An MCPEM approach to understanding inter-animal and inter-treatment changes with in-vivo striatal dopamine clearance in rats.

Robert R. Bies[1,2], Serge Guzy[3], Joshua Sokoloski[4], Laura Drewencki[4], Amy K. Wagner[4]

[1]University of Pittsburgh Department of Pharmaceutical Sciences, [2]Western Psychiatric Institute and Clinic Department of Psychiatry, Pittsburgh, PA USA [3]Xoma Inc. Albany CA USA, [4]Department of Physical Medicine and Rehabilitation, University of Pittsburgh, Pittsburgh, PA USA.

ABSTRACT

Objective: To capture the between animal, between occasion, and between differences in striatal dopamine (DA) clearance kinetics using a nonlinear mixed effects approach.

Methods: Striatal DA concentration versus time profiles were measured using fast scan cyclic voltammetry (FSCV) and a stimulated release paradigm in naïve rats and rats subjected to experimental traumatic brain injury (TBI). Three stimulus responses (occasions), reported as DA concentration versus time, were collected both before and after treatment with either saline (vehicle) or 5mg/kg methylphenidate (MPH). Pdx-MCPEM was used to evaluate the DA clearance profiles for each stimulus response using a Vmax and Km (saturable) model parameterization with 200 EM evaluations and between 10,000 and 30,000 vectors in the likelihood space for each assessment.

Results: The system was best described using both between animal and inter-occasion variability to obtain the Vmax and Km values reflective of DA clearance after evoked release (>300 objective function value point reduction).

Conclusions: A between-animal and within animal (between occasion) structure best described differences across this experimental design using two induced conditions and three different treatments. Specific deviations from this structure will guide the implementation of the structure of covariate relationships with treatment and injury status.

PURPOSE

To capture between animal and between occasion variations in striatal dopamine clearance kinetics using a nonlinear mixed effects approach under multiple different treatment conditions.

INTRODUCTION

•Traumatic brain injury (TBI) affects over 50,0000 people a year in the U.S. and is the cause of 60% of the traumatic deaths.

•Disruption of the dopamine system thought to be an important factor in resulting neurobehavioral deficits from TBI.

•MPH is beneficial in treating some patients with TBI and has been shown in our model of experimental TBI to promote cognitive recovery.

•DA release and clearance can be measured in synaptic terminals using FSCV, with oxidation of evoked DA, resulting in a current reading that can be converted to a DA concentration (micromolar).

•Previous work suggests that increases in evoked overflow DA with MPH administration are less after TBI than that observed in naïve rats.

METHODS

•Adult male Sprague-Dawley rats (Hilltop Laboratories) were used (Naïve N=7, TBI N=7).

•All experiments involving animals were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee.

•Under isoflurane anesthesia, injured animals were subjected to the controlled cortical impact (CCI) injury model of TBI using the device described by Dixon et.al. (1991).

•At 14 days post-injury, animals were re-anesthetized with chloral hydrate and underwent the FSCV procedure in conjunction with electrical stimulation of the medial forebrain bundle (MFB) to assess striatal DA neurotransmission.

•Electrical stimulation of the MFB consisted of pulses of 2 ms width at a frequency of 60 Hz for 2 s every 10 minutes with a 280 µA biphasic constant current pulse.

•Striatal DA clearance assessed with FSCV in multiple individuals with multiple treatments (none, saline, MPH) under naïve and TBI conditions (producing 6 different conditions).

•A basic Michaelis-Menten structure was used to probe the resulting DA vs. time profiles under these different conditions. (Vmax*C/Km/C).

•Between animal and between occasion variability was tested across individuals on the Vmax and Km parameters.

•10,000-30,000 vectors used with 200 EM iterations to determine the solution for Vmax and Km and provide the objective function (-2LL).

•Datasets were separated by treatments for individual condition assessment.

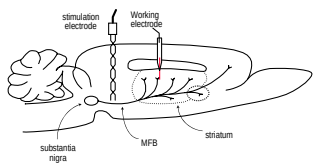


Figure 1: Illustration of experimental procedure

RESULTS

•Modeling all of the experimental conditions produced stable results with respect to the Monte Carlo oscillation in objective function as well as the final parameter values determined.

