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Microdialysis monitoring of methylphenidate in blood and brain correlated with changes in dopamine and rat activity

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Abstract

Methylphenidate (MPD), also called Ritalin, changes the extracellular levels of dopamine (DA) in the brain. This study coupled multiple-site microdialysis sampling with appropriate analytical methods to simultaneously profile the MPD concentration in blood and brain, while monitoring changes in the extracellular level of DA in the striatum of awake and freely moving rats. The animals' activity was also recorded. The maximum concentration of MPD in the blood and brain occurred during the first 20 min of sampling. The maximum DA concentration was reached in the first 20 min and gradually returned to the basal level after 3 h. The activity peak correlated well with the MPD and DA peaks and remained elevated for about 2.5 h. The ability to obtain and correlate data in this manner has the potential to reduce the number of animals required for a given study and to minimize interanimal variation.

Keywords: Methylphenidate; Ritalin; Dopamine; In vivo microdialysis sampling; Locomotor activity

1. Introduction

Methylphenidate (MPD), also called Ritalin, is widely prescribed for attention-deficit disorder and hyperactivity attention-deficit disorder [1,2]. It has been shown to increase the extracellular dopamine (DA) concentration in the striatal region of the rat's brain by blocking DA reuptake [3].

Several recent studies have examined the pharmacodynamics of MPD. Microdialysis sampling in rat brains has been used to monitor the effect of MPD on extracellular DA [4–7]. The influence of MPD on locomotor activity and stereotyped behaviors has been investigated by several groups [8–10]. Kuczenski and colleagues compared the effect of MPD on extracellular DA and stereotypic behavior in rats with that of amphetamine [11]. Aoyama et al. have published several articles on the pharmacokinetics and pharmacodynamics of MPD [3,12,13]. The stereospecific distribution of MPD in rat brain was studied by dissection of brains, harvested immediately following collection of a blood sample at specific times after dosing

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[12]. In another study, the pharmacokinetics of MPD were determined in serial blood samples in one group of rats, while MPD concentration and changes in DA concentration were monitored in alternate microdialysis samples from the striatum of another group. Yet another group of rats was dosed with MPD for the study of locomotor activity [3].

Microdialysis sampling is accomplished by implanting a probe consisting of a hollow fiber dialysis membrane, affixed with inlet and outlet tubing, into the site of interest. As perfusion fluid (termed the perfusate) is pumped through the probe, small molecules diffuse across the membrane and are collected for analysis. This technique makes possible the investigation of biochemical events in the extracellular fluid of virtually any tissue [14–18].

Microdialysis can be used simultaneously in multiple tissues [19–21]. Since microdialysis does not remove fluid from the animal, long-term continuous sampling is possible. Each animal can serve as its own control by sampling before and after some event, such as administration of a dose. As a result, fewer animals are required to obtain pharmacokinetic and pharmacodynamic information.

In this article, we illustrate these advantages by conducting multiple-site microdialysis sampling to simultaneously determine MPD concentration in brain and blood, monitor changes in extracellular DA in the striatum and track lateral motion. This was accomplished by implanting microdialysis probes in the brain and jugular vein. Following the surgery, the rat was placed in an animal containment system which prevents tangling of fluid lines and records direction and duration of the animal's lateral motion. In comparison to previous work in this area, where multiple rats were used, our research was successful in obtaining blood, brain and motion data simultaneously in the same rat from a single dose. This method decreases the number of rats required to collect the same sets of experimental data. Interanimal variation is also reduced because each rat serves as its own control. Our experiments were repeated in five rats, and the results compare favorably to what has been reported in the literature.

2. Methods and materials

2.1. Chemicals

Reagent grade sodium chloride, potassium chloride, calcium chloride, sodium dihydrogen phosphate, sodium citrate, diethylamine hydrochloride, 1-octanesulfonic acid, disodium EDTA, and potassium phosphate were purchased from Sigma (St. Louis, MO) and used as received. HPLC grade acetonitrile and tetrahydrofuran, and reagent grade phosphoric acid were used for mobile phase preparation. All solutions were made using distilled, deionized water (NANOpure, Barnstead, Boston, MA).

