Oral Administration of Methylphenidate Blocks the Effect of Cocaine on Uptake at the *Drosophila* Dopamine Transporter

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Supporting Information

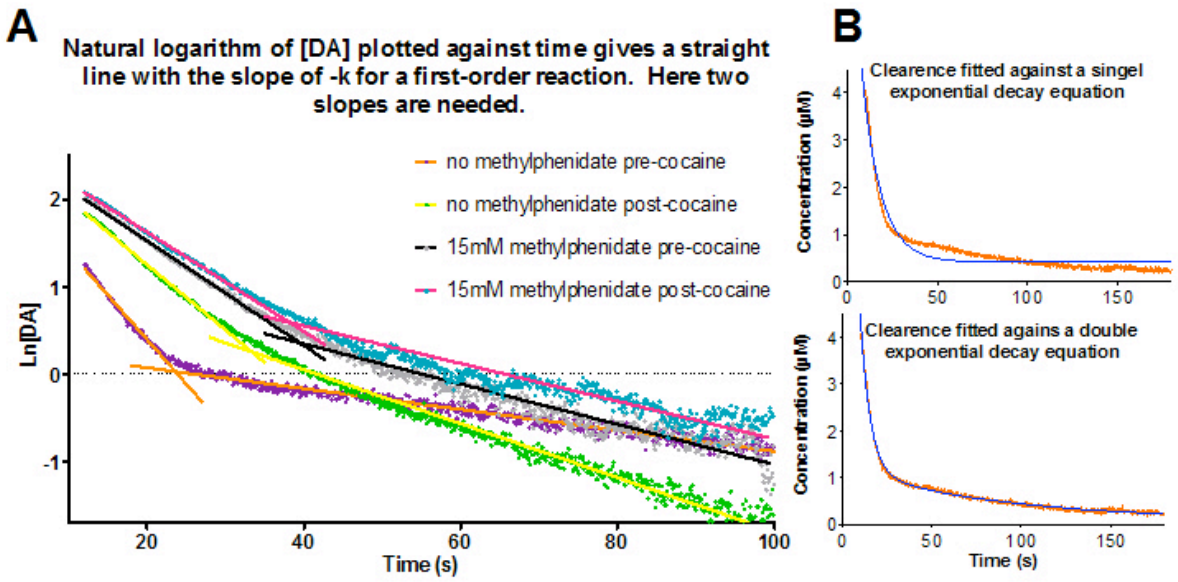

Figure 1. The rate law for a reaction of first order with respect to reactant A is r = -d[A]/dt = k[A] where k is the first-order rate constant and t time. By integrating the first-order rate law as $\ln[A] = -kt + \ln[A]_0$ and plotting ln[A] against time, if the reaction is of first order, a straight line with the slope –k is obtained. The clearance of dopamine from two flies, with methylphenidate feeding (blue and gray) and without (green and purple) methylphenidate feeding, before and after cocaine was plotted as the natural logarithm against time. As can be seen from **(A)** the reaction can be modeled by two first order equations having two straight lines on each trace with the slope $-k_{fast}$ for the first part of the curve and $-k_{slow}$ for the second part of the curve. For r2 values for the lines see Table 1 below. The integrated first-order rate law can be written as an exponential decay equation that can be fitted to the clearance. In **(B)** top part, the clearance has been fitted to a single exponential decay and in the bottom part to a double exponential fit. As can be seen the double exponential gives a better fit.

Table 1. The specific details for trendlines in Figure 1A.

