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(+)-Methamphetamine-induced spontaneous behavior in rats depends on route of (+)METH administration

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Abstract

These studies examined the role of (+)-methamphetamine ((+)METH) administration route on spontaneous behavioral activity vs. time relationships, and pharmacokinetic mechanisms for differences in effects. Male Sprague–Dawley rats (n=6 per administration route) received saline and three doses (0.3, 1.0 and 3.0 mg/kg) of (+)METH in a mixed-sequence design by intravenous (iv), subcutaneous (sc) or intraperitoneal (ip) administration. Locomotion and stereotypy were quantified by video-tracking analysis. The effects of (+)METH on spontaneous behavior were dose- and route-dependent. In particular, total locomotor activity was greatest following 3.0 mg/kg intraperitoneally (P<0.05) and stereotypy ratings were greatest following 3.0 mg/kg subcutaneously (P<0.05). In addition, the duration of locomotor effects was greatest after 3.0 mg/kg subcutaneously (P<0.05). Serum pharmacokinetic parameters were determined in separate rats given 3.0 mg/kg by subcutaneous and intraperitoneal administration (n=4 per administration route). The (+)METH elimination half-life was not different between the routes, but the (+)METH AUC was greater (P<0.05), and the (+)METH and (+)-amphetamine (AMP) maximum concentrations occurred later following subcutaneous than after intraperitoneal dosing (P<0.05), increasing and prolonging drug exposure. In conclusion, the overall pattern of (+)METH effects on locomotor activity depend on dose and the route of administration, which affects serum concentration and the time course of behavioral effects.

Keywords: Methamphetamine; Amphetamine; Administration route; Locomotor activity; Stereotypy; Pharmacokinetics; Dose–response relationships; Rat

1. Introduction

Rapid administration of (+)-methamphetamine ((+)METH) or (+)-amphetamine ((+)AMP) by the intravenous (iv) route of administration is popular with persons who abuse these drugs (Hall et al., 1996; Peters et al., 1997; Pennell et al., 1999). Reported percentages of users who inject (+)METH intravenously range from just over 10% (Domier et al., 2000) to 67% (Darke et al., 1994). High percentages of (+)METH abusers also utilize other routes of administration in addition to iv injection, including nasal insufflation or snorting (91%) and smoking (28%; Domier et al., 2000). These routes of (+)METH administration are popular because they are associated with rapid input of the drug, which leads to immediate and intensely pleasurable effects compared with routes of administration that result in slower onset, such as oral ingestion (Cho, 1990).

While many illicit users begin taking (+)METH by routes of administration that have slower onset, as many as 40% make the transition to injection, and subsequently rarely revert to slower routes. Indeed, once started, only 9% of users report a transition away from iv injection (Darke et al., 1994). It is difficult to identify all of the reasons a person begins using iv (+)METH (Crofts et al., 1996); however, a

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major reason they continue using this method of self-administration is because of the intense rush of effects that occurs. Indeed, the rapid onset of effects experienced when stimulants are taken by rapid routes of administration is reported to be an important determinant of the reinforcing properties of these drugs. For instance, the euphorigenic (Fischman and Schuster, 1982) and reinforcing (Balster and Schuster, 1973) effects of iv cocaine are greater after rapid infusion of a given dose. In addition, Cook et al. (1993) suggest the rapid onset of subjective effects that occur when (+)METH is smoked is a major reinforcement for subsequent use.

Because of the high risks associated with administration of (+)METH to humans, animal models are needed that mimic clinically important aspects of (+)METH use. Rats are probably the most commonly used species to study (+)METH pharmacology. Nevertheless, species differences in pharmacokinetics and responses necessitate careful consideration of the experimental design to maximize mimicry of human use patterns (e.g., doses and administration routes). Although use of iv dosing gives the best control of dosing patterns in animal models, establishing and maintaining iv access in rats is difficult. As a result, many investigators have used routes of administration such as subcutaneous (sc) or intraperitoneal (ip) in animal studies of (+)METH pharmacology. However, these two routes of administration have never been reported to be used by humans.

The purpose of this study was to test the hypothesis that route of (+)METH administration determines the dose–response relationships and time course of selected central nervous system effects for (+)METH. We systematically quantified the spontaneous locomotor activity and stereotypy responses to (+)METH using a range of doses (0.3–3.0 mg/kg) given by three routes of administration (iv, sc and ip). The range included lower doses (0.3 and 1.0 mg/kg) that cause primarily locomotor activity and a higher dose (3.0 mg/kg) that causes primarily stereotypy (Grilly and Loveland, 2001). Finally, we determined serum pharmacokinetic parameters following a 3.0 mg/kg (+)METH dose given by sc and ip routes of administration to assess pharmacokinetic mechanisms for these differences in effect among the routes. We compared these pharmacokinetic parameters to results recently obtained in this laboratory of 3.0 mg/kg iv (+)METH doses (Milesi-Halle et al. submitted for publication).

2. Methods

2.1. Drugs and chemicals

(+)Methamphetamine and [^{3}H](+)methamphetamine were obtained from the National Institute on Drug Abuse (Rockville, MD). The tritiated (+)METH ((+)-[2′,6′-^{3}H] methamphetamine, 23.5 Ci/mmol) was synthesized by the Research Triangle Institute (Research Triangle Park, NC) with the radiolabel at the 2 and 6 positions of the aromatic ring, which are metabolically stable sites. All drugs were dissolved in 0.9% sterile saline, which also served as the vehicle control; all injections were made in a volume of 1.0 ml/kg. Concentrations were expressed as the freebase form. All other reagents used in these studies were obtained from Fisher Scientific (Springfield, NJ), unless otherwise specified.

2.2. Animals

Male Sprague–Dawley rats (250–300 g) implanted with a jugular venous catheter were purchased with the catheter in place (Hilltop Laboratory Animals, Scottsdale, PA) and used for all experiments. Catheters (Dow Corning silastic tubing, 0.020 in. i.d. by 0.037 in. o.d.) were placed via a midline ventral incision into the right jugular vein under ketamine/xylazine anesthesia. The catheter was then tunneled subcutaneously to an exit point in the mid-scapular area. After flushing with heparinized saline, the distal end of the catheter was buried in the subcutaneous tissue for transport of the animal to our facility. Three days after arrival from the vendor, the rats were lightly anesthetized with halothane and the catheter was externalized and flushed with heparinized saline (25 U per flush). Subsequently, the catheter was flushed every other day to ensure patency (Valentine and Owens, 1996).

