Pharmacology Biochemistry & Behavior, Vol. 29, pp. 249–255. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Stimulant Effects of Apomorphine and (+)-Amphetamine in Rats With Varied Habituation to Test Environment

EVALYNN J. MAZURSKI AND RICHARD J. BENINGER

Department of Psychology, Queen's University, Kingston, Canada K7L 3N6

Received 20 April 1987

MAZURSKI, E. J. AND R. J. BENINGER. *Stimulant effects of apomorphine and (+)-amphetamine in rats with varied habituation to test environment.* PHARMACOL BIOCHEM BEHAV **29**(2) 249–255, 1988.—The stimulants (+)-amphetamine and apomorphine are known to increase motility and induce stereotypy in rats. The present study examined the effects of an habituation period immediately prior to injection on these stimulant effects. Male Wistar rats received doses of either drug including 0, 0.01, 0.1, 0.25, 1.0, 2.0 and 4.0 mg/kg in a random order. Activity was assessed in 6 automated chambers where horizontal and vertical activity were tabulated hourly for 4 hours. Initially all rats had equal exposure to the chambers over 5 days. In the subsequent drug phase, habituated rats were placed in the chambers for the 1-hr period prior to each injection whereas non-habituated rats were in their home cages at the corresponding time. (+)-Amphetamine stimulated horizontal activity, although under either condition the effect was not seen until the second hour post-injection, but lasted until the fourth hour. Vertical activity was similarly enhanced, but with habituation there was a significant stimulant effect in the first hour as well. With apomorphine the habituation period resulted in an absence of a significant stimulant effect. Non-habituated rats showed a significant stimulant effect with the highest dose only on vertical activity in the first hour and a stimulant effect with horizontal activity in the second hour. It is suggested that the relative novelty of the environment affected the behavioral response to apomorphine but not to (+)-amphetamine. Furthermore, the activating effects accompanying drug administration should be taken into account as a factor affecting responses to drugs. Such environmental factors may be of particular importance when considering drugs with a short duration of action.

Apomorphine (+)-Amphetamine Horizontal activity Vertical activity Habituation

THE behavioral effects of various pharmacological treatments are known to vary as a function of a number of parameters. Not only is there individual variation in response to a fixed dosage of a drug, but the individual's response itself may fluctuate on different occasions [7, 9, 15]. For example, such variables as the drug history, environmental context, and the internal state of the organism may modify drug effects.

An important factor in determining drug effects is the familiarity of the environment in which the drug is administered. Although not often studied in its own right, there seems to be an interaction of some drug effects with familiarity. For example, Russell and Pihl [14] determined that stereotypy induced by a particular dose of amphetamine was lessened in a novel environment. This was suggested to be due to an increase in exploration in the novel environment that interfered with stereotypy. Beninger [2] also found that two drugs which interact with the serotonergic systems produced different activity profiles depending on the familiarity of the testing environment. Thus, underlying factors contributing to the response to a drug include the drug itself as well as behavior invoked by the particular environment. Perhaps by examining this latter factor, a more uniform description of the behavioral effects produced by various pharmacological agents may be possible.

The psychomotor stimulant effects of dopamine agonists have been extensively documented (e.g., [1, 5, 6, 8, 16]). Typically, lower doses of the agonists enhance general locomotor activity, whereas higher doses tend to induce stereotyped behaviors [12]. It is apparent however, that not all stimulants produce similar behavioral profiles. For example, (+)-amphetamine typically induces locomotion, rearing and sniffing in the rat, whereas apomorphine induces locomotion, sniffing, licking and gnawing [8]. Again, variations in the intensity or duration of these effects may be related to internal and external variables.

The present study examined possible interactive effects of familiarity and dose of (+)-amphetamine or apomorphine on activity responses measured in automated monitoring chambers. The relative novelty of the environment was manipulated by varying the location of the rats prior to each drug administration.

