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Amphetamine-Induced On- and Off-Wall Rearing in Adult Laboratory Rats

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RUSSELL, K. H., M. GIORDANO AND P. R. SANBERG. *Amphetamine-induced on- and off-wall rearing in adult laboratory rats.* PHARMACOL BIOCHEM BEHAV **26**(1) 7–10, 1987.—Recently, Bauer [1] reported that amphetamine did not increase rearing behavior in adult rats. This result is at variance with many earlier reports demonstrating that amphetamine does increase rearing behavior. Because Bauer's automated measure only detected rearing when it occurred on the wall of the cage, it is possible that amphetamine only increased rearing behavior that occurred off the wall, which was not measured in his study. Bauer also included non-habituated animals in his study which might account for the discrepant results. The present experiment was performed to determine if, indeed, there was a difference between amphetamine induced on- and off-wall rearing and to determine to what extent habituation affected both types of rearing. The findings demonstrated that both types of rearing increased following d-amphetamine administration for both habituated and non-habituated animals. However, at the highest dose of d-amphetamine studied (4 mg/kg) rearing occurred mainly on the wall. Additionally, rearing behavior was found to increase following amphetamine when measured in the Digiscan Animal Activity Monitoring system which detects, but does not discriminate between, both on- and off-wall rearing.

Rearing d-Amphetamine Automated behavior Digiscan

FOR many years it has been consistently shown that amphetamine increases rearing in rats in a dose-dependent fashion (e.g., [3]). However, these results are at variance with those of a recent report by Bauer [1]. In the Bauer study, d-amphetamine administration did not increase rearing behavior in adult rats. The method used by Bauer was only sensitive to rears that occurred on the wall, since the apparatus required that the animal touch the metal walls in order to complete a circuit with the floor. Therefore, Bauer's methodology did not take into account the occurrence of off-wall rears. One reason for the discrepancy between Bauer's results and those of earlier researchers could be that amphetamine primarily increases rearing behavior that occurs away from the wall of the test cage. Another possible explanation is that Bauer used animals that had not been previously habituated to the open-field apparatus. As Glick [2] has shown animals can respond differently to dopaminergic drugs in habituated or non-habituated environments. Therefore, the present study was performed to differentiate between the effects of amphetamine on rearing that occurred on and off the wall of an open-field in habituated and non-habituated animals.

METHOD

Subjects

Seventy-one adult male Sprague-Dawley rats (400–600 g) were housed individually in stainless steel cages (24×11×20 cm). Food and water were available ad lib throughout the study except during the one hour observation period. The antibiotic, oxytetracycline, was administered prophylactically to the water of all rats. Preliminary studies have indicated that oxytetracycline alone does not influence open-field behavior as measured in Digiscan Monitors. The colony room had a twelve hour light/dark cycle which changed at 8:00 and 20:00 hours, respectively. Testing was performed between the hours of 10:00 and 14:00.

Apparatus

Four Digiscan-16 Animal Activity Monitors (Omnitech Inc., Columbus, OH) were used, as described elsewhere [6]. The printed activity variable "number of vertical movements" was used in this study, as earlier research has demonstrated its validity as a measure of rearing [5]. The

