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Automated Multivariate Measurement of Spontaneous Motor Activity in Mice: Time Course and Reliabilities of the Behavioral Measures

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OSSENKOPP, K -P , L K MACRAE AND G C TESKEY *Automated multivariate measurement of spontaneous motor activity in mice Time course and reliabilities of the behavioral measures* PHARMACOL BIOCHEM BEHAV **27**(3) 565–568 1987 —A variety of automated procedures have been developed to measure certain aspects of spontaneous motor activity in small animals The present study used a Digiscan Animal Activity Monitor to measure six different aspects of spontaneous motor behavior in male CF-1 mice The Digiscan system uses infrared beams and computer analysis to quantify various behavioral variables The mice were tested for 1 hour on 2 different days of the week for 3 consecutive weeks Both the temporal changes in the measured variables and the test-retest reliabilities were examined in a group of 30 mice Statistical analysis of the data revealed significantly higher mean values for total movement time, average distance travelled, and horizontal activity on the first test session relative to the second session ($p<0\ 01$) The other 3 measures, total distance travelled, number of movements, and average speed, did not vary significantly across test sessions All 6 behavioral variables showed good test-retest reliabilities and these could be increased by aggregating the data on a weekly basis The present results indicate that the measures obtained from the Digiscan system are reliable and that the animals should first be habituated to the test apparatus in order to obtain stable baseline activity values

Motor activity Open-field Automated monitor Reliabilities Aggregation Time course Mice Digiscan

THE measurement of locomotor behavior is central to obtaining information about an animal's behavior in general and important in indexing the behavioral effects of putative toxic substances, therapeutic agents, and other experimental manipulations Measurement of spontaneous motor activity can range from simple direct observation to sophisticated automated procedures which can provide measurements of a number of behavioral variables Reviews of various procedures used to measure motor activity in general psychobiological research or psychopharmacological investigations (e g , [3,11]) have described various techniques ranging from the popular open-field test to automated photocell cages, stabilimeters, and running wheels, among others

The open-field test has attained the status of one of the most widely used instruments in animal behavior analysis (cf [16]) Quantification of behavior in this apparatus is typically obtained via direct visual observation or use of a video recording procedure [6,16] The number of different behaviors that have been measured in the open-field test has increased to over 20 [16], but only a few have been shown to be reliable for rodents [4, 5, 9, 15] In an effort to avoid many of the problems associated with direct visual observation of behavior in the open-field test, several automated "open-field" instruments have been designed and tested (e g , [1,12]) These instruments use an array of infrared photobeams and beam interruptions are then interpreted by a computer to simultaneously reveal many aspects of the spontaneous activity of the test animal in the apparatus The Digiscan Animal Activity Monitor (Omnitech Electronics, Inc , Columbus, OH) is one such automated computer based system which has proven useful in a variety of experiments (e g , [7, 13, 14]) However, before full use can be made of the Digiscan system, this new technique needs to be standardized by comparing results obtained in this instrument with the more traditional direct observation procedures In addition, information about the reliability and generalizability of the automated behavioral measures is also needed Evidence for the validity of the automated measures, when compared to direct observation of behavior, has already been provided for rearing response measures [14] and stereotypic behavior [13] In the present study we examined the time course and reliabilities of several behavioral

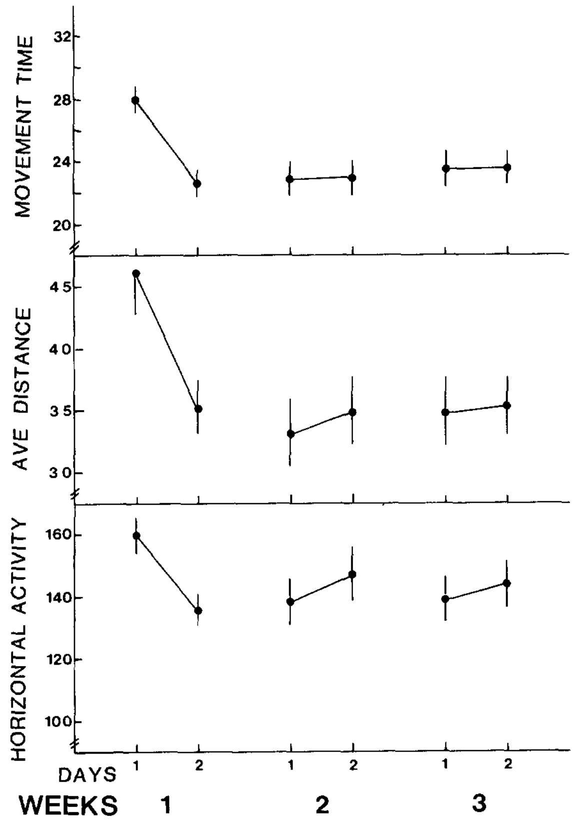

FIG 1 Mean response values for movement time (seconds), average distance travelled per movement (inches, 2 54 cm/inch), and horizontal activity (number of photobeam interruptions) as a function of test sessions Response values were cumulated in 10 minute time bins and then averaged for the 60 minute test session The error bars are standard errors of the mean

measures obtained in the automated Digiscan system when mice were repeatedly tested We also examined the effects of score aggregation [9] on the test-retest reliabilities of the measures