Sample	Trendline interval	r2-value
0 mM pre-cocaine	12-23 seconds	99.0
	23-100 seconds	96.2
0 mM post-cocaine	12-30 seconds	99.7
	30-100 seconds	98.1
15 mM pre-cocaine	12-35 seconds	99.8
	35-100 seconds	93.7
15 mM post-cocaine	12-30 seconds	99.7
	30-100 seconds	92.6

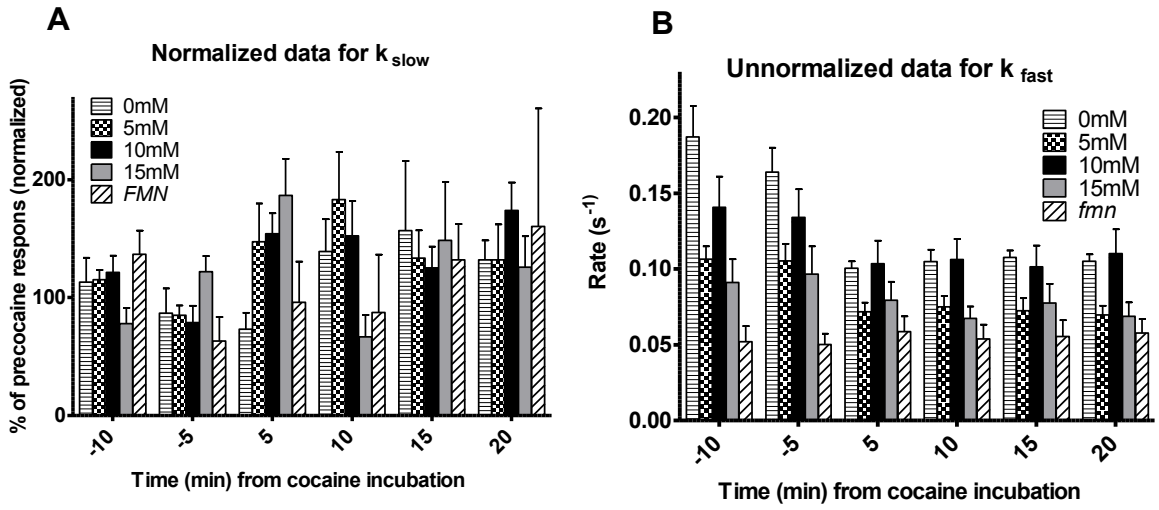

Figure 2. Oral methylphenidate treatment effect on cocaine uptake inhibition after fitting the clearance of exogenously applied 1.0 mM dopamine to a double exponential decay equation. Fitting the clearance rate for dopamine to the equation gave the rate constant k_{slow} and k_{fast} (s^{-1}). **(A)** Normalized k_{slow} shows no correlation between the different concentrations of methylphenidate feeding. **(B)** Unnormalized data for k_{fast}.

