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Digiscan Activity: Automated Measurement of Thigmotactic and Stereotypic Behavior in Rats

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SANBERG, P R , S A ZOLOTY, R WILLIS, C D TICARICH, K RHOADS, R P NAGY, S G MITCHELL, A R LAFOREST, J A JENKS, L J HARKABUS, D B GURSON, J A FINNEFROCK AND E J BEDNARIK *Digiscan activity Automated measurement of thigmotactic and stereotypic behavior in rats* PHARMACOL BIOCHEM BEHAV **27**(3) 569–572, 1987 —Visual measures of stereotypy, margin time (thigmotaxis or wall-hugging), and center time were correlated with automated measures using a revised 16 beam version of the Digiscan Animal Activity Monitor System Rats were injected with d-amphetamine (1 25, 2 5, 5 0 and 10 0 mg/kg), scopolamine (1 25 and 2 5 mg/kg) or saline and drugs were found to increase center time and decrease margin time in a dose-dependent manner, with the maximum effect occurring with 1 25 and 2 5 mg/kg, respectively At higher doses, an opposite effect was observed Extremely high correlations between visual and automated recordings of both margin time and center time were found Since thigmotaxic or wall-hugging behavior has been used as an indicator of emotionality in rats, the results of the present study suggest that these two locomotor variables may be useful additions to the Digiscan multivariate analysis of locomotor behavior It was also found that a redefinition of stereotypic behavior improved its correlation with visual measurements compared to earlier studies

Digiscan Thigmotaxis Wall-hugging Emotionality d-Amphetamine Scopolamine Locomotor behavior Stereotypy

THE open-field paradigm is a commonly-used technique for monitoring behavior in laboratory animals [1, 6, 9, 13, 16, 18] This technique has been used for small animals to measure ambulation [1], stereotypic behavior [3], emotionality [6], thigmotaxis or wall-hugging [11], and rearing [8] The effectiveness of using computerized automated systems in recording the motor activity of rodents has been successfully demonstrated [10, 14, 15], and has gained support as a means of reducing error due to observer biases and equipment calibration [2,15]

Until recently, some open-field behaviors, such as thigmotaxis, have eluded automation The thigmotactic tendencies of the rat in open-field situations have long been of interest [2, 4, 11, 13] Findings by Royce [13] suggested that thigmotaxis and penetration to the center of an open-field serve as indicators of emotionality in rats Hall [6] defined emotionality as a group of organic, experimental, and expressive reactions denoting a general upset or excited state of the animal Stimulant drugs are known to exert specific effects upon the amount of time spent in, or the number of entries into, the center of an open field by a rat [2,4]

Previous studies have demonstrated that d-amphetamine-treated animals tended to exhibit more entries into the center of the field, while scopolamine-treated rats rarely moved away from the periphery of the cage [2,4] It has been suggested that scopolamine interferes with an animal's habituation to a novel environment and thus inhibits exploratory behavior [5,7] This inhibitory effect may be related to an increase in emotionality in the rat, and subsequently produce a decrease in entries into the center of an open field The importance of thigmotaxis in psychopharmacological research suggests the need for its incorporation into automated systems of animal activity Recently, the Digiscan-16 Animal Activity Monitoring System (Omnitech Electronics, Columbus, OH) has added measures of margin and center times within the test cage The present study determined the validity of these measures