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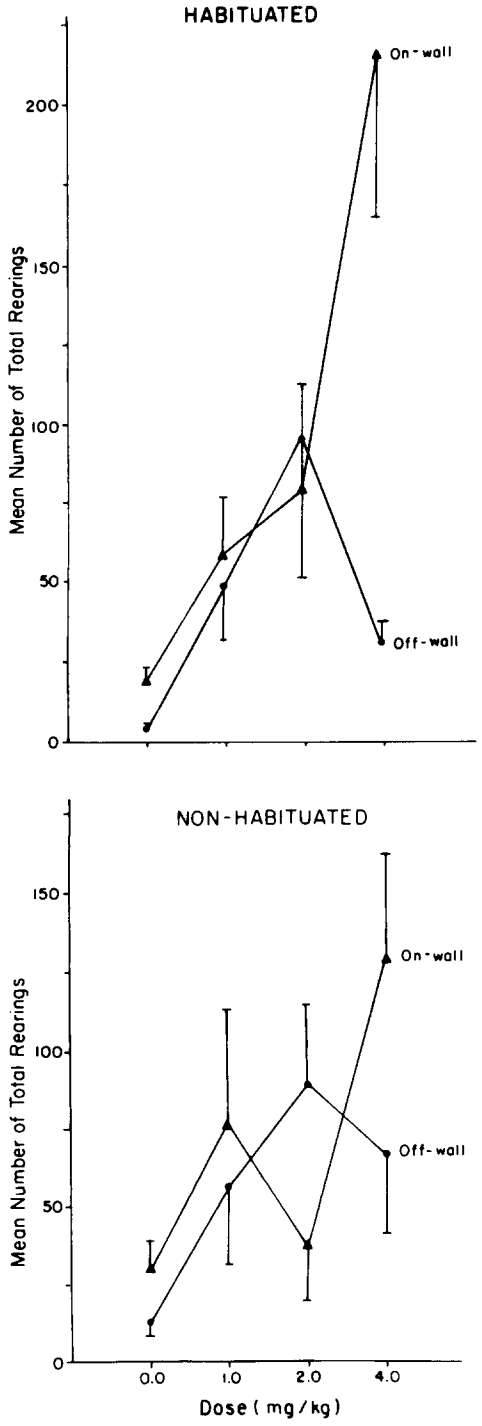

FIG. 1. Mean values of on- and off-wall rearing for habituated and non-habituated rats injected with various doses of d-amphetamine. Values of on-wall rearings and off-wall rearings are shown separately. The standard error for each mean is shown; $*p<0.001$ and $p<0.05$, respectively.

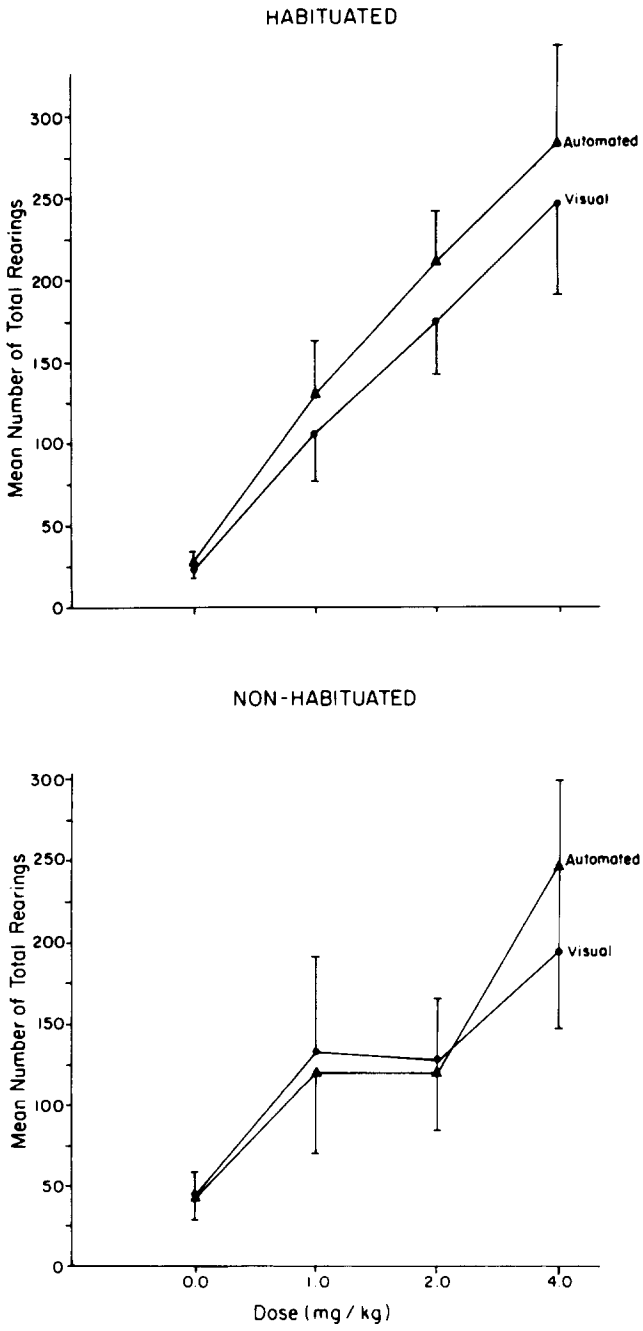

FIG. 2. Mean values of total rearing for habituated and non-habituated rats injected with various doses of d-amphetamine. Values for the automated and visual recordings are shown separately ($r=0.98$, $p<0.001$, for habituated animals), and for non-habituated animals ($r=0.95$, $p<0.01$). The standard error for each mean is shown; $*p<0.001$ for both conditions.

vertical beams were set at 6.6 in. above the floor of the cage. Each cage, whose sides were made of clear Plexiglas, contained cedar chips in the bottom. The monitors were located in a separate room with masking noise generated from a white noise amplifier.