METHOD

Subjects

Forty-eight male Wistar rats had free access to food (Purina Rat Chow) and water for the duration of the study. They were housed in individual wire mesh cages (20×20×25

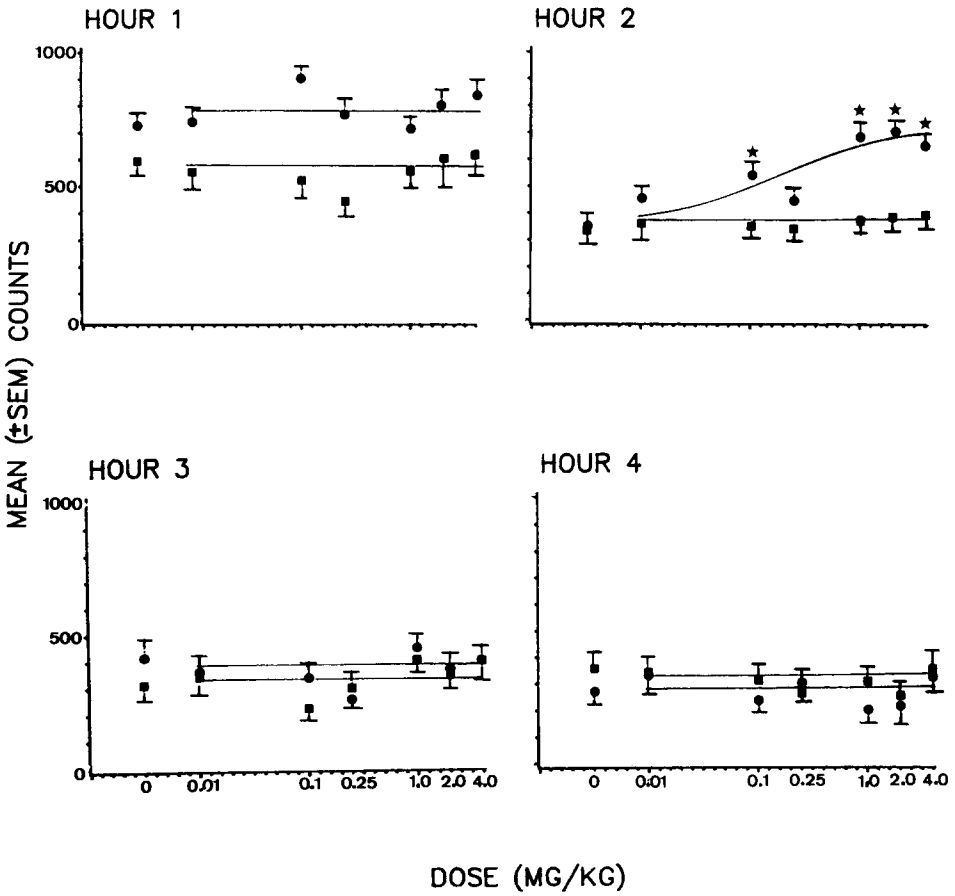

FIG. 1. Mean (±SEM) horizontal activity scores for each dose of apomorphine at each hour post-injection for habituated (■) and non-habituated (●) groups. A significant group effect ($p<0.01$) warranted separate curves for the two groups. *Indicates significant difference ($p<0.05$) from appropriate vehicle condition.

cm) in a climactically controlled environment (21±1°C) kept on a 12 hr light (0600–1800 hr)/dark cycle.

Apparatus

Activity was measured in 6 identical Plexiglas chambers (41×50×37 cm) encased in Styrofoam painted flat black. Each chamber was lit by a 2.5 W light mounted on the ceiling, and ventilated by a small fan which also provided constant background noise. Two sets of 7 infrared emitters and detectors, at 5 and 15 cm above the wire rod floor, were located in each chamber. Beam interruptions were recorded separately for each tier of beams by a Cromemco microcomputer. Further details of the apparatus have been presented elsewhere [3].

Procedure

Initially all rats received a daily 1-hr preexposure session to the activity chambers for 5 days. Each rat was always placed in the same chamber at approximately the same time of day.

On the second day following the fifth preexposure session the drug phase began. There were seven sessions, one every second day. Half of the rats were initially placed in the chambers for one hour, removed for injection, and immediately replaced for a further 4 hours. The other rats did not receive this habituation but rather were simply taken from the home cage, injected and placed in the chambers for 4 hours. Under each condition half of the rats (n=12) received (+)-amphetamine sulphate (Smith Kline & French) and the others received apomorphine hydrochloride (Sigma) in doses calculated from the salt of 0, 0.01, 0.1, 0.25, 1.0, 2.0, and 4.0 mg/kg. Each rat received all 7 doses of the drug in a randomly preselected order. However, groups of three rats each had the same order of administration, resulting in 4 different orders for each drug and condition.