Amphetamine has also been reported to have marked ef-

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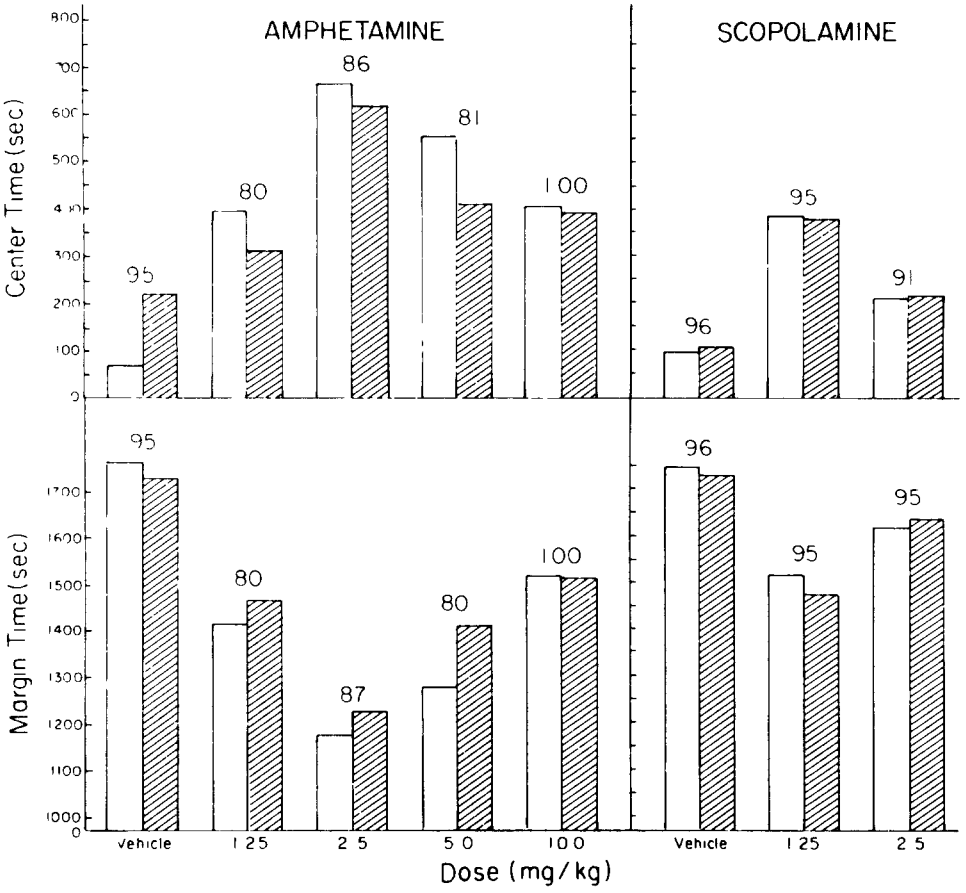


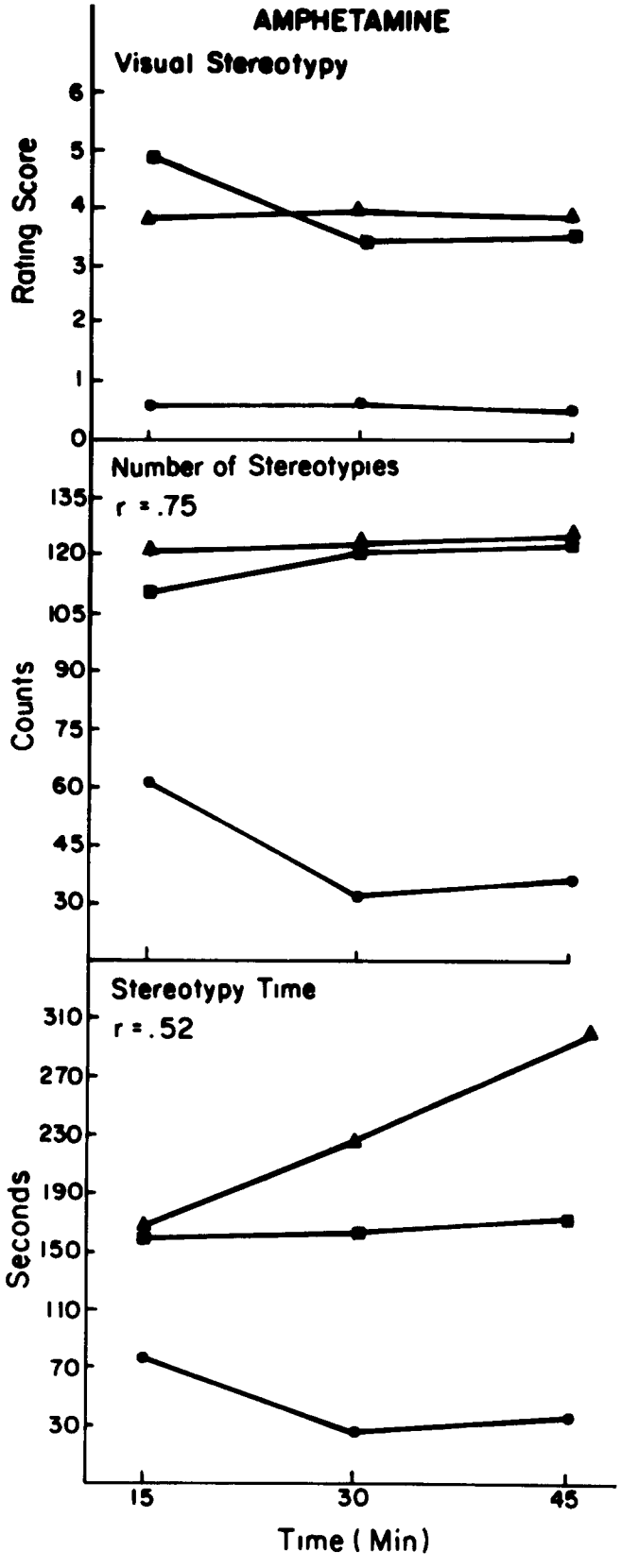
FIG 1 Mean time spent in the margin or center of Digiscan-16 Animal Activity monitors in response to d-amphetamine, scopolamine or saline (vehicle) Open bars represent visual observation measurements and hatched bars represent the automated Digiscan measures Correlation coefficients compare the respective automated to visual measures