Rats were housed in an animal care facility with a 12-h light and dark cycle (7:00 AM–7:00 PM) and an ambient temperature of 22 °C. Experiments were performed during the light phase of the cycle and were begun at 8:00 AM on each study day. All experiments and the surgical procedure were performed in accordance with the Guide for the Care and Use of Laboratory Animals, as promulgated and adopted by the National Institutes of Health. All animal protocols were approved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee (Little Rock, AR).

2.3. Protocol for behavioral activity studies

Behavioral activity (locomotion and stereotypy) was recorded with a videoimaging system described in previous studies (e.g., Riviere et al., 1999; Hardin et al., 2002). One week before the experiments, rats were placed in the study chambers (60×45×40 cm, l×w×h) daily for 4–6 h to allow habituation. On each experimental day, the rats were placed in the chambers 1 h before the administration of saline or (+)METH to acclimatize them to their surroundings. Baseline levels of behavior were determined during the 30 min prior to drug or saline administration (−30 to 0 min).

Rats (n=6 per group) received saline and (+)METH by one route of administration (iv, sc or ip). Saline (1.0 ml/kg) or (+)METH (0.3, 1.0 or 3.0 mg/kg in the same volume) was administered to each rat on separate occasions in a mixed-

sequence, repeated-measures design. The rats were temporarily (~2 min) removed from the study chamber and gently restrained in the lap of an investigator for each injection to facilitate safe drug administration. To maintain consistency among the groups, each animal was kept out of the chamber for 2 min, regardless of the time required for injection. In the iv group, injections were administered via the jugular catheter over a 15-s period beginning at time t=0 (t_0). The injection was followed by an equal volume of saline to flush the catheter. In the sc group, injections were given at time t_0 under the skin of the back just distal to the scapulae. In the ip group, saline or (+)METH was given at time t_0 into the right lower quadrant of the peritoneal cavity. Experiments were conducted on Monday and Thursday, or Tuesday and Friday to allow subjects 72 to 96 h between treatments. We have not observed a loss of habituation to these chambers with this dosing regimen previously.

2.4. Analysis of locomotor activity and stereotyped behavior

Analysis of videotaped locomotor behavior was conducted as described in our previous studies of (+)METH- and phencyclidine-induced locomotor activity (Riviere et al., 1999; Hardin et al., 2002) using computer digital imaging and video-tracking software (EthoVision, Noldus Information Technology, Sterling, VA). The locomotor activity parameters used in this study were the total distance each rat traveled, and the number of times each animal stood on its hind legs (rearing events).

The distance traveled (in cm) and numbers of rearing events were quantified in consecutive 2-min time intervals. These values were summed over the entire duration (exclusive of the first 4 min after injection to control for handling artifact) of each experiment to yield the total distance traveled and the total number of rearing events. The locomotor activity duration of action was defined as the time from (+)METH administration (t_0) until the behaviors returned to baseline. The return to baseline behavior was defined as the time at which locomotor activity was less than the mean +1 S.D. of the baseline (i.e., −30 to 0 min) for two consecutive 2-min intervals (Hardin et al., 1998).

Stereotyped behavior was evaluated using a rating scale devised in our laboratory based on several previous reports (e.g., Ellinwood and Balster, 1974; Sturgeon et al., 1979; Segal and Kuczenski, 1994). With this scale, the behavior of each animal was evaluated during a 30-s sampling period for the presence or absence of various stereotyped behaviors including oral stereotypies (chewing, licking and biting), focused sniffing, and repetitive head movements. Observations were made from the same videotapes used to quantify locomotor activity. The scale is purposely qualitative because the camera angle (directly above the study chambers) made it difficult to quantify different types of stereotyped behavior (e.g., chewing or biting). A single camera angle made it possible, however, to facilitate simultaneous data collection on four rats with our equipment.

Stereotypy Rating Scale

- 0=Sleeping or not moving.
- 1=Time engaged in locomotion greatly exceeds time engaged in stereotyped behaviors such that there is very little to no stereotypy observed (e.g., horizontal locomotion for 25 to 30 s of the 30-s sample).
- 2=Time engaged in locomotion was less than that of a rating of 1, but exceeds time engaged in stereotypy.
- 3=Time engaged in stereotypy was less than that of a rating of 4, but exceeds time engaged in locomotion.
- 4=Time engaged in stereotypy greatly exceeds time engaged in locomotion, such that there is very little to no horizontal movement observed (e.g., stereotypy for 25 to 30 s of the 30-s sample).

The videotapes recorded for the locomotor activity studies in which rats received 3.0 mg/kg (+)METH by all three routes were randomized. A single blinded individual rated each of the 18 (three routes of administration by six rats per route) tapes for stereotyped behavior occurring during 30-s samples. The rater was well trained in these measures. He had helped to define the scale, tested it, refined it and retested prior to performing these studies. Ratings were performed at two time points before (+)METH administration (−11 and −1 min) and at 10-min intervals from 10 to 420 min after (+)METH administration.

The time course of stereotyped behavior was analyzed with two parameters. The onset of stereotyped behavior was designated as the beginning of the first 10-min interval in which the rating was 2 or greater. The duration of stereotyped behavior was defined as the time from onset of stereotyped behavior until the first of two consecutive 10-min intervals in which the rating was 1 or 0. Analysis of only the 3.0 mg/kg dose groups for stereotyped behavior was performed for two reasons. Previous reports have indicated that these behaviors only occur at doses of above approximately 2.0 mg/kg (Segal and Kuczenski, 1987). Doses below about 1.0 mg/kg sc are primarily associated with locomotor activity (Grilly and Loveland, 2001). In addition, preliminary observations of the videotapes at doses of 0.3 and 1.0 mg/kg showed no measurable stereotyped behavior with our methods.

2.5. Pharmacokinetic experiments

The pharmacokinetics of (+)METH were determined following administration of 3.0 mg/kg (+)METH by the sc and ip routes of administration in separate groups of rats (n=4/administration route). The pharmacokinetic parameters of (+)METH following a 3.0 mg/kg iv dose in male rats were recently studied by our group (Milesi-Halle et al., submitted for publication) and reported elsewhere so these experiments were not repeated. The methods for the studies

with iv administration were very similar to the current studies.