Drug

d-Amphetamine sulfate (Sigma Chemical) was administered (IP) by the same experimenter at the following doses: vehicle control (0.0), 1.0, 2.0 and 4.0 mg/kg. The drug was diluted in physiological saline and injected in a volume of 1 ml/kg.

Procedure

Two experiments were conducted following similar procedures with the exception that animals were habituated to the test chamber in the first study and not in the second. In the first study thirty-nine rats were randomly assigned to one of four experimental groups. At 11:00 or 12:30 hours, each rat was placed into an activity monitor and habituated for one hour. Immediately following habituation animals were injected (IP) with either vehicle control (0.0), 1.0, 2.0, or 4.0 mg/kg of d-amphetamine and placed into an activity monitor. Visual and automated recordings of rearing behavior began immediately after injection and lasted one hour. For visual monitoring an experimenter was assigned to watch two animals and record rearing behavior as being on or off the wall. Prior to the beginning of the study, rearing behavior was operationally defined as being any vertical movement that raised the forepaws of the animal above the first level of infrared sensors within the activity monitor (6.6 in.) providing that the animal's hindpaws remained on the floor. Rearing was manually recorded as on-wall if the animals touched the wall anytime during a rearing movement. At the conclusion of the observation period, collection of data ceased and the animals were replaced into their home cages. In the second study thirty-two animals were randomly assigned to one of four treatment groups. At 12:00 or 13:30 hours, naive rats were injected (IP) with either vehicle control (0.0), 1.0, 2.0 or 4.0 mg/kg of d-amphetamine and immediately placed into an activity monitor. Automated and visual recordings of rearing behavior were recorded as described above.

Statistics

Two-way analysis of variance was used to evaluate the effects of the type of rearing (on-off wall), method of data collection (visual-automated) and drug dose, on the number of vertical movements. Tukey's HSD post-test comparisons were made for each dose group to evaluate differences between on- and off-wall rears, and between visual and automated measurements [4]. Scheffe's contrasts were then used to discriminate among drug dose effects [8]. Finally, a Pearson's correlation coefficient was determined for the automated and visual measures of total number of rears.

RESULTS

The means of the visually observed on- and off-wall rearing for all drug doses are shown in Fig. 1. Highly significant main effects of drug dose were found for habituated animals, $F(3,70)=10.15$, $p<0.001$, as well as on-off rearing, $F(1,70)=10.67$, $p<0.002$. A significant dose × on-off rearing interaction was also encountered, $F(3,70)=9.50$, $p<0.001$. Post-hoc comparisons revealed that the 4 mg/kg group had significantly more on-wall than off-wall rears ($p<0.001$). Significant main effects of drug were also found for non-habituated animals, $F(3,56)=3.67$, $p<0.05$. No significant effect was found for on-off rearing nor was the interaction between dose and on-off rearing significant.

Figure 2 shows the means for each dose group of total rears as obtained with the automated and the visual (combined on- and off-wall) measurements. For habituated animals a highly significant main effect across drug doses was found, $F(3,70)=18.68$, $p<0.001$. No other main effect or interaction was significant. Post-hoc contrasts of these results showed that regardless of the method of measurement used, the 4 mg/kg group had significantly more total rears than the 0.0 mg/kg or the 1.0 mg/kg group ($p<0.05$). The 2.0 mg/kg group differed significantly from the 0.0 mg/kg group ($p<0.05$). A significant main effect was also found for non-habituated animals, $F(3,56)=7.137$, $p<0.001$, with no other main effect or interaction reaching significance. Post-hoc contrasts again showed the 4 mg/kg group had significantly more total rears than the 0.0 mg/kg group ($p<0.001$) and the 2 mg/kg group ($p<0.05$). The 2.0 mg/ kg dose group was significantly different from the 0.0 mg/kg animals ($p<0.05$).