Stock solutions (1 mM) of the following neurotransmitters (from Sigma) were prepared in 0.1 M perchloric acid with 0.1% cysteine for stabilization: norepinephrine (NE), epinephrine (EPI), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-hydroxytyramine (DA), 5-hydroxyindole-3-acetic acid (5-HIAA), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), 3-methoxytyramine (3-MT), and 5-hydroxytryptamine (5-HT). Stock solutions were stored at −20 °C. Standards were prepared by dilution with Ringer's solution, as needed.

Ringer's solution (155 mM Na^+, 5.5 mM K^+, 2.3 mM Ca^{2+}, 165.1 mM Cl^-) was prepared and used for vascular probe perfusion. Sterile lactated Ringer's solution (130 mM Na^+, 4.0 mM K^+, 3.0 mM Ca^{2+}, 110 mM Cl^-, 28 mM lactate, pH 6.2) was purchased from McGraw (Irvine, CA) and used for brain probe perfusion.

MPD (M-2892) was obtained from Sigma, and a 1 mg/ml stock was prepared in NANOpure water. For a standard curve, further dilutions were made in Ringer's solution. Stock and standard solutions were stored at 4 °C.

2.2. Analytical method for MPD

Standard solutions and jugular dialysates were analyzed using reverse-phase liquid chromatography with UV absorption detection. The system consisted of a PM-80 pump, LC-26 vacuum degasser, UV–VIS 116A detector set at 210 nm

(two ranges, 0.1 and 0.001 AUFS), DA-5 CHROMGRAPH Interface, and a Pollen 8® online injector (BAS, West Lafayette, IN). Data was collected and analyzed using CHROMGRAPH software (BAS).

A C-8, 5 μ (Brownlee SPHERI-5 RP-8), 4 × 100 cm column preceded by a C-8, 7 μm, 1.5 × 3.2 cm guard column was used. In-line filters (2 μm) were placed before and after the injection valve. The mobile phase consisted of 27% acetonitrile and 73% potassium phosphate buffer, 50 mM, pH 3.5. The mobile phase was filtered through a 0.22 μm nylon filter and continuously vacuum degassed during use. The flow rate was 1.5 ml/min. Column, guard column, and mobile phase were all at room temperature. The retention time of MPD was 2.2 min.

The Pollen 8®, 10 port valve injector was fitted with matching 55 μl loops and a single injection port. Jugular dialysates were analyzed online every 10 min with a perfusion flow rate of 1 μl/min. Standards (range 0.25–100 μg/ml) were analyzed online line using the at the same flow rate as the in vivo samples. For brain dialysates, 5 μl manual injections were compared to manual injections the same volume of MPD standards.

2.3. Analytical method for neurotransmitters

Neurotransmitter standards and brain dialysates were analyzed using a reverse-phase liquid chromatography with electrochemical detection. The solvent delivery system consisted of a dual piston pump, pulse dampener and a flow splitter utilizing a 500 psi flow restrictor followed by 75 cm PEEK tubing (ID 0.0025 in). The pump flow rate was set to deliver 70 μl/min to the column.

A Unijet C-18, ODS, 5 μ, 1 × 150 mm column preceded by a Unijet guard column (C-18 ODS, 3 μm, 1 × 14 mm) was used to achieve separation of the neurotransmitters. The mobile phase, optimized for resolution of DA, consisted of 12 mM sodium dihydrogen phosphate, 29 mM sodium citrate, 10 mM diethylamine hydrochloride, 2 mM 1-octanesulfonic acid, and 2 μM disodium EDTA. The pH was adjusted to 3.1 with phosphoric acid [22]. After filtration and vacuum degassing, 64.9 ml of acetonitrile and 8.1 ml tetrahydrofuran were added to each liter of the aqueous portion of the mobile phase.

The electrochemical flow cell was a Unijet cell with a glassy carbon working electrode (3 mm) with an incorporated thermodynamic reference electrode combined with an LC-4C amperometric detector operated at 650 mV, 0.15 Hz filter and 20 nA range (BAS). The incorporated electrode has a 100 mV offset relative to Ag–AgCl [23]. Thus, the effective working potential was 750 mV versus Ag–AgCl. Data collection and analysis was by CHROMGRAPH software (BAS).

The injection volume was 10 μl in all cases and made manually. An equimolar mixture (200 nM each) of the eight neurotransmitters was used for daily optimization checks and maintained on ice between injections. A standard curve was generated from DA standards (range 1–100 nM) prepared fresh the day of the assay, and DA concentrations in the dialysate samples were calculated from the linear fit.