To perform the current studies, rats were placed in metabolism cages (Nalgene Supply, Rochester, NY), with free access to water. Food was withheld for 12 h prior to (+)METH dosing. Each animal received (+)METH (3.0 mg/kg at t_0) using the same administration methods as described for the locomotor activity studies. For the ip administration group, each (+)METH dose was co-administered with 670 μCi/kg of [^{3}H](+)METH (≈200 μCi/rat) as a tracer. For the sc administration group, the [^{3}H]-labeled (+)METH was omitted. Blood samples (100–300 μl) were collected from the jugular venous catheters before the (+)METH injection and at 1 or 2, 5, 10, 20, 30, 60, 120, 180, 240, 300 (ip only) and 360 min after the injection. The volume of blood collected during the entire experiment was <10% of the total blood volume. After collection of each blood sample, an equal volume of sterile saline was administered to maintain a constant intravascular volume. Hematocrit determinations were performed at selected time points to ensure a stable red blood cell concentration. The blood was allowed to clot, and the serum was collected after centrifugation. The serum samples were frozen at −80 °C until analysis.

2.6. Analysis of (+)METH and (+)AMP concentrations in serum samples

The serum concentrations of (+)METH and (+)AMP in the ip administration group were determined as previously reported using a solid phase extraction procedure for (+)METH and (+)AMP from serum (Byrnes-Blake et al., 2003), followed by high performance liquid chromatography separation of (+)METH and (+)AMP. Liquid scintillation spectrometry was then used to quantify the (+)METH

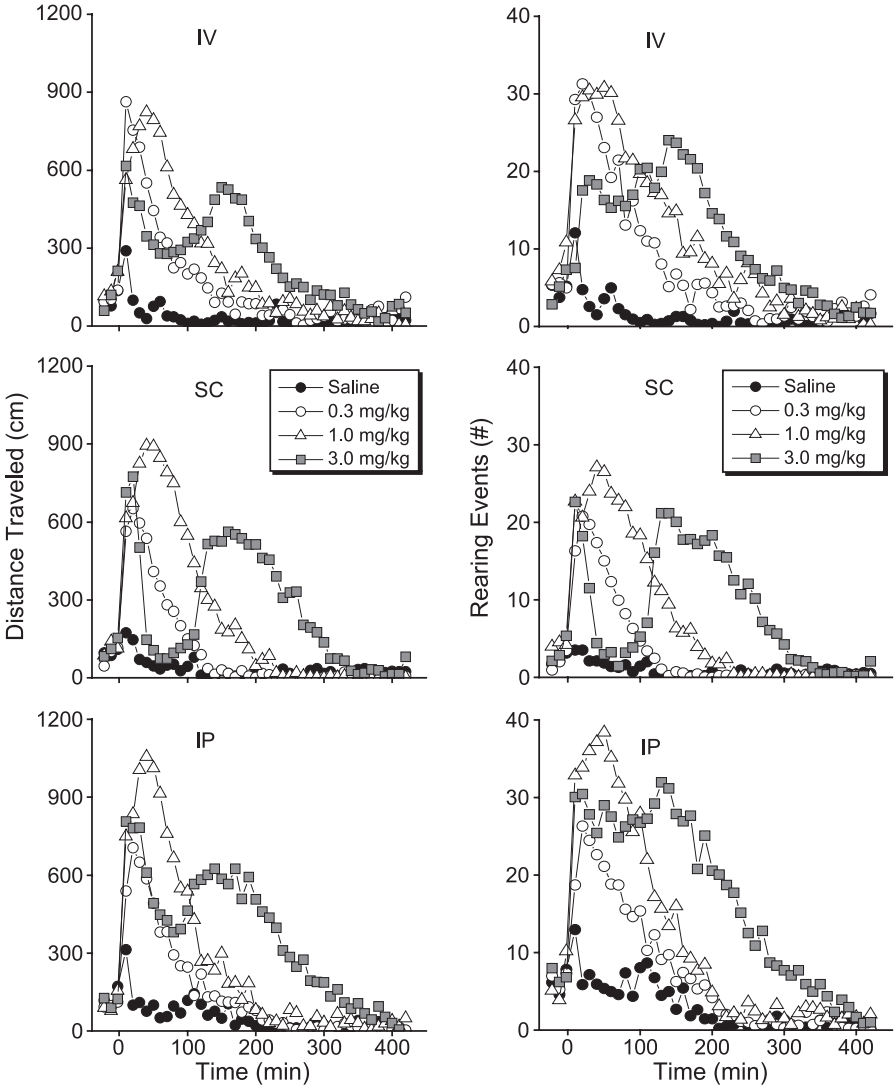

Fig. 1. Average distance traveled (left panel) and number of rearing events (right panel) over a 420-min (7-h) period following saline and three increasing (+)METH doses in rats (n=6 per administration route). Error bars are not shown to avoid cluttering the graphs. Although behavioral analysis was performed using data averaged over 2-min time increment, the data are plotted as 10-min averages, to more clearly show the trends in the effect vs. time curves. Saline or (+)METH was injected at time t=0 in all rats. Note the apparent bimodal effects of the 3.0 mg/kg (+)METH dose following all three routes of administration.

and (+)AMP concentrations in each fraction, respectively (Laurenzana et al., 2003). The analytical recovery of (+)METH and (+)AMP through the complete procedure (extraction and HPLC analysis) was ~80–90%.

The serum concentrations of (+)METH and (+)AMP in the sc administration group were determined using an LC/MS/MS technique developed recently in this laboratory (Hendrickson et al., 2004). Briefly, (+)METH and (+)AMP were extracted from serum using solid-phase-extraction followed by a 5-min isocratic reversed-phase separation and MS/MS quantitation. Limits-of-quantitation for (+)METH and (+)AMP were 0.3 ng/ml using 100 μl of serum. The accuracy of the method was within ±10% of the actual values ranging 0.3–1000 ng/ml. The within-day and between-day precision was <10% (CV). Cross-validation using the radioimmunoassay method (Byrnes-Blake et al., 2003) showed excellent agreement.

