

52

STIMULANT EFFECTS OF (+)-AMPHETAMINE ARE INFLUENCED BY METHODOLOGICAL VARIABLES

EVALYNN J. MAZURSKI AND RICHARD J. BENINGER

Dept Psychol, Queen's University, Kingston, Canada

(Final Form, August, 1987)

Abstract

Mazurski, Evalynn J. and Richard J. Beninger. Stimulant effects of (+)-amphetamine are influenced by methodological variables. Prog. Neuro-Psychopharmacol. & Biol. Psychiat. 1988, 12:323-329

1. The locomotor effects of (+)-amphetamine were examined in two groups of rats placed in chambers of different sizes.
2. Prior to drug exposure, rats in large chambers were more active, as measured on a rating scale.
3. Under amphetamine, ratings discriminated the 2.5 mg/kg dose from both saline and 0.25 mg/kg without observed differences between the latter two. Scores recorded automatically in the large chambers showed enhanced horizontal activity with both doses, although they did not differ amongst themselves.
4. Methodological variables, including the size of apparatus and recording technique may influence observed effects of amphetamine, and suggest these variables be taken into account when studying drug effects.

Key words: (+)-amphetamine, horizontal and vertical activity, rating scales, size of test cage

Abbreviations: (+)-amphetamine (AMPH), dopamine (DA)

Introduction

The behavioral effects of various drugs are affected by a number of variables, including drug history, conditioning, type of environment and age (Beck et al., 1986; Beninger, 1984; Herz and Beninger, 1987; Mazurski and Beninger, 1987; Russell and Pihl, 1978). The locomotor activity induced by the DA agonist AMPH may be affected by such factors. For example, a large variability across laboratories in the size and type of apparatus utilized to measure activity exists, raising the possibility that different techniques may provide dissimilar results. Earlier research from our laboratory, using large test chambers (41x50x37 cm), found a high level of ambulatory activity in non-drugged rats, which initially masked stimulant effects of AMPH (Herz and Beninger, 1987; Mazurski and Beninger, 1987). Possibly the size of the chamber accounted for this finding.

The method utilized to measure activity may also be a factor which influences observed effects of AMPH. Methods include observer rated activity and automated techniques which detect various aspects of the animal's movement within the particular apparatus. An important issue is the comparability of results produced by these various methods.

The present study examined the effects of AMPH on rats in small and large activity monitoring boxes. Rating scores and automated scores in the large chambers were also compared to determine if similar results would be obtained with each method. It was hypothesized that observed drug-induced effects may vary with the size of the chamber and the assessment technique.

Methods

Animals. Eighteen male Wistar rats, obtained from Charles River Canada, had free access to food (Purina Rat Chow) and water for the duration of the study. They were individually housed in a climatically controlled environment ($21 \pm 1^\circ\text{C}$) kept on a 12 h light (0600-1800h)/dark cycle.

Drugs. (+)-Amphetamine sulphate (Smith Kline and French) was dissolved in distilled water. Non-drug injections were 0.9% saline. All injections were administered i.p. at a volume of 1.0 ml/kg body weight.

Apparatus. Activity was monitored in two types of chambers, varying primarily with respect to size. The 3 large chambers (41x50x37 cm) were each enclosed in Plexiglas, and surrounded by styrofoam painted black. Two sets of infrared beams, at 5 and 15 cm above the wire rod floor, automatically assessed horizontal and vertical activity, respectively. A 2.5 W light was mounted on the ceiling of each chamber, and a small fan provided ventilation and masking noise. A Plexiglas window in the front of each chamber allowed observation of the animal. The 3 small chambers (25x25x16 cm) were also constructed of Plexiglas, with the side and back walls painted black and a wire rod floor. The front wall was used for viewing the interior. The front of the small chambers faced the larger chambers, which provided the only light.