Table 1
Time table of animal experimental procedures

Day	Experimental procedure(s)
1	Implantation of intracerebral guide cannula Recovery in home cage
5	Implantation of jugular probe and femoral dosing cannula Placement of brain probe into guide cannula Recovery in RATURN® system
6	Pre-dose determination of basal DA concentration
7	Placebo dose followed by monitoring DA for 4 h MPD (20 mg/kg) i.v. dose—DA and MPD monitored for 4 h
8	In vivo delivery of MPD to each probe

2.4. Surgical procedures

All animal procedures were performed according to animal use statements approved by the University of Kansas Institutional Animal Care and Use Committee. Each animal experiment was conducted following the sequence summarized in Table 1. Male Sprague–Dawley rats weighing

350–375 g were lightly pre-anesthetized by halothane inhalation followed by intramuscular injection of a ketamine–xylazine mixture (90 and 10 mg/kg, respectively). An intracerebral cannula (BAS) was implanted, following the manufacturer's instructions, into the striatum using the stereotaxic coordinates of Paxinos and Watson [24]. The animal was allowed to recover for 3 days before a second surgery to insert a vascular probe (10 mm active membrane, BAS) and a femoral vein dosing cannula was implanted. A brain probe (4 mm active membrane, BAS) was inserted into the intracerebral guide. The dosing cannula and probe conduits were externalized at the back of the neck, and the animal was tethered in the RATURN® awake animal containment system.

The animal was allowed to recover overnight before basal concentrations of neurotransmitters were measured, at which time a minimum of four consistent sample values were obtained. The following day, a 4 h placebo period was immediately followed by a 4 h dose period. To check probe viability, an in vivo delivery of 5 μg/ml MPD occurred 24 h later. Both jugular and brain probes were analyzed for MPD online, and compared to standards analyzed in the same fashion. Activity data were monitored during each phase using the RATURN® software and analyzed for counts and duration.

2.5. Sampling protocols

Ringer's solution (155 mM Na^+, 5.5 mM K^+, 2.3 mM Ca^{2+}, 165.1 mM Cl^-) was prepared and used for vascular probe perfusion. Sterile lactated Ringer's solution (130 mM Na^+, 4.0 mM K^+, 3.0 mM Ca^{2+}, 110 mM Cl^-, 28 mM lactate, pH 6.2) was purchased from McGraw and used for brain probe perfusion. The perfusion flow rate for both probes was 1 μl/min during the experimental collection periods.

The animal was allowed to recover overnight before basal concentrations of neurotransmitters were measured. Brain dialysates were collected every 20 min into capped vials in the refrigerated (4 °C) Honeycomb fraction collector (BAS®). Samples were removed as collected and were immediately injected (10 μl) for the determination of DA. Sampling continued until a minimum of four samples showed consistent values. The average of these samples was used as the basal concentration to calculate the relative DA concentration during the administration of the MPD dose.

The following day, a placebo dose of 2 ml sterile saline was administered via the femoral dosing cannula. Brain dialysis samples were collected and 10 μl was injected for the determination of DA. After 4 h, a 20 mg/kg dose of MPD dissolved in 1 ml of sterile saline, followed by an additional 1 ml of sterile saline, was administered via the femoral cannula. At the end of 4 h, a 20 mg/kg dose of MPD dissolved in 1 ml of sterile saline followed by an additional 1 ml sterile saline was administered via the femoral. Brain dialysates were collected as described above with the remaining portion of the samples frozen for later determination of MPD. Dialysate from the vascular probe was injected online at 10 min intervals (except Rat 1, 20 min intervals) to determine the MPD concentration. The frozen brain dialysates were thawed, vortexed and injected (5 μl) for the determination of MPD.

To check probe viability, an in vivo delivery of 5 μg/ml (1 μl/min) was conducted on the day following the dose. Dialysates from both vascular and brain probes were injected online to determine the concentration of MPD remaining in the samples. The 5 μg/ml MPD standard was also injected online, and the extraction efficiency by delivery (EE_{del}) for each probe was calculated according to the following equation:

$$EE_{del}(\%) = \frac{[MPD]_{in} - [MPD]_{out}}{[MPD]_{in}} \times 100\%,$$

where $[MPD]_{in}$ is the concentration in the perfusate entering the probe and $[MPD]_{out}$ is the concentration in the collected dialysis sample.