2.7. Pharmacokinetic data analysis

Model-independent pharmacokinetic analysis of (+)METH and (+)AMP concentration–time data collected after sc and ip (+)METH administration was performed using a non-compartmental analysis routine (WinNonlin v. 3.0, Pharsight, Mountain View, CA). The best-fit lines were determined visually. The (+)METH and (+)AMP elimination half-life ($t_{1/2\lambda z}$) for each rat were determined individually from the slope (λz) of the linear terminal portion of the log concentration–time curve. The bioavailability (F) of (+)METH was then estimated using the equation $F=(AUC_{ev}/AUC_{iv})(Dose_{ev}/Dose_{iv})$ where AUC_{ev} is the average AUC_0^∞ from the current studies of sc or ip (+)METH administration, and AUC_{iv} is the average AUC_0^∞ after 3.0 mg/kg iv (+)METH administration (Milesi-Halle et al., submitted for publication). The elimination clearance (Cl_T) for each animal was then estimated using the equation $Cl_T=F\times Dose/AUC_0^\infty$. The V_{dss} for each animal was then calculated using the equation $V_{dss}=Cl_T/\lambda z$ (Rowland and Tozer, 1995).

The maximum serum (+)METH and (+)AMP concentrations (C_{max}-(+)METH and C_{max}-(+)AMP, respectively) and the time to maximum serum (+)METH and (+)AMP concentrations (T_{max}-(+)METH and T_{max}-(+)AMP, respectively) were determined from the individual concentration–time curves histories and presented as the mean±S.D. Finally, the molar ratio of AMP to METH AUC was obtained by dividing the nmol min/ml AUC values for (+)AMP by the nmol min/ml AUC values for (+)METH.

2.8. Statistics

All values are expressed as mean±S.D. Statistical comparisons of locomotor activity within each group (i.e., within each route of administration) were performed using repeated-measures ANOVA, with post hoc analysis for all pairs using the Student–Newman–Keuls test. Comparison of locomotor activity at each dose with different route of administration was performed using a one-way ANOVA with post hoc analysis for all pairs using the Student–Newman–Keuls test. Stereotypy ratings were compared between administration route using the Kruskal–Wallis one-way ANOVA for ranks with post hoc analysis for all pairs using the Student–Newman–Keuls test. The pharmacoki-

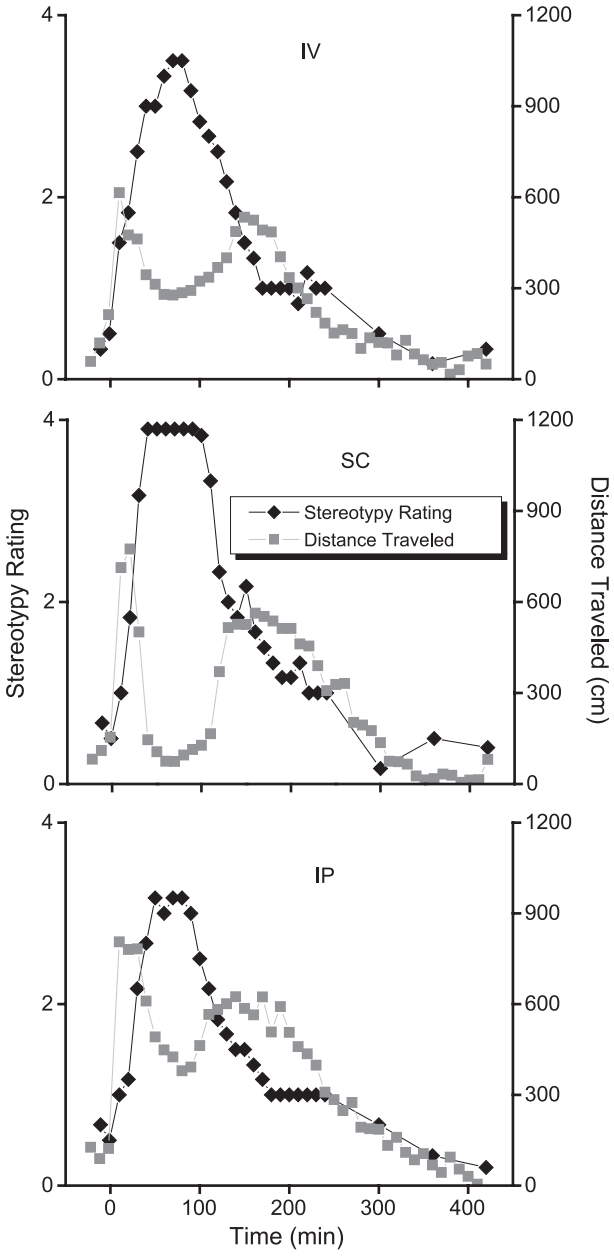

Fig. 2. Average stereotypy rating at each 10-min interval for all three routes of administration over a 420-min (7-h) period following 3.0 mg/kg (+)METH administration. The distance traveled that occurred with these routes is also shown superimposed as gray symbols to demonstrate the relationship between locomotion and stereotypy at this (+)METH dose. Error bars are not shown to avoid cluttering the graphs. (+)METH was injected at time t=0 in all rats. Stereotypy ratings were statistically significantly higher in the sc group vs. the iv and ip groups (P<0.05).

netic AUC, C_{max}-(+)METH, C_{max}-(+)AMP, T_{max}-(+)METH and T_{max}-(+)AMP values for (+)METH and (+)AMP were compared with two-tailed, unpaired *t*-tests. The level of significance was set at $P<0.05$. Statistical analysis was performed using the SigmaStat (v 2.01) software package (Jandel Scientific, San Rafael, CA).

3. Results

3.1. General experimental observations

The repeated doses of (+)METH were well tolerated in all rats. No directly observable toxicity was noted in any of the animals. We selected 3.0 mg/kg as our highest dose because rats have tolerated this dose well in our laboratory, and we have previously observed significant evidence of toxicity with iv doses of 5.6 mg/kg and above (e.g., self-mutilation).

3.2. Time course of (+)METH-induced locomotion and stereotypy

The onset of locomotor effects occurred soon after (+)METH administration with all dose/route combinations (Fig. 1). The time course and magnitude of locomotion, however, varied significantly with dose and route. Following 0.3 and 1.0 mg/kg by all routes of administration, the effect vs. time relationships were single modal, right skewed curves. Following 3.0 mg/kg, the averaged effect vs. time curves were bimodal for all routes of administration. That is, there was a short burst of activity followed by a return toward baseline values, followed by a prolonged increase in activity.