(+)-Amphetamine was dissolved in distilled water, as was apomorphine although the latter had 1 mg of ascorbic acid added per ml to act as an anti-oxidant. Both drugs were injected IP at a volume of 1 ml/kg.

RESULTS

Preexposure

Activity levels derived from each of the four groups during the five preexposure sessions were very similar. Individual repeated measures analyses of variance (ANOVA's) ex-

TABLE 1

F-VALUES, DEGREES OF FREEDOM AND CORRESPONDING SIGNIFICANCE LEVELS FROM THREE-WAY ANOVA, AND SUBSEQUENT TWO-WAY ANOVAs FOR EACH APOMORPHINE GROUP

	Horizontal		Vertical	
	F	*df*	F	*df*
Three-Way ANOVA				
Time	107.28†	3,66	49.97†	3,66
Dose	5.58†	6,132	2.26*	6,132
Group	15.42†	1,22	23.88†	1,22
Time × dose	2.44†	18,396	3.28†	18,396
Time × group	15.11†	3,66	18.98†	3,66
Dose × group	1.49	6,132	2.19*	6,132
Time × dose × group	1.65*	18,396	2.73†	18,396
Non-Habituated Group				
Time	134.00†	3,33	42.30†	3,33
Dose	4.08†	6,66	2.58*	6,66
Time × dose	4.21†	18,198	3.47†	18,198
Habituated Group				
Time	19.40†	3,33	8.13†	3,33
Dose	2.97†	6,66	0.68	6,66
Time × dose	0.59	18,198	0.93	18,198

*$p<0.05$; †$p<0.01$.

TABLE 2

F-VALUES, DEGREES OF FREEDOM AND CORRESPONDING SIGNIFICANCE LEVELS FROM THREE-WAY ANOVA, AND SUBSEQUENT TWO-WAY ANOVAs FOR EACH (+)-AMPHETAMINE GROUP

	Horizontal		Vertical	
	F	*df*	F	*df*
Three-Way ANOVA				
Time	83.37†	3,66	53.41†	3,66
Dose	39.98†	6,132	12.99†	6,132
Group	0.34	1,22	0.17	1,22
Time × dose	12.54†	18,396	3.77†	18,396
Time × group	1.39	3,66	1.94	3,66
Dose × group	0.34	6,132	1.35	6,132
Time × dose × group	0.93	18,396	0.66	18,396
Non-Habituated Group				
Time	56.02†	3,33	34.79†	3,33
Dose	21.72†	6,66	7.52†	6,66
Time × dose	9.02†	18,198	1.28	18,198
Habituated Group				
Time	30.31†	3,33	19.18†	3,33
Dose	19.03†	6,66	6.89†	6,66
Time × dose	4.81†	18,198	3.32†	18,198

†$p<0.01$.

amining total activity counts in the 5 sessions were conducted separately for the horizontal and vertical activity indices for each group and each drug (data not presented). Significant session effects were seen in all four groups for vertical activity and reflected the tendency for activity on this measure to decrease over sessions. The horizontal activity counts did not show significant effects under any condition, suggesting that horizontal activity within the 1-hr period did not vary across sessions.

Drug Phase

The data from the drug phase were analysed separately for each drug and for each of the two activity measures. Thus three-way mixed design ANOVA's were conducted with 4 times (1 hour periods), 7 doses and 2 groups (habituated and non-habituated) as the factors. The dose by time effects were also examined for each group, followed by analyses of simple main effects of the doses at each time. Dunnett's tests comparing each dose to the vehicle condition were conducted where there were significant dose effects.

Apomorphine. Table 1 shows the results of the statistical analyses conducted on the data from the apomorphine groups. The corresponding activity scores for horizontal and vertical activity for rats given apomorphine with or without the daily habituation, at each of the four hours following drug injection are shown in Fig. 1 and 2, respectively. As there were significant group, time by group and time by dose by group effects with horizontal activity it was apparent that habituation affected activity as well as the response to apomorphine. Generally, the scores were lower for the habituated group and appeared less variable. The non-habituated rats showed significant time, and time by dose effects. Tests of simple main effects determined that there were significant dose effects only at the second, $F(6,66)=10.32$, $p<0.01$, and third, $F(6,66)=2.82$, $p<0.05$, hours. Subsequent Dunnett's test revealed that in hour two, the three highest doses, as well as the 0.1 mg/kg dose, showed significantly more activity than the vehicle condition. Tests in the third hour revealed no significant differences from the vehicle condition. The habituated group showed only significant time and dose effects. Furthermore, it can be seen that a relatively flat curve was observed at each hour.