Procedure. All rats were handled frequently prior to the beginning of the study. They were randomly separated into two groups of 9 rats; one group was tested in the large chambers and the other in the small. All rats received 60 min habituation sessions over 5 days. The drug phase then began, wherein each rat received 0, 0.25, and 2.5 mg/kg of AMPH in a randomized order immediately prior to 2-h sessions conducted every second day.

During all sessions activity was rated by an observer, and in the large chambers, was also automatically recorded by number of beam interruptions. Automated scores

were cumulated every 10 min and ratings were taken at 10- min intervals beginning at 5 min into the session. The rater was blind with respect to drug dosage. Intermittently, a second rater also scored the rats, to determine inter-rater reliability.

The rater examined a rat for 20 s, determined the most frequent behavior and was allowed 10 s to record the score. The scale was comprised of the following scores: (1) asleep, (2) inactive, (3) in place activities, (4) normal alert active, (5) hyperactive, (6) slow patterned exploration, (7) fast patterned exploration, (8) restrictive repetitive movements, (9) dyskinetic-reactive movements (adapted from Ellinwood and Balster, 1974).

Statistical Analyses. Data were analysed using analyses of variance (ANOVA's) with an accepted level of significance of $p < 0.05$. Newman Keuls tests compared across doses where appropriate. Correlations were conducted with the Pearson-Product Moment correlation coefficient and significance was accepted at $p < 0.05$.

Results

Habituation. Ratings were analysed with a three-way ANOVA using time, session and chamber size as the factors. There were significant effects of time ($F=59.99$, $df=5,80$, $p < .01$), session ($F=7.83$, $df=4,64$, $p < .01$) and chamber size ($F=7.43$, $df=1,16$, $p < .025$). The time effect demonstrated a reliable decrease in activity across the session, whereas the session effect suggested the presence of inter-session habituation. The significant chamber size effect reflected the higher ratings in the large size chambers; the means (\pm SEM) for the two sizes were 3.02 (± 0.05) and 2.76 (± 0.05), respectively. The correlation coefficient between raters was found to be highly significant ($r=0.89$, $df=22$, $p < .01$).

The automated activity measures taken from the large chambers were analysed in two-way ANOVA'S with time and session as the factors. Horizontal activity showed a significant time effect ($F=6.69$, $df=5,40$, $p < .01$), illustrating the tendency to lesser activity across the session. Vertical activity showed significant time ($F=77.38$, $df=5,40$, $p < .01$), session ($F=10.29$, $df=4,32$, $p < .01$) and time by session effects ($F=5.15$, $df=20,160$, $p < .01$), suggesting the presence of inter- and intra-session habituation.

AMPH. Figure 1 shows the mean ratings for each dose in the two sizes of chamber. A three way ANOVA with time, dose and size of chamber as the factors, showed significant effects of dose ($F=189.16$, $df=2,32$, $p < .01$), time ($F=8.61$, $df=11,176$, $p < .01$), dose by time ($F=8.72$, $df=22,352$, $p < .01$) and dose by time by size ($F=1.98$, $df=22,352$, $p < .01$). Newman Keuls tests indicated that the 2.5 mg/kg dose was significantly higher than the 0.25 mg/kg and saline doses, whereas the latter two did not differ. The time by dose interaction demonstrated the tendency for ratings of

saline and 0.25 mg/kg to decrease across time, whereas with 2.5 mg/kg, ratings initially increased, then later began to decrease. The three-way interaction suggested that the time by dose effect differed depending on chamber size. Thus, the time by chamber size effect was studied at each dose. A significant interaction ($F=2.13$, $df=11,176$, $p<.025$) was found only with the 2.5 mg/kg dose, and examination of those data suggested that higher ratings occurred earlier in the small chambers, and later in the session in the large chambers. The inter-rater correlation of ratings was again highly significant ($r=0.94$, $df=16$, $p<.01$).