The decrease in locomotor activity after 3.0 mg/kg (+)METH was associated with the onset of stereotypy, which occurred 20 to 30 min after (+)METH administration (Fig. 2). The most profound decrease in locomotor activity occurred in the sc group, which also exhibited the greatest stereotypy. As stereotypy waned, a post-stereotypy hyperactivity phase ensued with all routes of administration. Because hyperactivity persisted beyond the stereotypy phase, the duration of increased locomotion included the period of stereotypy. Thus, the duration of locomotor activity was defined as the time from onset of increased locomotor activity until activity returned to baseline after the second locomotor phase.

Within each route of administration, there were significant dose-dependent increases in the duration of (+)METH induced locomotor effects, and there were significant differences in duration of action across routes of administration (Table 1). Specifically, the duration of increased distance traveled was significantly greater after sc than iv dosing ($P<0.05$) and the duration of increased rearing behavior was significantly greater after sc than after iv and ip dosing ($P<0.05$). Conversely, while there was a trend toward an increase in the duration of stereotypy (sc>iv>ip; Table 1) with route of administration, these differences did not reach statistical significance.

3.3. Total locomotor activity and stereotypy

The total (+)METH-induced locomotor responses were dependent on the dose following all three routes of administration (Fig. 3). When equal doses were compared with different routes of administration, the total responses did not vary for distance traveled after 0.3 and 1.0 mg/kg (+)METH. However, the total number of (+)-METH-induced rearing effects was significantly lower after administration by the sc route vs. the other routes after 1.0 mg/kg ($P<0.05$). In addition, total responses for both locomotor activity parameters were significantly greater following ip administration of 3.0 mg/kg (+)METH than after iv or sc administration of the same dose ($P<0.05$).

The nadir of the horizontal locomotor activity that occurred with the 3.0 mg/kg (+)METH doses was associated with several stereotyped behaviors that included focused sniffing, repetitive head movements, and oral stereotypies

Table 1
Average duration of action for increased distance traveled, numbers of rearing events and stereotypy of the rats (n=6 per route of administration) after three increasing doses of (+)METH

Route	Duration of action (min)								
	iv			sc			ip		
Effect	(+)METH dose (mg/kg)								
	0.3	1.0	3.0	0.3	1.0	3.0	0.3	1.0	3.0
Distance	82±34*	115±26*	212±17‡	70±20*	132±24†	263±23a,‡	91±38*	127±16*	233±33‡
Rearing	91±31*	116±26*	229±22‡	72±20*	129±30†	268±18b,‡	81±40*	134±18*	206±30‡
Stereotypy			118±22			130±33			91±37

Data are presented as the mean±S.D.
[a] $P<0.05$ vs. 3.0 mg/kg, iv.
[b] $P<0.05$ vs. 3.0 mg/kg, iv and ip.
* $P<0.05$ vs. saline with the same route of administration.
‡ $P<0.05$ vs. saline, 0.3 and 1.0 mg/kg with the same route of administration.
† $P<0.05$ vs. saline and 0.3 mg/kg with the same route of administration.

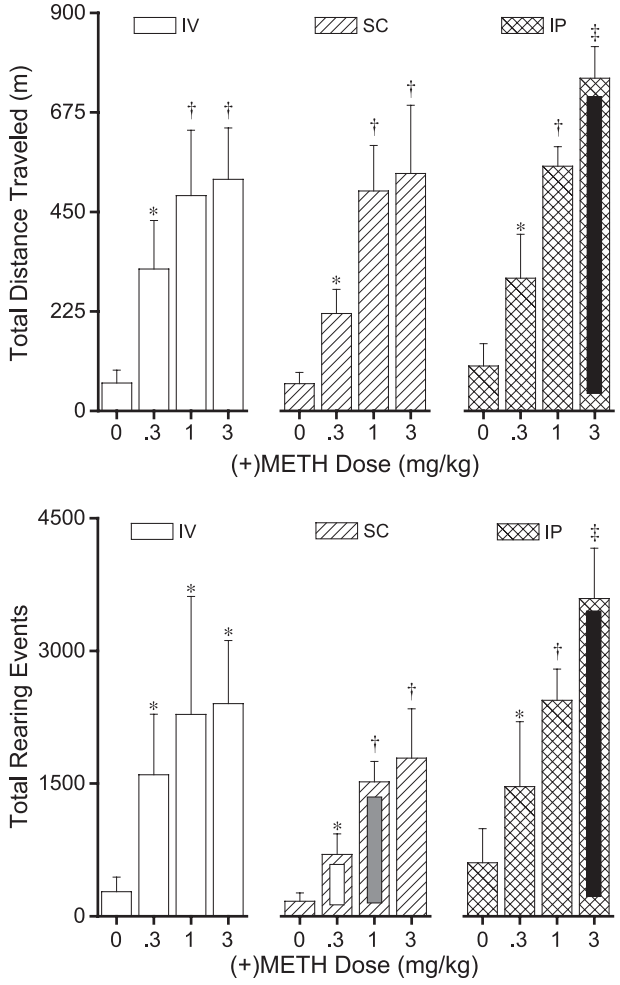

Fig. 3. Average total distance traveled (upper panel) and total number of rearing events (lower panel) of the rats (n=6 per route of administration) after saline and three increasing doses of (+)METH. Data are presented as the mean±S.D. *P<0.05 vs. saline with the same route of administration. †P<0.05 vs. saline and 0.3 mg/kg with the same route of administration. ‡P<0.05 vs. saline, 0.3 and 1.0 mg/kg with the same route of administration. The white bar in the 0.3 mg/kg sc distance group signifies P<0.05 vs. 0.3 mg/kg, iv. The gray bar in the 1.0 mg/kg sc rearing group signifies P<0.05 vs. 1.0 mg/kg, iv and ip. The black bar in the 3.0 mg/kg ip group signifies P<0.05 vs. all other dose/route combinations.

(chewing, licking and biting). Statistical analysis of the entire experiments from −11 to 420 min indicated the sc group had significantly greater stereotypy ratings than either the ip or iv groups (P<0.05).