Activity data were collected throughout the experiment using the RATURN® system software. The software monitors horizontal motion of the rat by means of right and left optical sensors. The software records left and right counts (triggers of the respective optical sensors), duration of each count, total counts, total duration, maximum duration and average duration. For the purposes of this study, the total counts and total duration

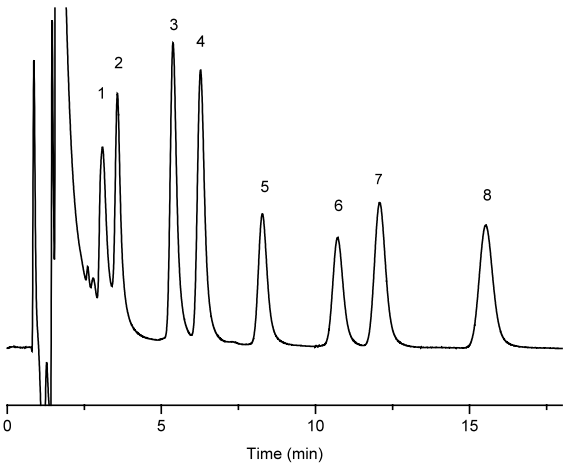

Fig. 1. Chromatogram of equimolar (200 nM each) mixture of eight neurotransmitters: (1) NE, (2) EPI, (3) DOPAC, (4) DA, (5) 5-HIAA, (6) HVA, (7) 3-MT, (8) 5-HT.

were blocked in 20 min intervals relative to the time of the administration of the MPD dose.

3. Results and discussion

3.1. Analytical parameters

MPD concentrations were linear in the range of 1–100 μg/ml (0.371–37.1 μM). The limit of quantitation in dialysates was 1 μg/ml (371 nM) for the 5 μl injected. The relative standard deviation for manual injections of the 1 μg/ml standard was less than 2% ($n = 10$).

A chromatogram of the eight neurotransmitter standard mixtures (200 nM each) is shown in Fig. 1. Elution order was confirmed by the injections of individual standards. DA concentrations were

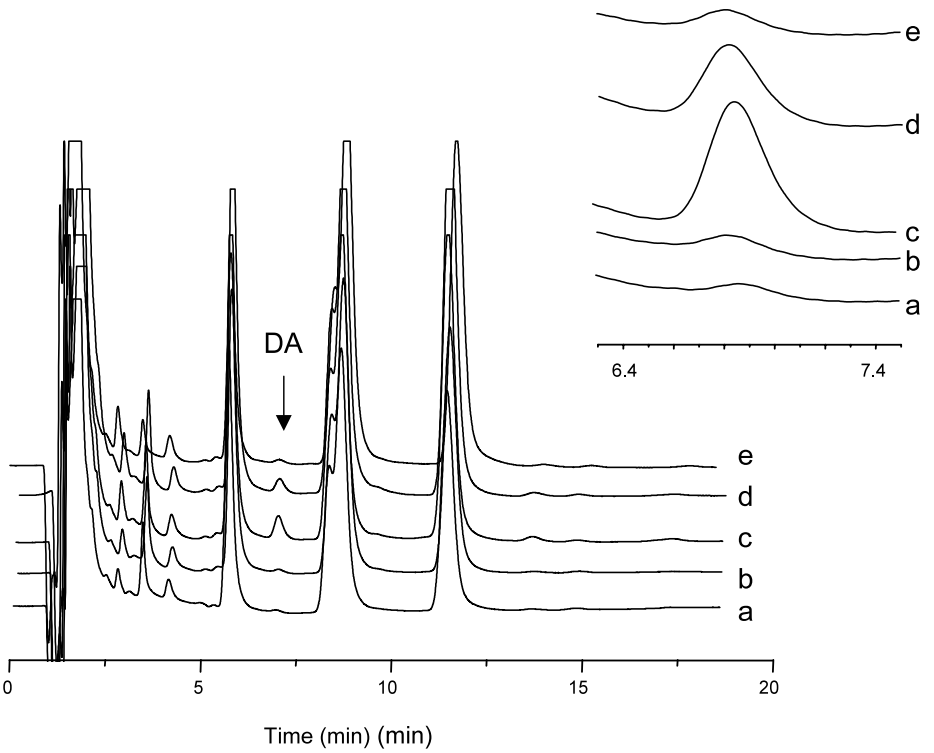

Fig. 2. Series of chromatograms of brain dialysates before and after a 20 mg/kg i.v. dose of MPD. The inset shows an enlargement of the DA peak region. Samples were collected at the following times relative to dose administration: (a) pre-dose, (b) 0–20 min, (c) 20–40 min, (d) 40–60 min, (e) 180–200 min.

