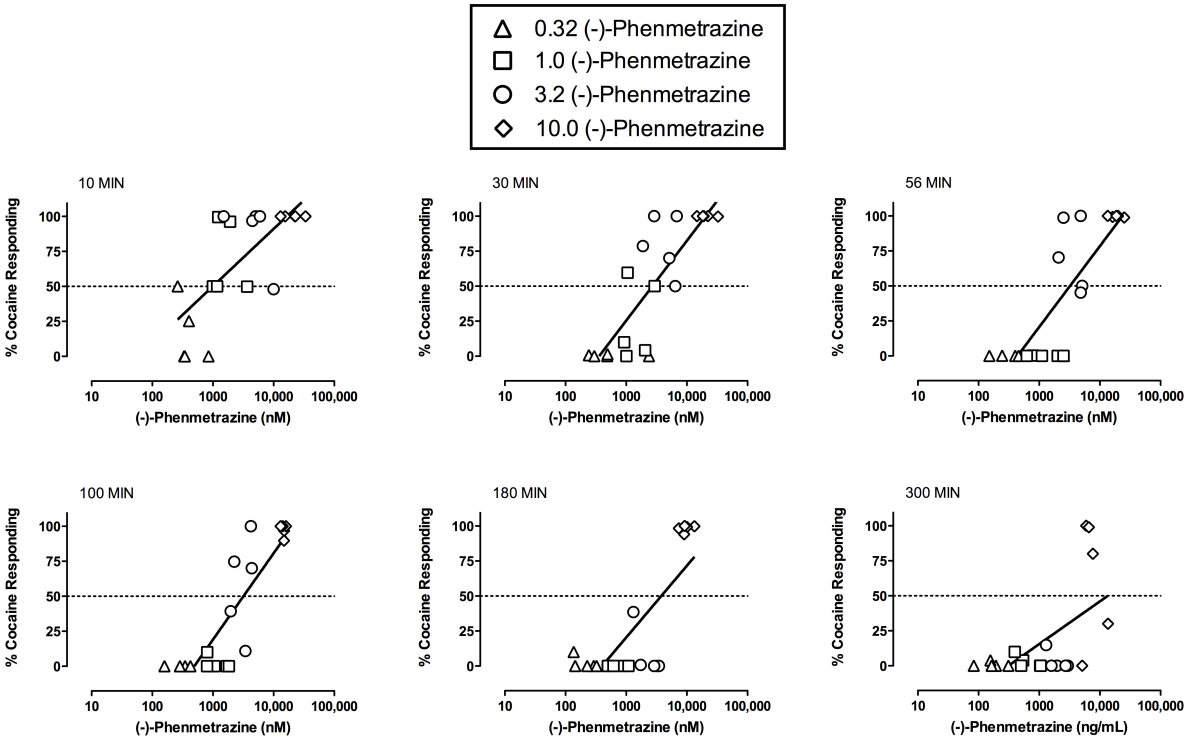
mg/kg significantly increased rates of responding from 30 to 180 min. For (-)-phenmetrazine, there was no significant main effect of dose, time, or a significant interaction. For rates of responding after (+)-phendimetrazine, there was only a significant dose x time interaction ($F_{24,96}$=2.6, $p<0.05$). Post-hoc analysis indicated that 3.2 mg/kg (+)-phendimetrazine significantly increased rates of responding from 100-180 min. For rates of responding after (-)-phendimetrazine administration, there was a significant main effect of dose ($F_{4,16}$=6.9, $p<0.05$), time ($F_{6,24}$=2.7, $p<0.05$), and a significant interaction ($F_{24,96}$=4.2, $p<0.05$). Post-hoc analysis indicated that 10.0 mg/kg significantly increased rates of responding from 56 to 180 min, whereas 32.0 mg/kg significantly decreased rates of responding from 10 to 56 min.