METHOD

Subjects

Sexually mature male CF-1 mice (Charles River, Quebec) 25–30 g, were held in groups of 4 in standard polyethylene cages with stainless steel wire tops The colony room was maintained at 22±1°C and kept on a 12 hr light 12 hr dark cycle with the light on from 700 to 1900 hr Food (Purina pellets) and water were freely available

Apparatus

The automated activity monitoring system consisted of two Digiscan Animal Activity Monitors (Omnitech Model RXYZCM [16]) The monitors consisted of clear Plexiglas cages measuring 40×40×30 5 cm with infrared monitoring sensors mounted every 2 54 cm along the perimeter (16 beams along each side) and 4 5 cm above the floor of the cages Data were collected and analyzed by a Digiscan Analyzer (Omnitech Model DCM) which in turn transmitted the data to an Apple II Plus computer with disk drive, where it was stored

TABLE 1

MEAN CORRELATION COEFFICIENTS AND RANGES OBTAINED FOR THE INTERCORRELATIONS FROM THE 6 TEST SESSIONS AND FOR THE INTERCORRELATIONS FROM THE 3 AGGREGATED WEEKLY SCORES

Dependent Variable	Test Sessions $\bar{r}$	range	Weekly Aggregates $\bar{r}$	range	Increase r^2
HA	636	(40– 77)	763	(68– 84)	178
TD	562	(21– 76)	680	(54– 84)	146
MT	585	(31– 75)	730	(59– 86)	191
AS	494	(20– 75)	657	(62– 68)	188
NM	488	(26– 69)	577	(48– 66)	095
AD	555	(33– 71)	700	(64– 78)	182

The right hand column gives the increase in r^2 produced by the aggregation procedure Note the critical r for $p<0$ 05 is 0 361 for $df=28$

Procedure

Testing of the animals was carried out between 1300 and 1600 hr The Digiscan system was set to operate in the two animals per monitor operation mode In this mode Plexiglas partitions divided each monitor into 4 equal quadrants and one animal was placed in the left front quadrant of the monitor, with another animal in the right rear quadrant Thus, each mouse had 20×20 cm of floor space The Digiscan Analyzer then collected data for each animal separately The mice were tested for 1 hr at a time, 2 days per week (either Monday and Wednesday, or Tuesday and Thursday) for 3 consecutive weeks Each animal was tested on the same days of each week The test apparatus was subjected to normal room light levels during the test sessions

System-differentiated behavioral variables recorded for each test session were (a) horizontal activity (HA)—total number of beam interruptions, (b) total distance (TD)—distance travelled by the animal, (c) movement time (MT)—amount of time the animal was in motion, (d) average speed (AS)—average distance travelled per given time interval, (e) number of movements (NM)—number of separate horizontal movements executed by the animal with a minimum stop time of 1 second to separate movements, and (f) average distance (AD)—mean distance travelled per horizontal movement

Data Analysis

A data matrix was constructed for each of the six dependent variables consisting of total scores on each of the test sessions for each of the subjects A subject by treatments design analysis of variance test examined the effects of days within weeks (factor 1) and weeks (factor 2) on the mean response values for each of the variables Next, weekly aggregate scores (Day 1 + Day 2) were calculated for each subject and added to the data marix A zero-order product-moment correlation matrix was computed to examine the test-retest reliabilities of each of the variables for test session scores and for aggregated scores (weekly totals) Mean correlation coefficients were calculated for the 15 intercorrelations obtained from the 6 test sessions and for the 3

TABLE 2

MEAN CORRELATION COEFFICIENTS AND RANGES OBTAINED FOR THE INTERCORRELATIONS OF SESSION 1 DATA WITH DATA FROM SESSIONS 2–6 (FIRST COLUMN) AND FOR THE INTERCORRELATIONS FROM SESSIONS 2–6 (SECOND COLUMN)