Figure 2 shows vertical activity observed with apomorphine. Again there were significant group, time by group and time by dose by group effects, indicating that habituation played an important role in the response to the drug. The non-habituated rats showed significant time, dose, and time by dose effects. Subsequent analyses showed significant dose effects only in the first hour, $F(6,66)=3.42$, $p<0.01$. Post hoc tests determined that the 4.0 mg/kg dose exhibited a higher activity level than the control. In the habituated group there was only a significant time effect. Again the very flat nature of the curves with the habituated group is apparent.

(+)-Amphetamine. The data from the (+)-amphetamine groups were analysed in a similar manner and the results are presented in Table 2. The corresponding scores for horizontal and vertical activity for the habituated and non-habituated groups over the four hours are presented in Figs.

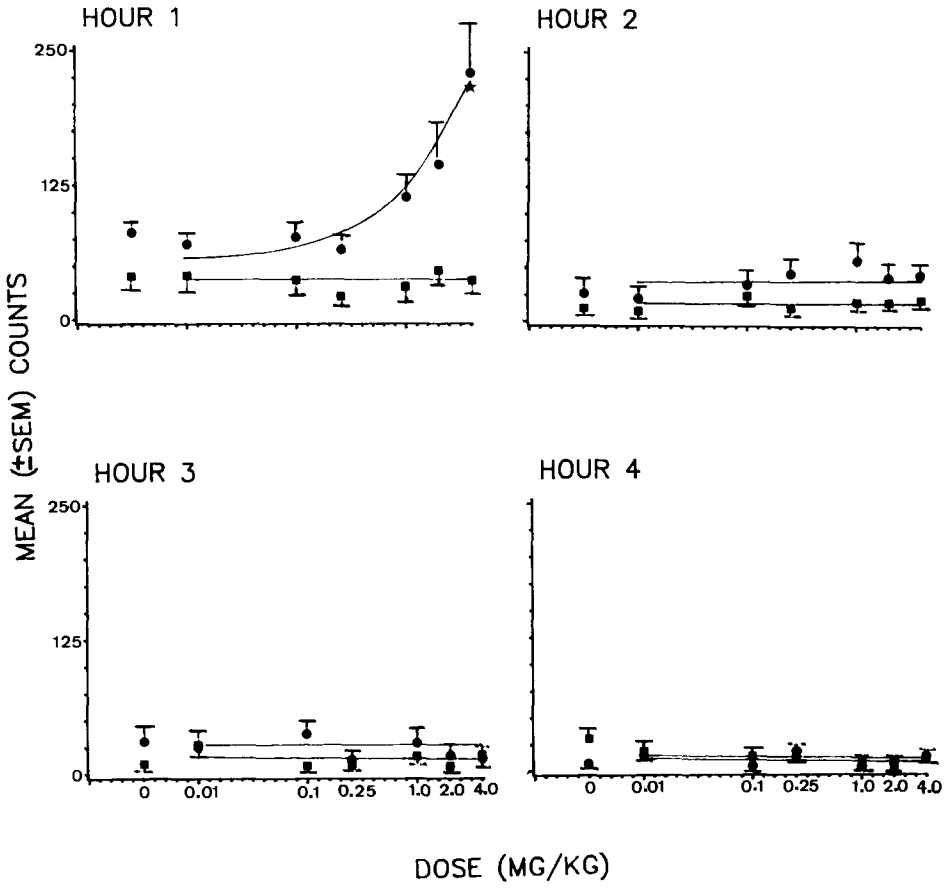

FIG. 2. Mean (±SEM) vertical activity scores for each dose of apomorphine at each hour post-injection for habituated (■) and non-habituated (●) groups. A significant group effect ($p<0.01$) warranted separate curves for the two groups. *Indicates significant difference ($p<0.05$) from appropriate vehicle condition.