Table 2
DA concentrations in brain microdialysis samples

[DA] nM	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
Basal[a]	5.11 ± 0.01	2.05 ± 0.82	N.Q.[b]	2.04 ± 0.02	6.04 ± 1.92
Placebo[c]	4.99 ± 1.06	2.45 ± 0.18	3.98 ± 0.024	N.Q.[b]	5.64 ± 0.72
Peak	28.64	38.74	23.75	9.18	23.24

Values are not corrected for microdialysis probe extraction efficiency.

[a] Average ± standard deviation of at least three samples per animal, determined on the day prior to dosing.

[b] DA was detectable but could not be reliably quantitated. Relative DA for Rat 3 is based on the average [DA] during 4 h following the placebo.

[c] Average ± standard deviation of at least 10 samples per animal, determined during 4 h after the administration of placebo.

linear in the range of 1–250 nM with a limit of detection ($S/N = 3$) of 1 nM in standards (10 μl injected). The limit of quantitation in brain dialysates was 2 nM (10 μl injected). Shown in Fig. 2 is a series of chromatograms from brain dialysates from a rat given a 20 mg/kg intravenous (i.v.) dose of MPD at $t = 0$. The inset is an enlargement of the DA peak region.

3.2. DA

In vivo extraction efficiency was not determined for DA. Concentrations of DA reported here are those determined in the brain dialysis samples. The average basal DA concentrations from five rats are shown in Table 2. Direct comparison to other reports in the literature is difficult because of the differences in experimental procedures (probe type, dimensions and implantation site, perfusion rate, and sample injection volume), perfusate composition, manner of reporting values and details presented. Values in the reports were converted to a common basis when sufficient information was given. The concentrations reported here are in good agreement with the average basal DA concentrations reported by several other groups studying MPD in rats [4,5,11,25]. The average uncorrected basal DA concentrations reported by these groups ranged from 1.9 to 6.5 nM, essentially the same range that we observed in this study (2.04–6.04 nM). The DA concentrations determined at 20 min intervals for 4 h following the administration of the placebo are also shown in Table 2. As can be seen, the placebo values agree well with the basal values and were stable over 4 h, indicating little or no reaction to the i.v. placebo administration.

The peak DA concentrations measured in the dialysates following the administration of a 20 mg/kg i.v. dose of MPD are shown in Table 2. Only Rat 4 shows a peak DA concentration that differs widely from the others. The percent DA (relative to average basal concentration) versus time from dose profiles of four animals is shown in Fig. 3. The basic pattern of these four profiles is not greatly different from one another. The maximum relative values of DA ranged from 380 to 600%. The maximum was reached within 40 min after the dose. DA gradually returned to basal concentration within 4 h. These results are similar in magnitude and pattern to the findings reported in the literature [3,7]. The increase in DA following the dose can be attributed to MPD since the administration of the placebo had no effect.

The inset of Fig. 3 includes all five animals. As can be seen, a much higher maximum relative DA was observed (about 1400%) in this animal (Rat 2) and the maximum was observed later, about 60 min after the dose. As with the other animals, relative DA returned to basal concentration within 4 h after the dose. There is no apparent explanation for this unusually high relative DA, since the other values from this animal (MPD concentrations, activity data) are very similar to those of the other animals.

3.3. MPD

In vivo extraction efficiency by delivery for MPD was determined for both probes in each

animal. The EE_{del} values for vascular and brain microdialysis probes in five animals are shown in Table 3. The concentrations of MPD in dialysates were corrected using the appropriate EE_{del}. While this correction provides only a first approximation of the concentration of MPD surrounding the probe, it does normalize the data for differences among probes of the same type.