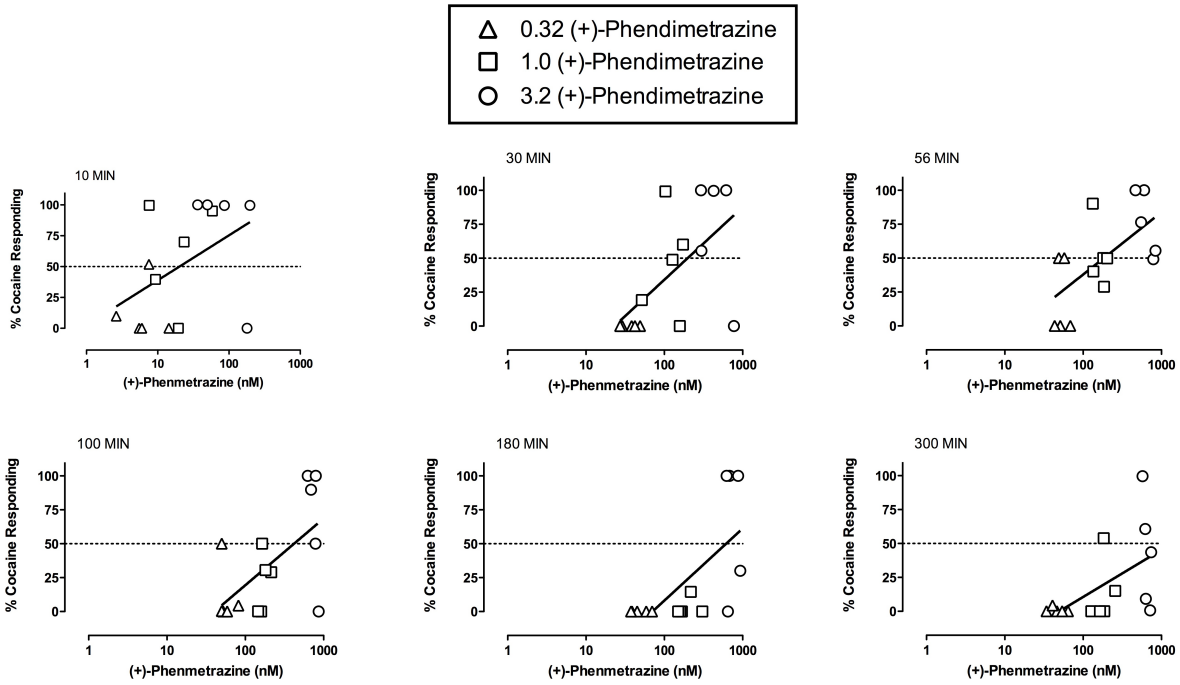
Supplemental Figure 3: Magnitude and time course of cocaine-like discriminative stimulus effects produced by racemic (±) phendimetrazine in rhesus monkeys (n=5). Ordinates: Rates of responding in responses per second. Abscissae: time in min or hr after administration (log scale). Symbols above "S" and "C" represent the group averages ± SEM for all training sessions preceding test sessions when the saline- and cocaine- associated keys were correct, respectively. Filled symbols represent statistical significance ($p < 0.05$) within a given time point compared to experimental sessions when saline was administered (dotted line). All points represent the mean ± SEM of 5 monkeys except where indicated by numbers in parentheses in the top panels. In these cases, responding was eliminated in some monkeys, and the parenthetic number indicates the number of subjects responding at levels sufficient to contribute to the data point. For percent cocaine-appropriate responding, there was a significant main effect of dose ($F_{3,12}$=36.3, $p<0.05$), time ($F_{6,24}$=10.1, $p<0.05$) and a significant interaction ($F_{18,70}$=7.8, $p<0.05$) for %CAR. Post-hoc analysis indicated that the 3.2 mg/kg significantly increased %CAR from 10-180 min, and 10.0 mg/kg significantly increased %CAR from 10-300 min. Full substitution was produced in three monkeys by 3.2 mg/kg, and in five monkeys by 10.0 mg/kg (±)-phendimetrazine. For rates of responding after (±)-phendimetrazine, there was also a significant main effect of dose ($F_{3,12}$=3.5, $p<0.05$), time ($F_{6,24}$=3.7, $p<0.05$), and a significant interaction ($F_{18,72}$=5.8, $p<0.05$). Post-hoc analysis indicated that 3.2 mg/kg significantly increased rates of responding compared to saline from 30 to 100 min, and 10.0 mg/kg significantly increased rates of responding only at 560 min.