3.4. Pharmacokinetics of sc and ip (+)METH administration

The measured (+)METH concentrations increased after 3.0 mg/kg (+)METH administration in all rats reaching a maximum at the 5 or 10 min blood sampling time points in the ip group and the 20 to 30 min time points in the sc group (Fig. 4; Table 2). The resulting average T_{max}-(+)METH was significantly different in the two groups (P<0.05). The C_{max}-(+)METH values were similar and were followed by a similar decline in both groups that resulted in virtually identical $t_{1/2\lambda z}$ values (approximately 51 min; harmonic mean) (Lam et al., 1985). (+)AMP concentrations also increased over time following (+)METH administration to peak at approximately 20 min after the (+)METH ip dose but significantly later at 76 min with sc dosing (P<0.05). However, neither the C_{max}-(+)AMP values nor the $t_{1/2\lambda z}$ values for (+)AMP (79 min for sc and 88 min for ip) were significantly different.

The (+)METH AUC values after sc dosing were similar to those we have previously observed for the same (+)METH dose given via the iv route (Milesi-Halle et al., submitted for publication). These AUC values were significantly different between the sc and ip groups (P<0.05), resulting in different bioavailability values (100% vs. 58%, respectively). However, the (+)AMP AUC values were not significantly different. Finally, while there was a trend toward an increase in the ip group, the

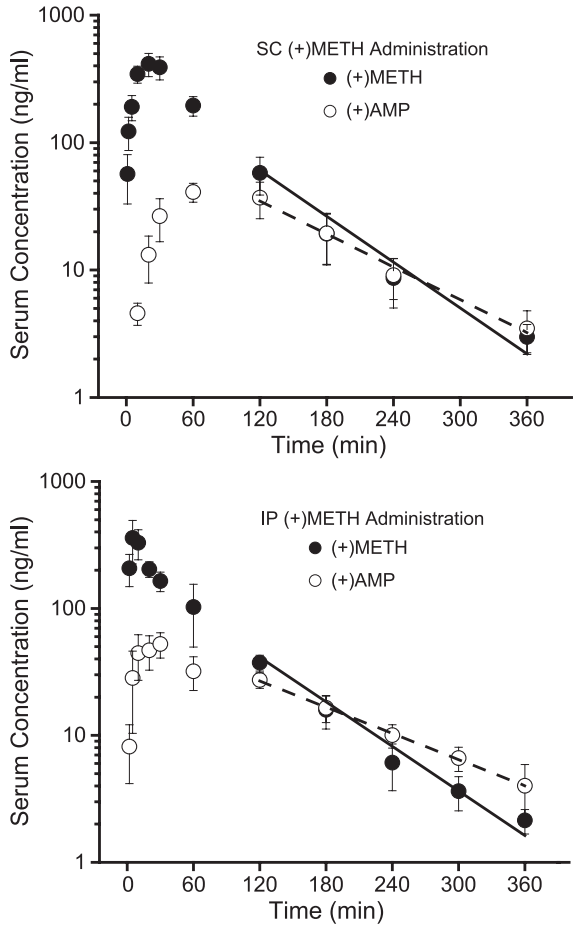

Fig. 4. Average concentration vs. time profiles for (+)METH (filled circles) and (+)AMP (open circles) following administration of (+)METH 3.0 mg/kg, sc (upper panel) and ip (lower panel). The solid line associated with the (+)METH data shows the average linear-regression fit to the terminal (+)METH log concentration vs. time data as determined by non-compartmental analysis. The dashed line associated with the (+)AMP data shows the average linear-regression fit to the terminal log concentration vs. time data as determined by non-compartmental analysis. All values are represented as the mean±S.D., n=4 rats per route of administration.

Table 2
Average (±S.D.) pharmacokinetic parameters of (+)methamphetamine and (+)amphetamine following a single 3.0 mg/kg (+)methamphetamine dose by two routes of administration

Parameter	(+)METH		(+)AMP	
	SC	IP	SC	IP
$t_{1/2\lambda z}$ (min)[a]	51.0±3.4[a]	50.8±2.2[a]	79±12[a]	87.7±11.4[a]
AUC (ng min/ml)	31,998±5416[b]	18,272±3920	7370±2116	7053±1518
V_{dss} (l/kg)	6.7±1.1	6.9±1.4	n/d[c]	n/d[c]
Cl_T (ml/min/kg)	94±17	95±19	n/d[c]	n/d[c]
T_{max} (min)	23±5[b]	7.5±2.8	76.2±29.7[b]	20±8.2
C_{max} (ng/ml)	432±67	382±116	42.7±9.6	48.3±15.5
Bioavailability (F)	100%[d]	58%[d]	n/a	n/a
(+)AMP/(+)METH AUC molar ratio	0.34±0.11[e]	0.46±0.09[e]	n/a	n/a

Pharmacokinetic parameters were obtained from a model-dependent analysis of the individual rat (+)METH and (+)AMP concentration–time curves ('n/a' is not applicable).

[a] Harmonic mean and "pseudo" standard deviation (Lam et al., 1985).

[b] $P<0.05$ vs. ip group.

[c] "not determined" since these data are for a metabolite of (+)METH.

[d] Calculated using IV AUC data from Milesi-Halle et al. (submitted for publication).

[e] The molar ratio of (+)AMP to (+)METH AUC was obtained by dividing the nmol min/ml AUC values for (+)AMP by the nmol min/ml AUC values for (+)METH.

(+)AMP/(+)METH AUC molar ratios were not significantly different (Table 2).

4. Discussion

The overall average locomotor effect vs. time relationships and the time course of effects were similar for all administration routes following the 0.3 and 1.0 mg/kg (+)METH doses (Fig. 1). In addition, observable stereotyped behavior was minimal at these lower doses. This finding is consistent with other reports that 1.0 mg/kg doses produces predominately locomotion (Grilly and Loveland, 2001) and that the transition from locomotion to stereotypy occurs at doses of 1.75 mg/kg (Segal and Kuczenski, 1987). Thus, the influence of route of administration on (+)METH-induced locomotor activity and stereotypy was small at low doses.

Conversely, there were significant route-dependent differences in effects after 3.0 mg/kg (+)METH, a dose that produced stereotypy and bimodal locomotor effects after all administration routes. In particular, stereotypy ratings and the duration of locomotor effects were highest after sc dosing (Table 1). While the duration of stereotypy was not significantly increased following sc dosing, there was an increasing trend in duration from ip to iv to sc.