The concentration time profiles for MPD in jugular and brain dialysate samples from five rats are shown in Fig. 4. The points are plotted at the initial time point of the sample collection time and values have been adjusted using the EE_{del} determined in vivo for the probe. The maximum jugular MPD concentrations observed in the dialysate samples for these rats average 37.7 ± 6.6 μM ($n = 5$). Based on the reports in the literature, this is a reasonable result. Wargin et al. reported maximum blood concentration of about 5000 ng/ml (18.5 μM) in rats following 10 mg/kg i.v. dose of MPD [26]. In this study, the average of the maximum MPD concentrations

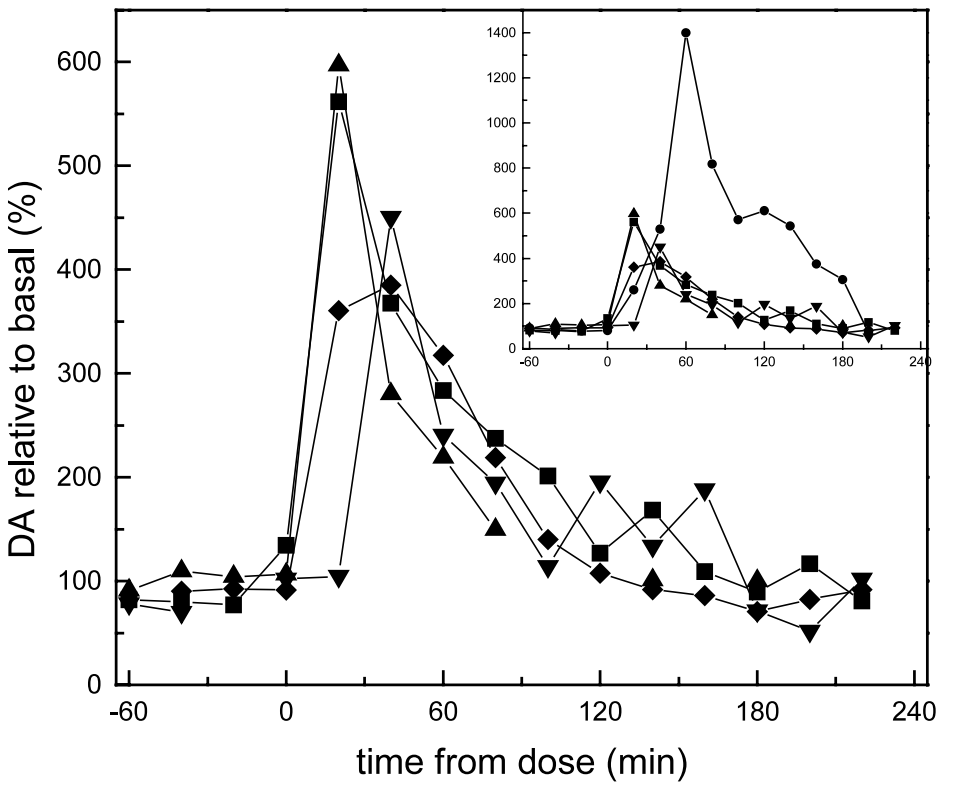

Fig. 3. Percent DA relative to basal level from four rats following 20 mg/kg i.v. MPD at $t = 0$. Inset shows percent DA for five rats: ■, Rat 1; ●, Rat 2; ▲, Rat 3; ▼, Rat 4; ◆, Rat 5. Percent DA for Rat 3 is based on average [DA] following placebo.

Table 3
In vivo EE_{del} for MPD

	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
IV-10 probe in jugular vein	42 ± 6	63 ± 6	58 ± 4	53 ± 3	56 ± 4
BR-4 probe in striatum	25 ± 9	27 ± 5	26 ± 11	18 ± 1	26 ± 4

Values are average ± standard deviation (by propagation of error) based on $n \geq 4$ samples.

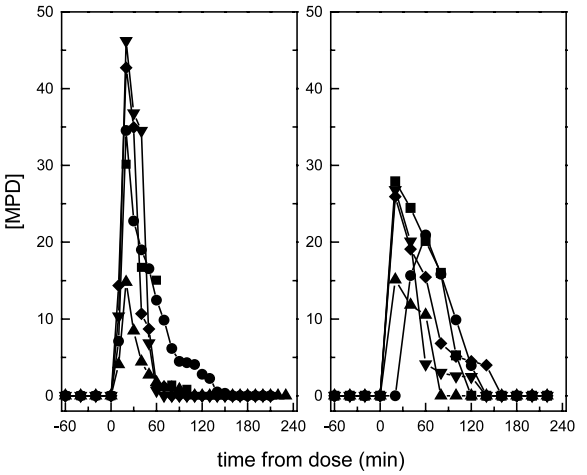

Fig. 4. Concentration–time profile of MPD in blood (left) and striatum (right) of five rats. Concentrations represent the unbound drug and have been corrected for probe EE_{del} determined in vivo. ■, Rat 1; ●, Rat 2; ▲, Rat 3; ▼, Rat 4; ◆, Rat 5.