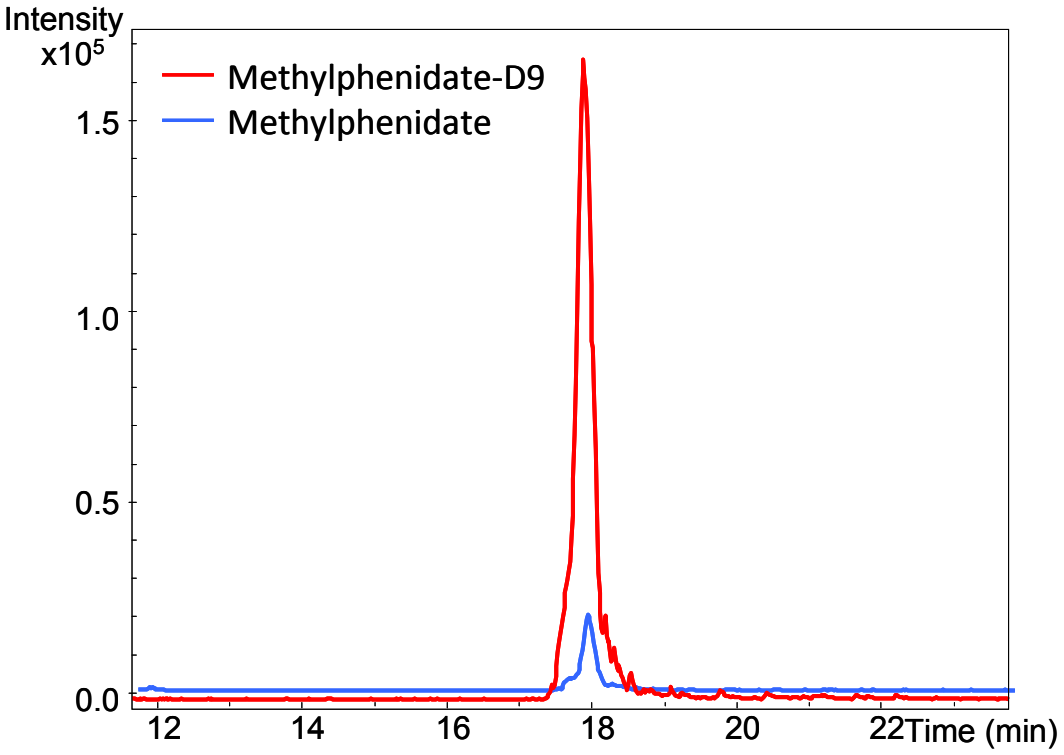

Figure 3. CE-MS electropherogram of methylphenidate and internal standard methylphenidate-d9 detected in 30 fly heads fed with 10 µM. Methylphenidate-d9 was spiked before the sample preparation; final concentration was 6.6 ng/µL. Separation was performed in 50 mM citric acid at voltage 20 kV, positive mode. MS conditions: ESI voltage 4500 V, positive mode, sheath liquid isopropanol-water (70:30, v/v) with flow rate 3 µL/mL. Selected ions for methylphenidate and methylphenidate-d9 are m/z 234 and m/z 243, respectively.

Table 2: Specific data for Figure 3. In vivo concentrations of methylphenidate in the fly head and standard error of mean (SEM) of n different independent samples. The flies were fed with different doses of methylphenidate from 0 to 15 mM.

Feeding concentration of methylphenidate (mM)	Averaged in vivo concentration of methylphenidate (µM)	SEM	n
0	0	0	0
5	80.1	5.1	12
10	205.6	29.1	12
15	241.6	24.9	10

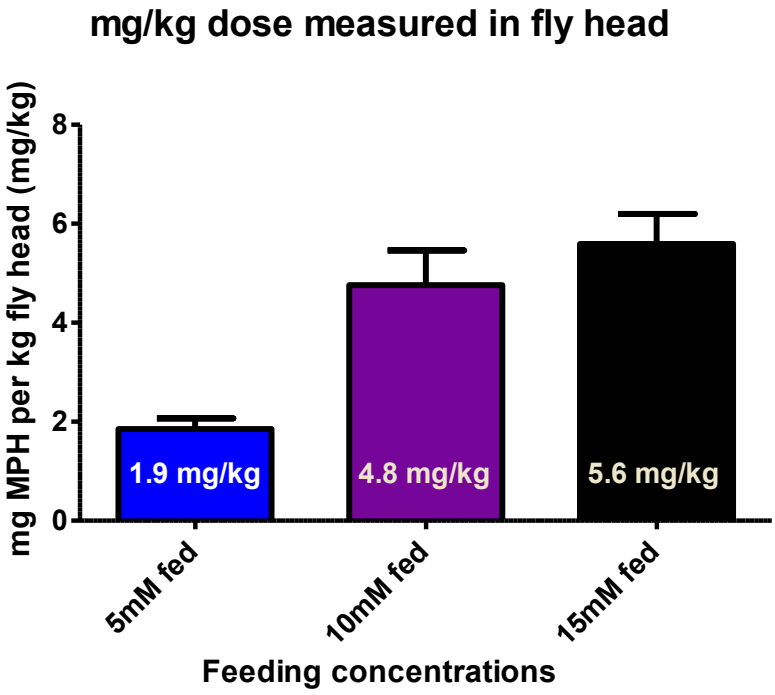

Figure 4. Measured *in vivo* methylphenidate doses per fly head in mg/kg for different feeding concentrations of methylphenidate. The therapeutic doses of oral methylphenidate, up to 1 mg/kg, induced significant DAT blockade of 50 to 75% in the human brain. The pharmacokinetics and bioavailability of methylphenidate has been examined in animal studies, in rats with doses of 1 to 10 mg/kg.