Dependent Variable	Session 1 $\bar{r}$	Session 1 range	Sessions 2–6 $\bar{r}$	Sessions 2–6 range	Increase r^2
HA	570	(40– 65)	669	(56– 77)	123
TD	422	(21– 56)	632	(50– 76)	221
MT	530	(31– 66)	613	(42– 75)	095
AS	350	(20– 53)	566	(42– 75)	198
NM	438	(26– 69)	513	(43– 59)	071
AD	516	(33– 68)	574	(37– 71)	063

The right hand column shows the increase in r^2 for the respective variables in the second column relative to those in the first column Note the critical r for $p<0\ 05$ is 0 361 for $df=28$

intercorrelations obtained from the 3 aggregated scores An alpha value of 0 05 was used for all hypothesis testing procedures and post-hoc tests for the analysis of variance were carried out with Newman-Keuls procedure [10]

RESULTS

Temporal Changes in the Dependent Variables

The results of the analyses of variance indicated that there were significant changes in the mean values of 3 of the variables as a function of test sessions The data for these 3 variables are presented in Fig 1

The analysis for movement time revealed a significant main effect of Days, $F(1,174)=4\ 07$, $p=0\ 045$, and a significant Days by Weeks interaction, $F(2,174)=4\ 33$, $p=0\ 015$ A significant main effect for the Weeks factor was obtained for the average distance variable, $F(1,174)=3\ 24$, $p=0\ 042$, as well as a significant Days by Weeks interaction, $F(2,174)=3\ 12$, $p=0\ 047$ For the horizontal activity variable the only significant effect was a Days by Weeks interaction, $F(2,174)=3\ 11$, $p=0\ 047$ In all cases the obtained effects were the result of significantly larger mean values on the first test session relative to the second test session ($p<0\ 01$), and for movement time and average distance the first test session values were also significantly greater than those for all the other test sessions ($p<0\ 05$)

Test-Retest Reliabilities of the Dependent Variables

The means and ranges of the correlation coefficients for both the test session comparisons and comparison of the aggregated scores are presented in Table 1 All of the variables exhibited significant average reliabilities for daily test sessions scores (range 0 488 to 0 636) Scores aggregated into weekly totals exhibited an increase in the average correlation value (r) for all six of the variables The increase in r^2 ranged from 0 095 to 0 191, demonstrating substantial gains due to score aggregation

Mean correlation coefficients for the daily test session scores were also calculated for comparison of the first test session to the other 5 sessions, and for the 10 intercorrelations from Sessions 2 to 6 The means and ranges for these correlation coefficients are presented in Table 2 Examination of Table 2 shows that the mean correlation coefficients are greater if the data from test session 1 are not used in computing the mean values of the correlations The improvement in strength of correlation is especially evident for the total distance and average speed correlations, since r^2 increased 0 221 and 0 198, respectively

DISCUSSION

The results of the present study demonstrate that all of the dependent variables obtained in the automated Digiscan activity apparatus exhibit substantial reliability across test sessions Even greater reliabilities were obtained when the data were aggregated on a weekly basis (two test sessions) A previous study using a Digiscan automated activity system obtained test-retest correlations of 0 69 for total distance, 0 73 for average distance, and 0 77 for average speed in 3 month old male rats retested at 3-day intervals [7] These values compare favourably with the ones obtained in the present study (cf Table 1) The increase in reliability for scores aggregated over two test sessions is also consistent with previous demonstrations of aggregation effects for activity data from rats tested in an open-field [9] The principle of aggregation is based upon the notion that the sum of a set of multiple measures is a more stable and unbiased estimator than any single measure from the set (cf [2]) By combining several measurements over time the error components are averaged out, producing a more stable measure Using such aggregated scores for calculation of correlation coefficients results in a better estimate of the strength of the true underlying relationships (cf [8, 9, 15])

The present data also demonstrate that scores on the first test session often differ substantially from those obtained on subsequent test sessions (see Fig 1 and Table 2) This "first-time encounter" effect with a particular stimulus situation has been noted previously [2,8] This effect may reflect an underlying neophobic reaction to the test environment, or an "emotional reactivity" factor, and if a stable baseline of activity levels is required, against which the effects of some experimental manipulation is to be compared, then pre-exposure to the apparatus is advisable in order to eliminate any major interaction of the "first-time encounter" effect with the effects of the experimental manipulation

In summary, the present study demonstrates that the automated measurement of spontaneous locomotor activity in mice by means of a Digiscan Activity Monitor is accomplished with system-differentiated variables that are reliable, and the reliability of these variables can be increased by application of the principle of aggregation Finally, since some of the variables seem to be influenced by a "first-time encounter" effect, the present data suggest that habituation to the test environment, prior to implementation of the experimental manipulation, is advisable if a stable activity level baseline is desirable for comparison purposes

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