Table 4
Pharmacokinetic parameters

C_0 (extrapolated)	0.0122 ± 0.0059 mg ml^{-1}
AUC ($t = 0$ to last observed)	0.4737 ± 0.1918 mg min ml^{-1}
K	0.0255 ± 0.0045 min^{-1}
$t_{1/2}$	25.7 ± 4.7 min

Values are average ± standard deviation for $n = 5$ rats.

observed in the brain dialysates was 23.3 ± 5.3 μM ($n = 5$).

Pharmacokinetic parameters for MPD were calculated using standard formulas for a non-compartmental model. For the jugular dialysate data, the concentration at $t = 0$ was back extrapolated from a linear regression slope using the 20–80 min samples. Area under the concentration (unbound fraction) time curve (AUC) from $t = 0$ to last time point observed, K, and t_2, was calculated from the EE-corrected jugular dialysate values. The average values obtained are reported in Table 4. Considering the dose administered here was 20 mg/kg, AUC reported here for a 20 mg/kg dose compares favorably with the combined AUCs for both enantiomers reported by Aoyama et al. [12] for a 2 mg/kg racemic dose, assuming that AUC should be proportional to the dose.

3.4. Activity monitoring

Table 5 shows total counts, total duration, and maximum single motion in 4 h blocks for each animal. The blocks are defined relative to the administration of the MPD dose. Rats are nocturnal animals and thus, more counts and total duration, although not necessarily longer maximum duration for any one movement, are to be expected during the night. The −8 to −4 h block corresponds to very early morning hours, the end of the rat's most active period. The placebo was always administered in the morning (approximately 8 AM) and the MPD dose given near noon. As can be seen from Table 5, the rats were much less active during the placebo (−4 to 0 h) than during the −8 to −4 h block. Increased activity, in terms of both counts and total duration, was observed during 4 h immediately following the dose administration. From 4 to 8 h after the dose, activity declined to levels about the same as those observed from −8 to −4 h. The rat's normal transition from inactivity to activity would begin during this final time block.

The maximum duration of a single continuous motion during each block is also shown in Table 5. It is worth noting that the maximum duration of a single continuous motion is 4.0 s or less, except during the dose. The duration of single continuous motions during the dose exceeded 1 min in some cases. Only Rat 5 did not show longer continuous motions during the dose than during other blocks. While this rat shows very low activity, the pattern of increased counts and total duration in response to the dose was still observed.

The activity data were also summed by 20 min intervals around the dose to provide temporal resolution blocks corresponding to the MPD and DA time points. Fig. 5 shows counts/20 min (left graph) and duration in s/20 min (right graph) for each rat versus time from dose. The greatest activity, both in counts and duration, generally occurred during the first 90 min after the dose. While Rat 5 shows very low activity, the pattern of increased counts and total duration in response to the dose was still observed.

Table 5
Horizontal motion in 4 h blocks relative to the administration of MPD dose

	Rat 1	Rat 2	Rat 3	Rat 4
Total counts				
−8 to −4 h (baseline activity)	23	54	136	78
−4 to 0 h (during placebo)	10	9	4	9
0–4 h from MPD dose	273	284	198	431
4–8 h after MPD dose	20	60	3	80
Total duration (s)				
−8 to −4 h (baseline activity)	26.5	50.0	98.5	52.0
−4 to 0 h (during placebo)	9.0	9.5	2.0	6.5
0–4 h from MPD dose	562.5	750.5	296.5	693.5
4–8 h after MPD dose	17.5	44	2.5	62.5
Maximum single movement (s)				
−8 to −4 h (baseline activity)	4.0	2.5	1.5	2.5
−4 to 0 h (during placebo)	1.5	1.5	0.5	2.0
0–4 h from MPD dose	8.3	58.5	89.5	39.0
4–8 h after MPD dose	2.0	2.0	1.0	2.0

Horizontal motion for Rat 5 (data not shown) was minimal, possibly due to mechanical failure of air conditioning.

It should be noted that the activity monitored was only horizontal locomotion. Rearing, sniffing and other stereotype behaviors were not recorded in this study.