3 and 4. The three-way ANOVA did not provide any significant effects incorporating the group variable, and thus suggested that horizontal activity did not vary as a function of habituation. Nonetheless, the habituated and non-habituated groups were analysed separately to assess dose and time effects in each. The horizontal activity in both the non-habituated and habituated groups showed significant time, dose, and time by dose effects. Thus, simple main effects of dose at each time were studied. The non-habituated rats had significant dose effects in the second, $F(6,66)=4.63$, $p<0.01$, third, $F(6,66)=14.40$, $p<0.01$, and fourth, $F(6,66)=8.31$, $p<0.01$, hours. In each case the 2.0 and 4.0 mg/kg conditions exhibited more activity than under the 0 mg/kg condition. The 1.0 mg/kg dose was significantly higher in the second and third hours as well. In the habituated group there were significant dose effects at each of the four hours (hour 1, $F=2.49$, $p<0.05$; hour 2, $F=8.64$, $p<0.01$; hour 3, $F=15.64$, $p<0.01$; hour 4, $F=6.66$, $p<0.01$; all $df=6,66$). However, post hoc tests determined that there were differences from 0 mg/kg only in the last three hours; again 2.0 and 4.0 mg/kg were higher in each case, whereas the 1.0 mg/kg dose yielded a higher score only at the second hour.

The vertical activity with (+)-amphetamine gave results comparable to those seen with horizontal activity (see Fig. 4). The results from the three-way ANOVA indicated that the group factor was not significant nor did it interact significantly with the other variables. Again each group was analysed individually for time and dose effects. With the non-habituated rats there was only significant time and dose effects, whereas the habituated group showed time, dose and time by dose effects. In each case the dose effect was examined at each time interval. The non-habituated rats had significant effects only in the second, $F(6,66)=4.63$, $p<0.01$, third, $F(6,66)=14.40$, $p<0.01$, and fourth, $F(6,66)=8.31$, $p<0.01$, hours. In each case the 4.0 mg/kg dose was higher. Furthermore, in the third hour the 2.0 mg/kg dose showed a significant enhancement from saline. The habituated rats, on the other hand, showed significant dose effects at each of the four hours (hour 1, $F=5.25$, $p<0.01$; hour 2, $F=2.80$, $p<0.05$; hour 3, $F=8.79$, $p<0.01$; hour 4, $F=4.37$, $p<0.01$; all $df=6,66$). In the first hour 1.0 and 2.0 mg/kg were higher than saline. Although there was an overall dose effect at hour two, $F(6,66)=2.80$, $p<0.05$, no individual dose had significantly higher scores than the vehicle. In both of the last two hours 4.0 mg/kg was higher, whereas only in the third hour