Other reports have shown that stereotypy is associated with sc administration of higher doses of (+)METH. Segal and Kuczenski (1997) show that profound oral stereotypy occurs following 4.42 mg/kg sc doses of (+)METH in rats. This study shows that a brief period of increased spontaneous locomotor activity precedes the onset of stereotyped behavior. Locomotor activity wanes as stereotypy increases. While our activity measurement system did not allow the same degree quantification of stereotyped behavior as other investigators (e.g., Segal and Kuczenski, 1987, 1997), it appears our findings are consistent with previous observations.

The observed increases in (+)METH effects probably relate to differences in pharmacokinetics between the two routes. (+)METH AUC was significantly greater following sc dosing, compared with ip, even though the C_{max}-(+)METH values were similar for both routes. This difference in AUC is related to differences in absorption and metabolism. With sc dosing, (+)METH absorption into the bloodstream was slower than via the ip route, as demonstrated by the significantly increased T_{max}-(+)METH. This, combined with a bioavailability of 100%, resulted in a prolonged duration of action. Although ip absorption of (+)METH is rapid compared with sc, hepatic first pass metabolism limited the dose of (+)METH absorbed (bioavailability=58%). Indeed, the molar ratio of the metabolite (+)AMP to (+)METH after 3.0 mg/kg ip was 46% compared with 34% after sc, and 29% after iv (+)METH (Milesi-Halle et al., submitted for publication). Others have also suggested that ip administration of (+)METH results in significant hepatic first pass metabolism that alters (+)METH and (+)AMP concentration time profiles (Sakai et al., 1983). This increase in (+)AMP formation appeared to reduce the overall exposure to (+)METH, effectively shifting the response from stereotypy to greater locomotor effects (as seen with lower (+)METH does), thus increasing distance traveled and rearing events.

The relative larger exposure to (+)AMP associated with ip dosing (as measured by the (+)AMP/(+)METH AUC ratio) could also have contributed to the observed increases in locomotion. It has been shown that (+)AMP produces different locomotor effects than (+)METH. Shoblock et al. (2003) have shown that three doses of (+)AMP given ip (1.0, 2.0 and 3.0 mg/kg) resulted in increased locomotor activity (measured by total photocell activity counts) when compared to the same (+)METH doses. They also demon-

strated differential neurochemical effects after ip administration of 2.0 mg/kg (+)METH or (+)AMP. While dopamine levels in the nucleus accumbens were similar after both drugs, there were differences in dopamine levels in the prefrontal cortex, and glutamate levels in both brain areas following (+)METH or (+)AMP administration. Thus, route-dependent differences favoring enhanced formation of (+)AMP after ip administration could have contributed to the differences in locomotor activity in this study.

The altered overall effect following 3.0 mg/kg ip in the current study may also be partially explained by variability associated with ip administration. Absorption from the peritoneal cavity may vary depending on the amount of food in the gut and needle placement. Indeed, there was a marked interindividual variation in the effect vs. time curves for rearing events for the rats in the ip group following 3.0 mg/kg. Three of the six rats in this group exhibited bimodal response patterns, while the other three rats exhibited only a single peak in activity (individual animals' results are not shown).

(+)METH elimination rates were similar in the two groups as measured by $t_{1/2\lambda z}$ values. These values were slightly lower than values reported in previous serum pharmacokinetic studies, (+)METH after 1.0 mg/kg, iv (63 min; Riviere et al., 1999) and 3.0 mg/kg, iv (73 min; Milesi-Halle et al., submitted for publication), but similar to those obtained in tissue pharmacokinetic studies of (+)METH (54 min; Riviere et al., 2000), and serum pharmacokinetic studies after 1.0 mg/kg, iv (49 min; Melega et al., 1995). The relatively small differences among these studies indicate that the current results are consistent with known values and that differences in $t_{1/2\lambda z}$ did not significantly contribute to the observed route-dependent differences in (+)METH effects.

Despite the route-dependent differences in drug exposure as reflected by (+)METH AUC values, other pharmacokinetic parameters appear to be similar among the groups. For instance, V_{dss} and Cl_T were similar among the two groups in the current study (Table 2), and these values were similar to those obtained in our study of the pharmacokinetics of (+)METH after iv administration (V_{dss}=6 l/kg; Cl_T=109 ml/min/kg; Milesi-Halle et al., submitted for publication). Nevertheless, there were effect differences between sc and iv administration, though the differences were not as great as between sc and ip.

An explanation for differences between iv and sc dosing has been proposed in another previous study. Cho et al. (1999) showed that neurochemical responses were different following sc stimulant administration when compared to iv dosing, and that the differences were associated with different pharmacokinetic values. These authors reported that caudate-putamen dopamine concentrations peaked within 2 min of iv administration of (+)AMP (3.6 mg/kg) and decreased immediately thereafter. In contrast, peak dopamine concentrations were delayed until 9 min after sc administration of 8.0 mg/kg (+)AMP and remained elevated for about 30 min, despite achieving approximately equal brain (+)AMP concentrations. The prolonged changes in neurotransmitter levels after sc (+)AMP were associated with prolonged stereotypy. Thus, the similar findings of extended stereotypy after sc (+)METH dosing in the current study could be due to prolonged elevations in brain dopamine concentrations.

Interestingly, the onset of stereotyped behavior in the Cho et al. (1999) study was similar after both routes of administration. We also observed similar onsets for stereotypy after the 3.0 mg/kg dose, and locomotor activity after all three doses across route. These findings suggest that the onsets of these different effects are not entirely due to the rate of rise of brain (+)METH concentrations. An explanation of the mechanism(s) of this finding is beyond the scope of this paper since brain concentration–time data were not collected; however, other authors have suggested this may be due to the complex neurochemical events which cause these effects. It is likely that time-dependent effects other than those that cause the behaviors measured here will inhibit or compete with the expression of these behaviors (Cho et al., 1999). Further studies are needed to explain these findings.

In summary, the results of the current study indicate there are significant route-dependent differences in (+)METH effects at doses high enough to result in profound stereotyped behavior. Because stereotypy appears to be associated with greater drug exposure than locomotor activity (Segal and Kuczenski, 1994; Shoblock et al., 2003), and the sc route had the greater AUC, the sc route of administration caused the most profound effect in the current studies. Route of administration should therefore be considered when interpreting dose–effect studies of stimulants in animals.

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References

Balster RL, Schuster CR. Fixed-interval schedule of cocaine reinforcement: effect of dose and infusion duration. J Exp Anal Behav 1973;20:119–29.