•Table 1 summarizes the n for each of the treatment conditions.

RESULTS

Table 1: Numbers of animals and occasions for each treatment group

Condition	N	# of occasions (maximum)
Naïve no injection	7	4
Naïve saline injection	3	3
Naïve methylphenidate injection	4	3
TBI no injection	7	3
TBI saline injection	3	3
TBI methylphenidate injection	4	3

Table 2:Individual treatment group analysis results

Condition	BSV (obj)	BOV (obj)	Vmax (micromol/s)	BSV Vmax	BOV Vmax	Km (micromolar)	BSV Km	BOV Km	Sigma
Naïve No Injection	-247.93	-291.98*	3.2	0.69	0.06	0.48	0.64	0.07	0.42
Naïve Saline Injection	-18.00*	-22.29	5.03	0.57	n/a	0.69	0.65	n/a	0.56
Naïve MPH Injection	-381.08	-567.10*	5.89	0.02	0.1	2.86	0.45	0.06	0.29
TBI No Injection	51.2	32.01*	5.46	0.65	0.04	0.28	2.11	0.06	0.58
TBI Saline Injection	-135.89*	-141.05	5.18	0.27	n/a	1.16	0.88	n/a	0.41
TBI MPH Injection	99.08*	101.68	6.02	0.65	n/a	0.38	1.6	n/a	0.68

*Denotes a significant change in the -2LL within the treatment condition tested (<0.01 for 2df)

Figure 2: Pred vs DV, Ipred vs DV for Naïve No Injection and Naïve Methylphenidate Injection

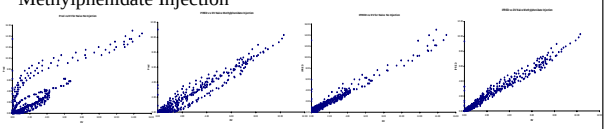


Figure 3: Ipred, Pred and DVversus time by ID for Naïve No Injection and Naïve Methylphenidate Injection.

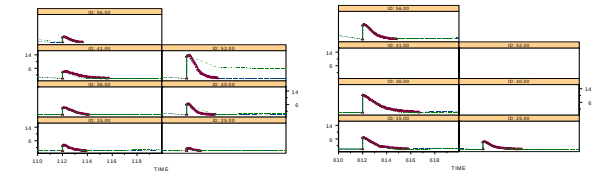


Figure 4: Pred vs DVand Ipred vs DV for TBI No Injection and TBI Methylphenidate Injection

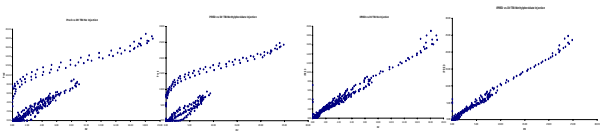
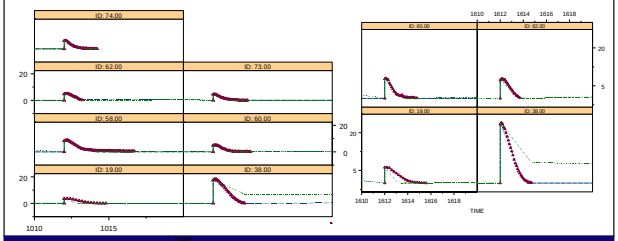


Figure 5: Ipred, Pred, DV vs Time for TBI No Injection and TBI Methylphenidate Injection



SUMMARY

•Most treatment conditions required both a BSV and BOV term to be adequately described here.

•Pred vs DV seems to suggest a mis-specification or variability in the system that is not seen with MPH administration in the naïve animal.

•Km and Vmax values for naïve rats are consistent with reported synaptosomal and in vivo values respectively taken from the literature for DA clearance under before and after treatment with competitive inhibitors of the DA transporter.

•Consistent with previous work, results suggest a TBI specific treatment response to MPH.

This project was supported by NIH Research Grant P41 EB-001975 funded by the National Institute for Biomedical Imaging and Bioengineering and by NIH Research grant K08HD40833 funded by the National Institute of Child Health and Development