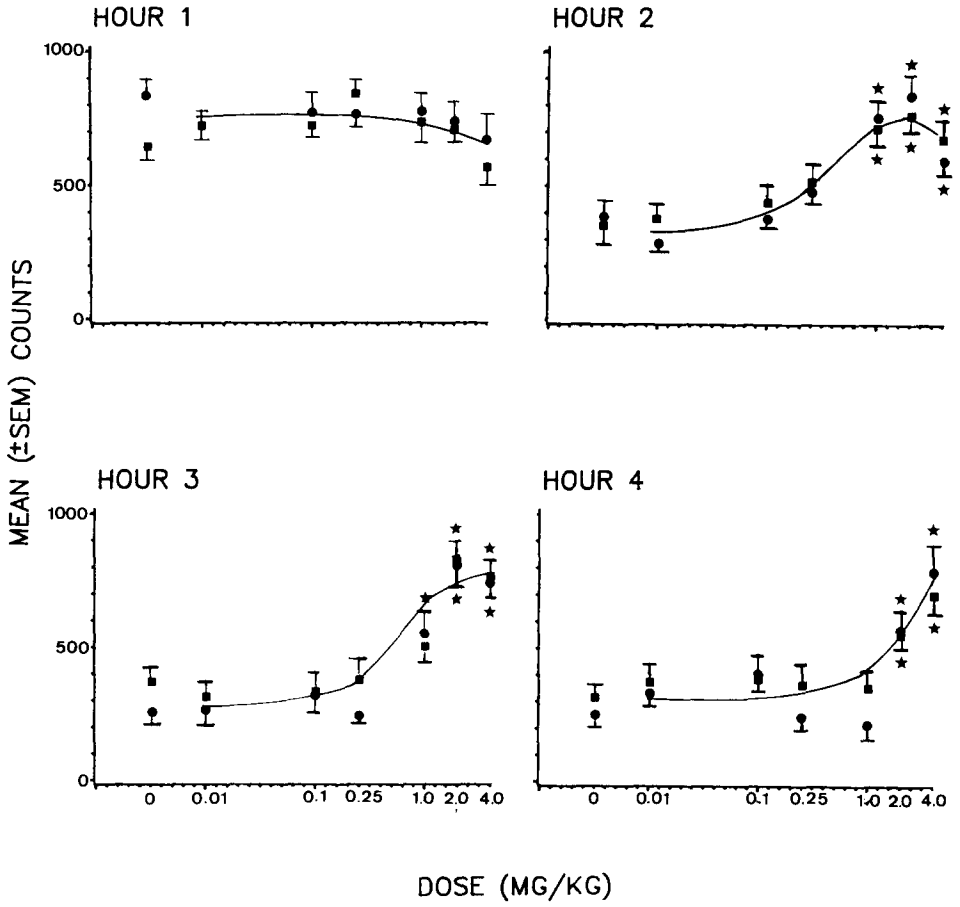

FIG. 3. Mean (±SEM) horizontal activity scores for each dose of (+)-amphetamine at each hour post-injection for habituated (■) and non-habituated (●) groups. A non-significant group effect allowed fitting one curve to the data for both groups. *Indicates significant difference ($p<0.05$) from appropriate vehicle condition.

did the 2.0 mg/kg dose significantly exceed the scores from the saline condition.

DISCUSSION

Pronounced stimulant effects on horizontal and vertical activity were observed with both drugs. However, it appeared that habituation greatly affected the response to apomorphine, but had little influence on activity resulting from administration of (+)-amphetamine. Thus, stimulant effects were observed with apomorphine only in the first two hours, and only under the condition without habituation. (+)-Amphetamine, on the other hand, yielded stronger and more durable effects that appeared independent of habituation. A dose-related stimulant effect continued into hour 4 with both horizontal and vertical activity. In general, the levels of activity seen with the various doses of (+)-amphetamine were similar under habituated and non-habituated conditions.

The absence of a marked stimulant effect in the first hour following drug administration is not congruent with previous reports showing stimulant effects within that time frame (e.g., [8,15]). One explanation for this finding may be related to the size of the activity monitors. Research in our laboratory has consistently found a high level of horizontal activity in rats after being placed in the chambers, regardless of drug treatment [3,4]. It is suggested that the large size of the chambers, particularly in comparison to the size of the home cage (about one-quarter the size of the monitors), produces a high level of activity which does not seem to readily decrease within 60 minutes. Thus, it is feasible that the drugs did produce behavioral effects within the first hour following administration, but the high level of activity in rats under the vehicle condition may have masked this effect. Perhaps vertical activity, which did show an effect in the first hour, is less susceptible to the effects of the size of the environment. Activity during preexposure sessions further suggests that vertical activity habituated quite readily, and thus stimulant effects could be more easily observed on this measure.

The briefer stimulant effect of apomorphine in comparison to that of (+)-amphetamine has been reported previously [5,8]. This is consistent with the present data as the apomorphine effects appeared to wane within 2 hours. The observation that the high dose of (+)-amphetamine still elicited a high level of horizontal and vertical activity in the fourth hour post-injection suggests that the stimulant action of this