fects on stereotypic behavior in rodents [8, 12, 15] Sanberg *et al* [14] tested the effects of 5 0 and 10 0 mg/kg d-amphetamine on stereotypic behavior in male Sprague-Dawley rats, using the earlier eight beam version of the Digiscan Animal Activity Monitor System Visual ratings of stereotypy, based on the stereotypy rating scale of Creese and Iversen [3], were significantly correlated with automated measures, and revealed that the drug produced significant increases in stereotypic behavior In the present study, the 16 beam version of the Digiscan system was also used to reinvestigate the behavioral effects of d-amphetamine in order to determine whether this newer system produced a more accurate measure of stereotypy

METHOD

Twenty-four male Sprague-Dawley rats (200 to 300 g) were maintained individually in stainless-steel cages (24×11×20 cm) and kept on a 12 hr light/dark cycle with food and water available ad lib Prior to behavioral assessment, the rats were placed singly in one of four Digiscan Animal Activity Monitor cages and allowed 30 min for habituation The animals were then injected intraperitoneally (IP) with either 1 25, 2 5, 5 0, or 10 0 mg/kg of d-amphetamine (Sigma, St Louis, MO), or 1 25 or 2 5 mg/kg of scopolamine (Sigma, St Louis, MO) or saline in a volume of 1 mg/kg, and replaced into the activity monitor A counter-balanced design was used in which each rat received every dose of d-amphetamine, scopolamine, and saline over the course of the experiment, based upon the drug and dosage administered in the first session A rest period of at least 48 hours was alloted between injections Four animals were tested in each experimental session, and there were two sessions per day (between 11 00 and 16 00 hours) A double-blind procedure was used throughout the study Measurement of motor activity began 15 min after injection, and continued for a period of 30 min, at which time the dependent measures were recorded on a silent printer

Stereotypic behavior, margin time, and center time were recorded in an automated manner by the Digiscan-16 system, and also by visual observation The Digiscan Animal Activity Monitoring System (Model RXYZCM, Omnitech Electronics, Inc , Columbus, OH) consists of vertical and horizontal infrared sensors, 16 per side, surrounding an acrylic activity monitor cage (40×40×30 5 cm) A similar Digiscan-8 system has been described in detail previously [15] In the present system, margin time was operationally defined as the animal's center of gravity being within 6 35 cm or less of the cage wall The remaining time in which the rat did not enter this area of the cage was considered center time For visual



observation, the center of gravity of each animal was marked with black dye on the center of its back in order to assist the observers in monitoring margin and center time One observer was randomly assigned to each rat, and measured margin time with a stop watch The time an animal spent in the margin was subtracted from the total observation time of 30 min in order to calculate center time

Stereotypic behavior was visually rated every 15 min during a 30 min period using the stereotypy rating scale of Creese and Iversen [3] The ratings were 0=asleep or stationary, 1=active, 2=predominantly active but with bursts of stereotyped sniffing or rearing, 3=stereotyped activity such as sniffing along a fixed path in the cage, 4=stereotyped sniffing or rearing maintained in one location, 5=stereotyped behavior in one location with bursts of gnawing or licking The raters were unaware of the drugs used The Digiscan measure of stereotypic behavior was defined as the time spent breaking the same beam pattern repeatedly (stereotypy time), and the number of episodes of this stereotypic behavior (number of stereotypies) Each episode was separated by at least one second