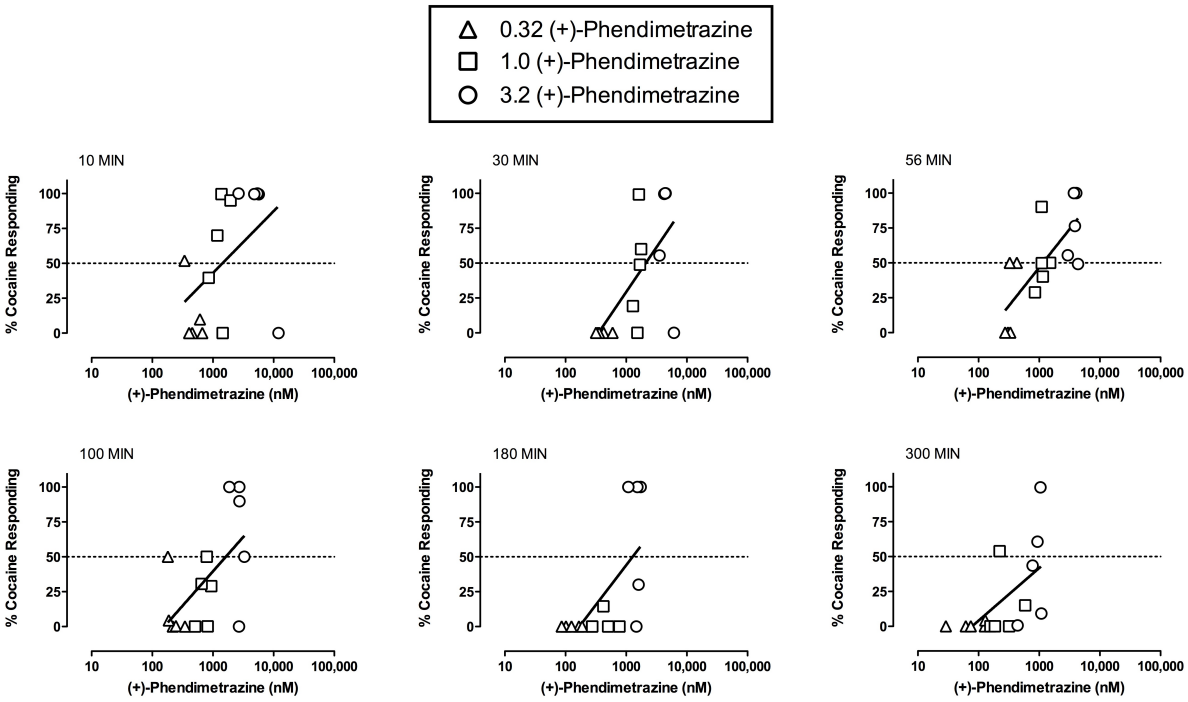
Supplemental Figure 4: Plasma levels (nM) of phenmetrazine and phendimetrazine as a function of time after administration of (-)-phenmetrazine (0.32 – 10.0 mg/kg), (-)-phendimetrazine (1.0 – 10.0 mg/kg) in rhesus monkeys (n=5). Ordinates: plasma levels in nM (log scale). Abscissae: time, in min, after drug administration (linear scale). For (-)-phenmetrazine, two-way repeated-measures ANOVA demonstrated a significant main effect of dose ($F_{3,12}$=191.5, $p<0.05$) and time ($F_{5,20}$=18.8, $p<0.05$). Post-hoc analysis indicated each (-)-phenmetrazine dose produced phenmetrazine plasma levels that were significantly different from each other at each time point. Furthermore, post-hoc analysis indicated that plasma (-)-phenmetrazine levels peaked between 10 – 30 min and then decreased over time such that plasma levels were significantly higher at 10 and 30 min vs. 300 min. For (-)-phendimetrazine, two-way repeated-measures ANOVA on plasma phendimetrazine levels demonstrated a significant main effect of dose ($F_{2,8}$=10.4, $p<0.05$) and time ($F_{5,20}$=15.5, $p<0.05$). Post-hoc analysis indicated that 10.0 mg/kg produced significantly higher plasma phendimetrazine levels compared to both 1.0 and 3.2 mg/kg at each time point. Furthermore, post-hoc analysis indicated that plasma (-)-phendimetrazine levels peaked between 10 – 30 min and then decreased over time such that plasma levels were significantly higher at 10 and 30 min vs. 300 min. For (-)-phenmetrazine plasma levels after (-)-phendimetrazine administration, two-way repeated-measures ANOVA demonstrated a significant main effect of dose ($F_{2,8}$=12.5, $p<0.05$) and time ($F_{5,20}$=77.7, $p<0.05$). Post-hoc analysis indicated that 10.0 mg/kg phendimetrazine produced significantly higher plasma (-)-phenmetrazine levels compared to both 1.0 and 3.2 mg/kg at each time point. Furthermore, post-hoc analysis indicated that plasma (-)-phenmetrazine levels were lowest at 10 min after (-)-phendimetrazine and were significantly increased from 30 – 300 min.