3.5. Correlation of MPD, DA and activity data

The relative DA versus MPD concentration in blood indicates increasing pharmacological response with time (counterclockwise hysteresis) in all five animals. This suggests a delay in equilibration between MPD in plasma and the striatum [27]. This is in agreement with most of the relative DA versus time profiles shown in Fig. 3. The maximum relative DA occurred later in three animals than the maximum MPD concentration in the jugular, although the MPD concentration peaked in the brain at the same time as in the jugular in four of the five animals.

All the data from a single animal experiment are overlaid in Fig. 6. As can be seen, for this animal the maximum MPD concentrations, duration of activity and relative increase in DA occur simultaneously. The plot illustrates the quantity and quality of information that can be obtained from a single animal using simultaneous microdialysis sampling.

4. Conclusions

Multiple-site microdialysis sampling can be conducted simultaneously with activity monitoring in a single animal, thus allowing the animal to serve as its own control. The increase in relative concentration of DA in the striatum is clearly due to the presence of MPD, as a similar increase did

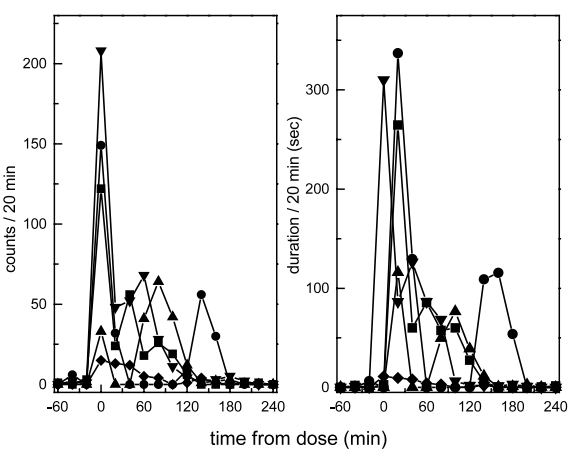

Fig. 5. Profile of horizontal motion relative to time of dose administration. Left graph in counts/20 min and right graph shows total duration of motion in s/20 min. ■, Rat 1; ●, Rat 2; ▲, Rat 3; ▼, Rat 4; ◆, Rat 5.

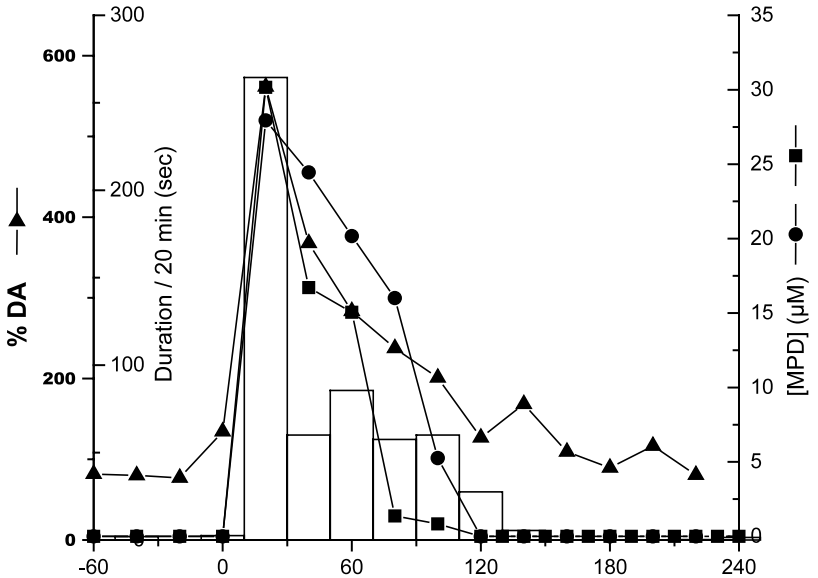

Fig. 6. Overlay of all data from Rat 1. Bars are total duration (s) per 20 min—scale inside left y-axis. Percent DA, ▲—scale outside left y-axis, bold. Concentrations of MPD in dialysates—■ for jugular and ● for brain—on right y-axis.

not occur with the administration of the placebo (vehicle only). Furthermore, the increase in DA correlated well with the peak concentration of MPD the brain. Likewise, increases in activity, both in number of horizontal movements and duration of motion, occurred in response to the dose since the placebo did not generate increased activity. The ability to obtain and correlate data in this manner has the potential to greatly reduce the number of animals needed for a given study.

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