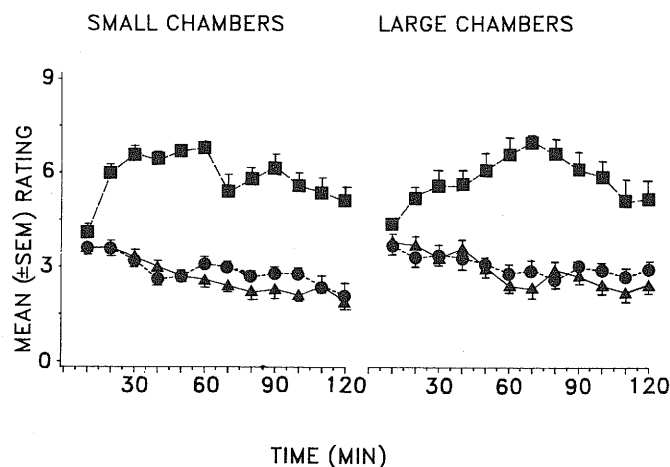


Fig. 1. Mean rating in small and large chambers per each 10 min of 120 min sessions with saline (▲), 0.25 (●) and 2.5 (■) mg/kg AMPH. Ratings ranged from (1) asleep to (4) normal, alert, active, through to (9) dyskinetic-reactive movements (see text).

Automated activity counts (fig 2) were analysed with dose and time as the factors. Horizontal activity revealed significant time ($F=6.53$, $df=11,88$, $p<.01$) and dose effects ($F=14.24$, $df=2,16$, $p<.01$). It was determined that 2.5 mg/kg and 0.25 differed from saline, but not from each other. Analysis of vertical activity produced significant time ($F=31.74$, $df=11,88$, $p<.01$) and dose effects ($F=8.66$, $df=2,16$, $p<.01$). No groups were found to differ significantly, although the difference between 0.25 mg/kg and 2.5 mg/kg approached significance.

The correlation of ratings and automated scores was also of interest. As both measures were taken only in the large chambers, comparisons could only be made there. At saline and 0.25 mg/kg significant correlations were found with both horizontal ($r=0.52$, and $r=0.44$, both $df=106$, both $p<.01$) and vertical activity ($r=0.46$, and $r=0.33$, both $df=106$, both $p<.01$). At 2.5 mg/kg however, there was no significant correlation of ratings with horizontal activity ($r=0.04$, $p>.05$) and a small but significant negative correlation ($r=-0.21$, $p<.05$) with vertical activity.