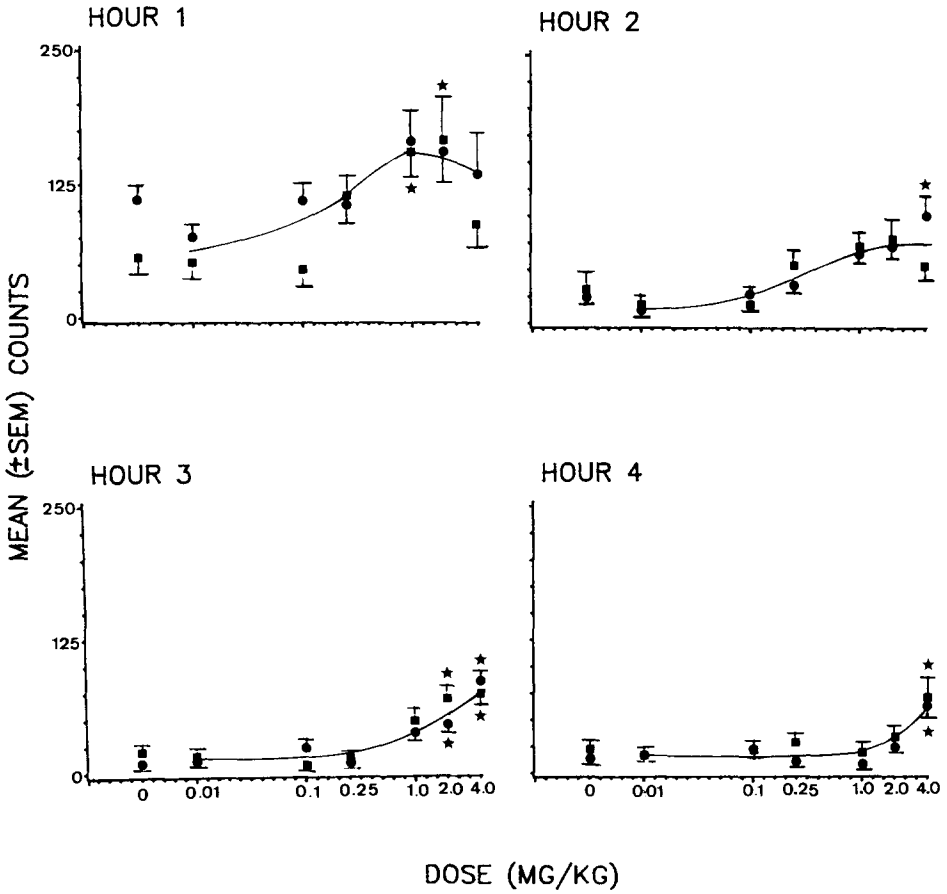

FIG. 4. Mean (±SEM) vertical activity scores for each dose of (+)-amphetamine at each hour post-injection for habituated (■) and non-habituated (●) groups. A non-significant group effect allowed fitting one curve to the data for both groups. *Indicates significant difference ($p<0.05$) from appropriate vehicle condition.

drug lasts at least 4 hr (cf. [3]). Lower doses of the drugs that produced stimulant effects tended to have shorter lasting effects in comparison to those seen with higher doses. This finding is consistent with typical dose-response effects [7].

Habituation played an important role in the activity seen with apomorphine, particularly in the first two hours post-injection. With horizontal activity scores seemed to be downshifted in the habituated rats, being consistently about 100 counts lower. Perhaps the most striking finding was the large difference observed in the first hour between the groups with respect to vertical activity, where only the non-habituated rats showed an increase in activity with the higher doses. These results are puzzling, yet in partial agreement with Fray *et al.* [8] who did not find an increase in rearing with apomorphine in rats habituated to the environment prior to drug administration. The difference between the two groups here may also be due in part to the fact that the non-habituated group, in addition to the habituation difference per se, was also handled more just before injection (i.e., weighed and carried to the test room). This may have further contributed to the high activity levels seen in the non-habituated group as apomorphine apparently enhances reactivity to environmental stimuli [10]. Perhaps the novelty and handling prior to injection together increased arousal in the non-habituated group. It is not possible to determine here which factor, habituation or handling prior to injection, influenced the results to a greater degree.

Previous studies have demonstrated a reduction of activity produced by low doses of apomorphine, which appears to be due to preferential stimulation of the dopamine autoreceptor [11]. However, no evidence of reduced activity was seen presently in either the habituated or non-habituated groups. The lack of hypoactivity may be the result of the sensitivity of the measuring system. That is, perhaps only relatively large fluctuations in activity are detected. It would be important to determine if other measuring techniques, such as rating scales, produce similar results.

There was a lack of differentiation in (+)-amphetamine effects with respect to the habituation period. Perhaps the stimulant effects of this drug were more pronounced or robust in comparison to apomorphine, and thus were less susceptible to environmental effects. Earlier research has shown that stereotypy with this drug can be manipulated by novelty [14]. However, it is possible that the variation in novelty here was perhaps not great enough to induce a very strong effect with (+)-amphetamine.

Apomorphine and (+)-amphetamine are both known dopamine agonists, although they differ in their modes of action. Apomorphine directly stimulates the receptor, whereas (+)-amphetamine enhances release and inhibits up-

take [5, 6, 13]. Furthermore, (+)-amphetamine is known to interact with other transmitters [13]. Perhaps these differences in pharmacological action may at least partially account for the variation in results between the drugs in the present study. Further studies examining other compounds are necessary however, to determine if the effects were unique to these drugs, or if they represent effects specific to their class of drugs.