Although the habituated and non-habituated studies were performed independently we compared the two studies statistically. No significant interaction was found for habituated vs. non-habituated animals. However, in all dose groups except 0.0 mg/kg habituated animals exhibited a tendency to rear more than non-habituated animals, although not enough to reach significance. The correlation between the automated and visual measures was also very high for habituated animals ($r=0.98$, $p<0.001$) and for non-habituated animals ($r=0.95$, $p<0.01$).

DISCUSSION

This study supported previous findings that d-amphetamine significantly increased rearing behavior in adult rats in a dose-dependent fashion (e.g., [3]). However, these results are not in accordance with a recent study by Bauer [1] reporting no significant effect of various doses of d-amphetamine on the rearing behavior of adult rats. The method of measurement used in the latter study was sensitive only to on-wall rearing. Thus, it may be that the many previous studies showing a significant effect of d-amphetamine on rearing were due to the fact that this drug only increased off-wall rearing, a behavior not detected by Bauer's instrument. Previously, Schiorring [7] indicated that both rearing in free air, as well as rearing at the wall, was significantly increased by amphetamine. The present studies also showed dose-dependent increases in both on- and off-wall rearing. Furthermore, at higher doses (4 mg/kg) the rats reared much more on the wall than in free air. Generally, at 4 mg/kg of d-amphetamine, stereotypy starts to appear, which could influence rearing. At 5 mg/kg Schiorring [7] reported no rearing behavior (either on- or off-wall) in the sterotypy phase of amphetamine-induced behavior. Thus, an animal may be sitting in the cage away from the wall, engaging in stereotyped movements, reducing the number of rears in this area. However, rearing is greatly increased when the animal approaches the wall, since it can probably use the wall for postural support.

Although Glick [2] indicated that locomotor activity of rats treated with amphetamine is influenced by habituation to the testing apparatus, we found no differences in the general pattern of amphetamine-induced rearing in habituated or

non-habituated animals. Animals that were injected with d-amphetamine and habituated to the open-field did show more total rears than non-habituated animals, but not enough to reach significance. Therefore, the present results do not support the possibility that Bauer's discrepant results are due to the use of naive animals.

Both kinds of measurements used in the present experiment, visual and automated, demonstrated a significant dose-dependent increase in the total number of rears. Additionally, both measures were highly correlated and no differences were found between them. This supports earlier research demonstrating the validity of the Digiscan system [5]. However, the present study would suggest that it may be of interest for automated systems to differentiate between on- and off-wall rearing. The difference between these two measures may be of value in discriminating various doses of certain drugs. The large difference between on- and off-wall rearing behavior presently found with higher doses of amphetamine suggests that these two behaviors may be underlined by different mechanisms. Further research is needed in order to discover what these mechanisms may be.

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REFERENCES

1. Bauer, R. H. Differential effects of d-amphetamine and scopolamine on the ontogeny of rearing. *Pharmacol Biochem Behav* **21:** 321–323, 1984.
2. Glick, S. D. Changes in amphetamine sensitivity following frontal cortical damage in rats and mice. *Eur J Pharmacol* **20:** 351–356, 1972.
3. Hitzemann, R., J. Curel, D. Hom and H. Loh. Effects of naloxone on d-amphetamine and apomorphine-induced behavior. *Neuropharmacology* **21:** 1005–1011, 1982.
4. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences*, 2nd edition. Belmont, CA: Brooks/Cole Pub. Co., 1982.
5. Sanberg, P. R., T. H. Moran and K. L. Kubos. Automated measurement of rearing behavior in adult and neonatal rats. *Behav Neurosci* **98:** 743–746, 1984.
6. Sanberg, P. R., S. H. Hagenmeyer and M. A. Henault. Automated measurement of multivariate locomotor behavior in rodents. *Neurobehav Toxicol Teratol* **7:** 87–94, 1985.
7. Schiorring, E. Amphetamine induced selective stimulation of certain behaviour items with concurrent inhibition of others in an open-field test with rats. *Behaviour* **39:** 1–17, 1971.
8. Spatz, C. and J. D. Johnston. *Basic Statistics*, 2nd edition. Belmont, CA: Brooks/Cole Pub. Co., 1981.