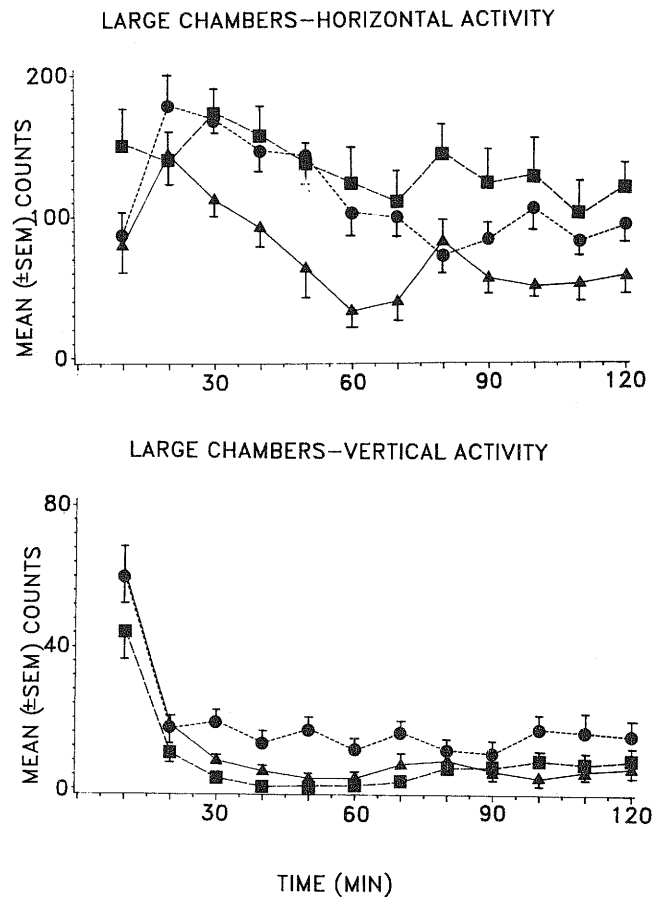


Fig. 2. Mean number of horizontal (upper panel) and vertical (lower panel) activity counts per each 10 min of 120 min sessions with saline (▲), 0.25 (●) and 2.5 (■) mg/kg AMPH.

Discussion

The present study determined that the size of chamber affected rats' activity, both in the absence of drugs and in response to AMPH, even though all variables (lighting, sound, floor and wall textures) were equated as closely as possible except chamber size. The two sets of chambers were not identical however, (i.e., there were no fans directly behind the small chambers, nor did they have their light source directly above each chamber) thus these factors may have also contributed to the results to some degree. The relative importance of these other factors however, could not be directly assessed here. With AMPH the size of chamber interacted with dose and time; an effect due primarily to the 2.5 mg/kg dose where

rats in the smaller chambers exhibited stimulant effects sooner. Ratings showed that the high dose appeared different from the low dose and saline. Automated activity measures did not discriminate the doses in the same manner. Horizontal activity showed both doses to produce more activity than saline. Vertical activity suggested that 0.25 mg/kg enhanced and 2.5 mg/kg depressed this measure, although differences were not significant. Thus, the size of the apparatus and recording technique appeared to have important consequences on the observed results. Earlier studies showed that novelty and type of environment were important determinants of AMPH induced stereotypy (Beck et al., 1986; Russell and Pihl, 1978). This study suggests that the relative size of the environment also has an important role.

Thus, apparatus variables and method of assessing activity can affect the results of activity studies. To interpret and compare data from various laboratories it is vital that these parameters be considered. Undoubtedly other factors (e.g., level of illumination, sound level, etc.) also affect behavior seen with AMPH and other drugs, and until these variables are fully examined, it should be assumed that they also may influence experimental results.

Conclusions

Activity of rats varied with chamber size. Chamber size also interacted with AMPH dose in its effects on activity. Rating scales and automated scores produced different activity profiles with AMPH.

Acknowledgements

AMPH was the generous gift of the Smith, Kline and French Co. 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.

References

- BECK, C.H.M., CHOW, H.L., and COOPER, S.J. (1986) Initial environment influences amphetamine-induced stereotypy: Subsequently environment change has little effect. *Behav Neural Biol* 46, 383-397.
- BENINGER, R.J. (1984) Effects of metergoline and quipazine on locomotor activity of rats in novel and familiar environments. *Pharmacol Biochem Behav* 20, 701-705.
- ELLINWOOD, E.H. and BALSTER, R.L. (1974) Rating the behavioral effects of amphetamine. *Eur J Pharmacol* 28, 35-41.
- HERZ, R.S. and BENINGER, R.J. (1987) Comparison of the ability of (+)-amphetamine and caffeine to produce environment-specific conditioning. *Psychopharmacology*, 92, 365-370.
- MAZURSKI, E.J. and BENINGER, R.J. (1987) Environment-specific conditioning and sensitization with (+)-amphetamine. *Pharmacol Biochem Behav*, 27, 61-65.

RUSSELL, R.L. and PIHL, R.O. (1978) The effect of dose, novelty, and exploration on amphetamine-produced stereotyped behavior. *Psychopharmacology* 60, 93-100.

Inquiries and reprint requests should be addressed to:

Evalynn J Mazurski,
Dept Psychology,
Queen's University,
Kingston, Canada, K7L 3N6