The indices of activity used in the present study incorporated all aspects of horizontal and vertical activity that could possibly be measured by interruption of a photocell beam. Behaviors including exploration, grooming, ambulating, and rearing and jumping were cumulated in horizontal and vertical activity counts, respectively. However, the system itself was non-discriminatory across these various behaviors, and thus activity on either measure could be comprised of any combination of the various behaviors included in each index. Thus, with the present data it was not possible to determine which particular aspects of behavior were individually altered by the treatments. Further refinements of the monitoring system, or data collected in conjunction with other methods of assessing activity, would allow for specification of behaviors (e.g., grooming, gnawing and jumping) which are modified under the present conditions.

In conclusion, the present data suggest that the behavioral effects of drugs may be affected by minor variations in procedure. Thus, when examining the stimulant effects of some drugs, it is important to note the test procedures surrounding those injections.

ACKNOWLEDGEMENTS

We would like to thank Smith Kline and French for the generous gift of (+)-amphetamine. This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Health to R.J.B. The authors thank D. C. Hoffman for her valuable comments on the manuscript.

REFERENCES

1. Anden, N., A. Rubenson, K. Fuxe and T. Hokfelt. Evidence for dopamine stimulation by apomorphine. *J Pharm Pharmacol* **19:** 627–629, 1967.
2. Beninger, R. J. Effects of metergoline and quipazine in locomotor activity of rats in novel and familiar environments. *Pharmacol Biochem Behav* **20:** 701–705, 1984.
3. Beninger, R. J., T. A. Cooper and E. J. Mazurski. Automating the measurement of locomotor activity. *Neurobehav Toxicol Teratol* **7:** 79–85, 1985.
4. Beninger, R. J. and R. S. Herz. Pimozide blocks establishment but not expression of cocaine-produced environment-specific conditioning. *Life Sci* **38:** 1425–1431, 1986.
5. Colpaert, F. C., W. F. M. VanBever and J. E. M. F. Leysen. Apomorphine: Chemistry, pharmacology, biochemistry. *Int Rev Neurobiol* **19:** 225–268, 1976.
6. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10:** 316–323, 1967.
7. Feldman, R. S. and L. F. Quenzer. *Fundamentals of Neuropsycopharmacology*. Sunderland, MA: Sinauer, 1984.
8. Fray, P. J., B. J. Sahakian, T. W. Robbins, G. F. Koob and S. D. Iversen. An observational method for quantifying the behavioral effects of dopamine agonists: Contrasting effects of d-amphetamine and apomorphine. *Psychopharmacology (Berlin)* **69:** 253–259, 1980.
9. Havemann, U., B. Magnus, H. G. Moller and K. Kuschinsky. Individual and morphological differences in the behavioral response to apomorphine in rats. *Psychopharmacology (Berlin)* **90:** 40–48, 1986.
10. Jansenn, P. A. J., C. J. C. Niemegeers and A. H. M. Jageneau. Apomorphine-antagonism in rats. *Arzneimittelforsch* **10:** 1003–1004, 1960.
11. Ljungberg, T. and U. Ungerstedt. Different behavioural patterns induced by apomorphine: Evidence that the method of administration determines the behavioural response to the drug. *Eur J Pharmacol* **46:** 41–50, 1977.
12. Lyon, M. and T. W. Robbins. The action of central nervous system stimulant drugs: A general theory concerning amphetamine effects. In: *Current Developments in Psychopharmacology, Vol 2*. New York: Spectrum, 1975, pp. 79–163.
13. Moore, K. E. The actions of amphetamine on neurotransmitters: A brief review. *Biol Psychiatry* **12:** 451–461, 1977.
14. Russell, R. L. and R. O. Pihl. The effect of dose, novelty, and exploration on amphetamine-produced stereotyped behavior. *Psychopharmacology (Berlin)* **60:** 93–100, 1978.
15. Sahakian, B. J. and T. W. Robbins. Potentiation of locomotor activity and modification of stereotypy by starvation in apomorphine treated rats. *Neuropharmacology* **14:** 251–257, 1975.
16. Segal, D. S. and M. A. Schuckit. Animal models of stimulant-induced psychosis. In: *Stimulants: Neurochemical, Behavioral, and Clinical Perspectives*, edited by I. Creese. New York: Raven, 1983, pp. 95–129.