RESULTS

The effects of amphetamine and scopolamine on margin and center time are illustrated in Fig 1 As shown, high correlations were found between visual automated measures of these two variables In addition, an analysis of variance demonstrated that there were no significant differences between automated and visual recordings over all doses of amphetamine and scopolamine All dosage levels of amphetamine were found to have significant effects on margin time, $F(4,140)=10\ 8$, $p<0\ 001$, and center time, $F(4,140)=11\ 8$, $p<0\ 001$ The same was true for the influence of scopolamine on margin time, $F(2,48)=6\ 8$, $p<0\ 002$, and center time, $F(2,84)=6\ 6$, $p<0\ 002$

Figure 2 depicts the effects of amphetamine on stereotypic behavior A significant correlation was found between visual and automated recordings of the number of stereotypies ($r=75$, $p<0\ 05$), and between visual and automated recordings of stereotypy time ($r=52$, $p<0\ 05$) Amphetamine, over all doses, had a significant effect on stereotypy measured visually, $F(2,42)=29\ 1$, $p<0\ 001$, number of stereotypies, $F(2,42)=11\ 68$, $p<0\ 001$, and stereotypy time, $F(2,42)=22\ 74$, $p<0\ 001$

DISCUSSION

Automated and visual recordings of thigmotactic behavior were found to be highly correlated across all dosage levels of d-amphetamine and scopolamine These high correlations demonstrated the reliability and validity of the 16 beam Digiscan System in recording thigmotaxis, and indicated the usefulness of automated monitoring techniques to measure margin and center time

The effects of amphetamine and scopolamine upon margin and center time appear to be dose-dependent Both drugs showed similar dose-response curves Intermediate doses of

FIG 2 Mean stereotypy response to d-amphetamine (5 0 mg/kg, squares, 10 0 mg/kg, triangles), and saline (circles) as measured by a visual rating scale (visual stereotypy) and Digiscan-16 Animal Activity Monitors (number of stereotypies and stereotypy time) Correlation coefficient compares the respective automated measure to visual stereotypy

d-amphetamine (2 5 and 5 0 mg/kg) produced the greatest increase in center time, while the lowest and highest doses of d-amphetamine (1 25 and 10 0 mg/kg) produced the greatest increase in thigmotaxis 1 25 mg/kg scopolamine resulted in increased center time, while 2 5 mg/kg resulted in increased margin time The results of the present investigation differ with those of Geyer *et al* [4], who reported a decrease in center entries over increasing dosage levels in scopolamine-treated animals at doses starting at 0 5 mg/kg A study by Stewart and Blain [17] reported that scopolamine produced an inverted U dose-response effect on activity, with activity peaking at 1 0 mg/kg It is therefore, possible that a general increase in activity brought about by scopolamine is responsible for the increased center entries at this dose The results of this study concerning the effects of amphetamine on thigmotaxis and center time are consistent with previous reports [2,4]

The 16 beam Digiscan analyzer appears to also produce accurate and reliable measurements of stereotypic behavior in rats Use of this system resulted in higher correlations between visual and automated recordings of stereotypy for amphetamine-treated rats than were found previously with the eight beam version [14] The present study did not reveal a significant difference between the 5 0 mg/kg and 10 0 mg/kg dosage levels of d-amphetamine with visual observation, however, the 16 beam Digiscan recorded a significant difference between these doses when measuring stereotypy time This finding indicated that the automated measure of stereotypy time may be more sensitive than visual observation to dose-response effects Furthermore, the use of standardized monitoring system eliminates experimenter bias which may influence the measurement of drug-induced stereotypy In the study by Sanberg *et al* [14], visual observation of stereotypic behavior resulted in a significant difference between the 5 0 and 10 0 mg/kg doses of d-amphetamine and it is possible that the discrepancy between the earlier study and the present findings were due to differences between observers

Overall, the present findings indicated that the 16 beam Digiscan is an improvement over the eight beam version in producing reliable, valid measures of stereotypy, margin time and center time The addition of the Digiscan program to measure thigmotactic behavior will prove useful in determining the effects of various drugs upon this behavior and hence their role in emotionality in rats The improvement of automated multivariate techniques to monitor locomotor behavior is certainly a step forward in exploring the behavioral pharmacology of movement

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