Byrnes-Blake KA, Laurenzana EM, Carroll FI, Abraham P, Gentry WB, Landes RD, et al. Pharmacodynamic mechanisms of monoclonal antibody-based antagonism of (+)-methamphetamine in rats. Eur J Pharmacol 2003;461:119–28.

Cho AK. Ice: a new dosage form of an old drug. Science 1990;249:631–4.

Cho AK, Melega WP, Kuczenski R, Segal DS, Schmitz DA. Caudate-putamen dopamine and stereotypy response profiles after intravenous and subcutaneous amphetamine. Synapse 1999;31:125–33.

Cook CE, Jeffcoat AR, Hill JM, Pugh DE, Patetta PK, Sadler BM, White WR, Perez-Reyes M. Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride. Drug Metabolism and Disposition 1993;21:717–23.

Crofts N, Louie R, Rosenthal D, Jolley D. The first hit circumstances surrounding initiation into injecting. Addiction 1996;91:1187–96.

Darke S, Cohen J, Ross J, Hando J, Hall W. Transitions between routes of administration of regular amphetamine users. Addiction 1994;89: 1077–83.

Domier CP, Simon SL, Rawson RA, Huber A, Ling W. A comparison of injecting and noninjecting methamphetamine users. J Psychoactive Drugs 2000;32:229–32.

Ellinwood Jr EH, Balster RL. Rating the behavioral effects of amphetamine. Eur J Pharmacol 1974;28:35–41.

Fischman MW, Schuster CR. Cocaine self-administration in humans. Fed Proc 1982;41:241–6.

Grilly DM, Loveland A. What is a "low dose" of D-amphetamine for inducing behavioral effects in laboratory rats? Psychopharmacology 2001;153:155–69.

Hall W, Hando J, Darke S, Ross J. Psychological morbidity and route of administration among amphetamine users in Sydney, Australia. Addiction 1996;91:81–7.

Hardin JS, Wessinger WD, Proksch JW, Owens SM. Pharmacodynamics of a monoclonal antiphencyclidine Fab with broad selectivity for phencyclidine-like drugs. J Pharmacol Exp Ther 1998;285:1113–22.

Hardin JS, Wessinger WD, Wenger GR, Proksch JW, Laurenzana EM, Owens SM. A single dose of monoclonal anti-phencyclidine IgG offers long-term reductions in phencyclidine behavioral effects in rats. J Pharmacol Exp Ther 2002;302:119–26.

Hendrickson HP, Milesi-Halle A, Laurenzana EM, Owens SM. Development of a liquid chromatography-tandem mass spectrometric method for the determination of methamphetamine and amphetamine using small volumes of rat serum. J Chromatogr B, Analyt Technol Biomed Life Sci 2004;806:81–7.

Lam FC, Hung CT, Perrier DG. Estimation of variance for harmonic mean half-lives. J Pharm Sci 1985;74:229–31.

Laurenzana EM, Byrnes-Blake KA, Milesi-Halle A, Gentry WB, Owens SM. Use of anti-(+)-methamphetamine monoclonal antibody to significantly alter (+)-methamphetamine and (+)-amphetamine disposition in rats. Drug Metab Dispos 2003;31(11):1320–6 [Nov].

Melega WP, Williams AE, Schmitz DA, DiStefano EW, Cho AK. Pharmacokinetic and pharmacodynamic analysis of the actions of D-amphetamine and D-methamphetamine on the dopamine terminal. J Pharmacol Exp Ther 1995;274:90–6.

Milesi-Halle A, Hendrickson H, Laurenzana E, Gentry W, Owens S. Sex- and dose-dependency in the pharmacokinetics and pharmacodynamics of (+)-methamphetamine and its metabolite (+)-amphetamine in rats. Submitted for publication.

Pennell S, Ellett J, Rienick C, Grimes J, 1999. Meth matters report on methamphetamine users in five Western Cities. Washington, DC: U.S. Department of Justice, National Institute of Justice; 1999. p. 24–5.

Peters A, Davies T, Richardson A. Increasing popularity of injection as the route of administration of amphetamine in Edinburgh. Drug Alcohol Depend 1997;48:227–34.

Riviere GJ, Byrnes KA, Gentry WB, Owens SM. Spontaneous locomotor activity and pharmacokinetics of intravenous methamphetamine and its metabolite amphetamine in the rat. J Pharmacol Exp Ther 1999;291:1220–6.

Riviere GJ, Gentry WB, Owens SM. Disposition of methamphetamine and its metabolite amphetamine in brain and other tissues in rats after intravenous administration. J Pharmacol Exp Ther 2000;292:1042–7.

Rowland M, Tozer TN. Clinical pharmacokinetics concepts and applications. Media, PA: Williams & Wilkins; 1995.

Sakai T, Niwaguchi T, Kimura R, Murata T. Distribution and excretion of methamphetamine and its metabolites in rats: II. Time-course of concentration in blood and distribution after multiple oral administration. Xenobiotica 1983;13:715–24.

Segal DS, Kuczenski R. Individual differences in responsiveness to single and repeated amphetamine administration: behavioral characteristics and neurochemical correlates. J Pharmacol Exp Ther 1987;242:917–26.

Segal DS, Kuczenski R. Behavioral pharmacology of amphetamine. In: Cho AK, Segal DS, editors. Amphetamine and its analogs psychopharmacology, toxicology and abuse. San Diego: Academic Press; 1994. p. 115–52.

Segal DS, Kuczenski R. Repeated binge exposures to amphetamine and methamphetamine: behavioral and neurochemical characterization. J Pharmacol Exp Ther 1997;282:561–73.

Shoblock JR, Sullivan EB, Maisonneuve IM, Glick SD. Neurochemical and behavioral differences between D-methamphetamine and D-amphetamine in rats. Psychopharmacology (Berl) 2003;165:359–69.

Sturgeon RD, Fessler RG, Meltzer HY. Behavioral rating scales for assessing phencyclidine-induced locomotor activity, stereotyped behavior and ataxia in rats. Eur J Pharmacol 1979;59:169–79.

Valentine JL, Owens SM. Antiphencyclidine monoclonal antibody therapy significantly changes phencyclidine concentrations in brain and other tissues in rats. J Pharmacol Exp Ther 1996;278:717–24.