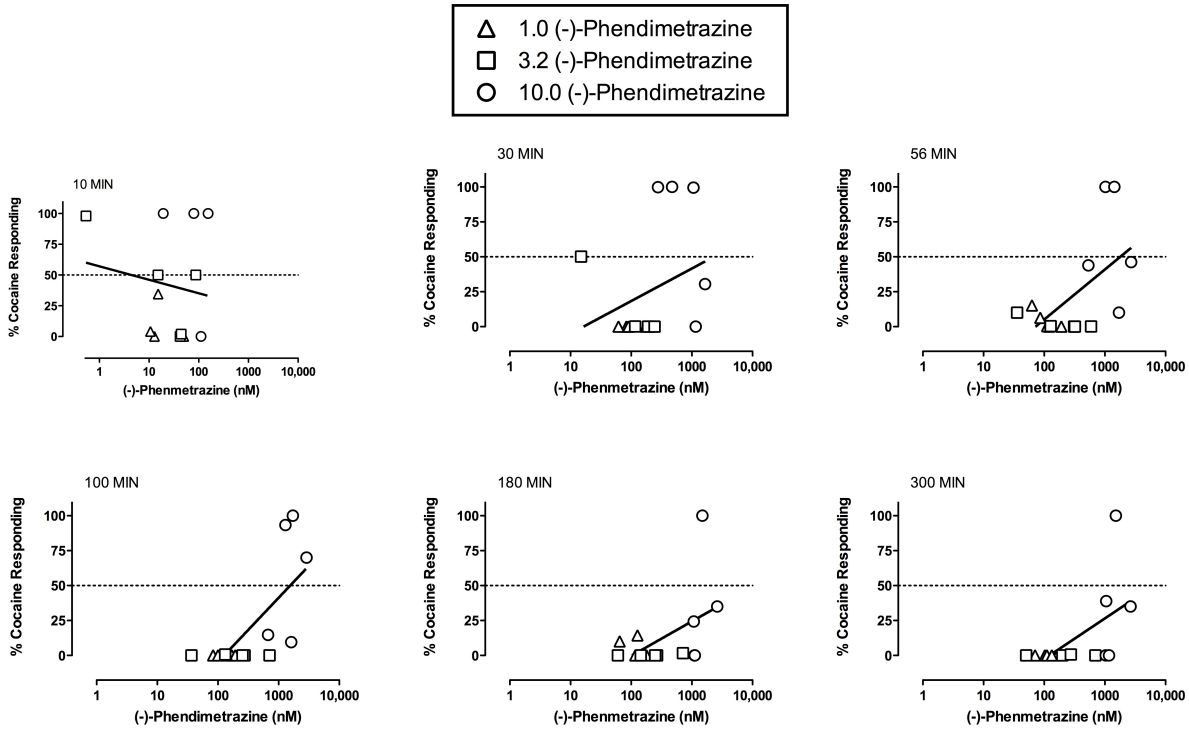
Supplemental Figure 5: Correlation between plasma (-)-phenmetrazine levels and percent cocaine-appropriate responding as a function of time after (-)-phenmetrazine administration in rhesus monkeys (n=5). Ordinates: percent cocaine-appropriate responding. Abscissae: plasma (-)-phenmetrazine levels in nM (log scale). Different symbols represent different doses of (-)-phenmetrazine in individual monkeys. In the presence of a significant correlation, the effective concentration, in nM, that produced 50% cocaine-appropriate responding (EC50) value was calculated using linear regression and the results of this analysis are shown in Supplemental Table 2.



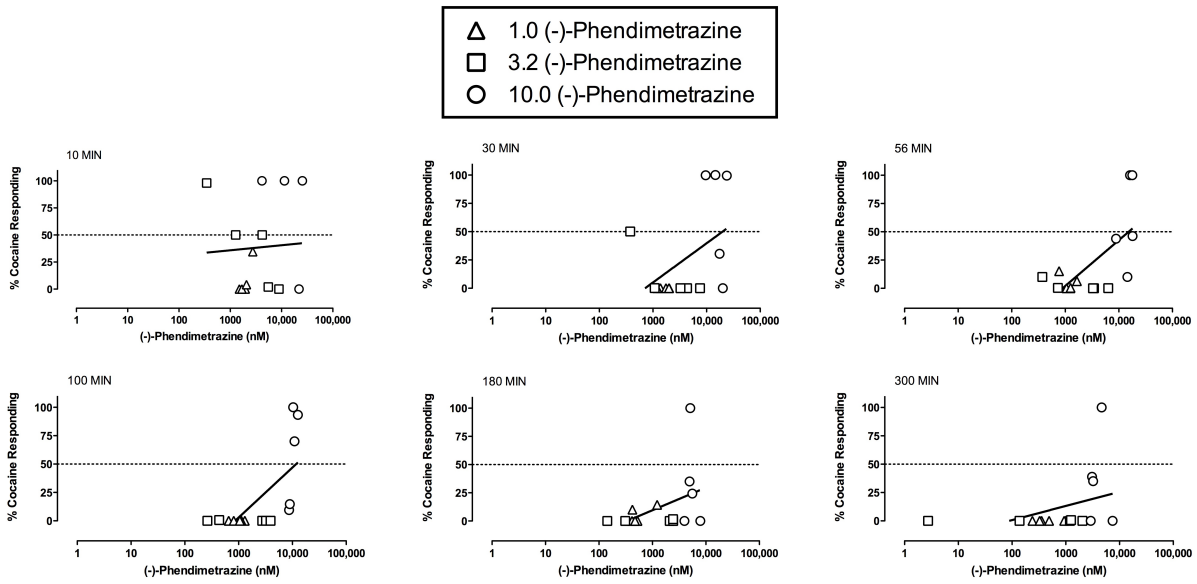
Supplemental Figure 6: Correlation between plasma (+)-phenmetrazine levels and percent cocaine-appropriate responding as a function of time after (+)-phendimetrazine administration in rhesus monkeys (n=5). Abscissae: plasma (+)-phenmetrazine levels in nM (log scale). Other details as in Supplemental Figure 5, except the results of this analysis are shown in Supplemental Table 3.



Supplemental Figure 7: Correlation between plasma (+)-phendimetrazine levels and percent cocaine-appropriate responding as a function of time after (+)-phendimetrazine administration in rhesus monkeys (n=5). Abscissae: plasma (+)-phendimetrazine levels in nM (log scale). Other details as in Supplemental Figure 5, except the results of this analysis are shown in Supplemental Table 3.



Supplemental Figure 8: Correlation between plasma (-)-phenmetrazine levels and percent cocaine-appropriate responding as a function of time after (-)-phendimetrazine administration in rhesus monkeys (n=5). Abscissae: plasma (-)-phenmetrazine levels in nM (log scale). Other details as in Supplemental Figure 5, except the results of this analysis are shown in Supplemental Table 3.



Supplemental Figure 9: Correlation between plasma (-)-phendimetrazine levels and percent cocaine-appropriate responding as a function of time after (-)-phendimetrazine administration in rhesus monkeys (n=5). Abscissae: plasma (-)-phendimetrazine levels in nM (log scale). Other details as in Supplemental Figure 5, except the results of this analysis are shown in Supplemental Table 3.