Pharmacology Biochemistry & Behavior, Vol. 6, pp. 351–353.

BRIEF COMMUNICATION

A New Device for the Rapid Measurement of Impaired Motor Function in Mice[1,2]

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(Received 8 December 1976)

COUGHENOUR, L. L., J. R. MCLEAN AND R. B. PARKER. *A new device for the rapid measurement of impaired motor function in mice.* PHARMAC. BIOCHEM. BEHAV. 6(3) 351–353, 1977. – Measurement of the ability of mice to balance on a rotating rod or cone is often used as a measure of impaired motor function. In most of these procedures the mice must be trained prior to the test. In the new screen test described in this paper, untrained mice are used in a 60 sec test which measures the ability of mice to either climb to the top of or cling to the bottom of a horizontal screen. The ED_{50} values obtained for failure to reach the top of the horizontal screen are similar to those obtained with the rotarod; the values for falling from the screen are somewhat higher. With both of the horizontal screen measures there were fewer control failures than in the rotarod procedure.

Mouse Motor function Rotarod

MEASUREMENT of the ability of mice to balance on a rotating rod or cone is often used as a measure of impaired motor function [1, 2, 3, 4, 5]. In most of these procedures, the mice must be trained prior to the test, a process which involves considerable time and which means a certain percentage of mice that cannot pass the test even with training must be eliminated from use in the experimental work. In our laboratory mice are trained in three sessions on the day before a test and in one session on the day of the test. Two days are required to perform just one test. Similar procedures are followed in other laboratories [1, 2, 4, 5].

The screen test described in this paper was developed as an alternative to the rotarod test. In this test, untrained mice are used and the test itself lasts only 60 sec. A great deal of time and effort are thus saved. The studies reported in this paper show that the newly developed screen test provides basically the same kind of information as the rotarod but at much less expense in terms of time, effort, and the number of mice required.

METHOD

For both the rotarod and horizontal screen tests, male Swiss-Webster mice which weighed approximately 20–28 g were used. The mice were fasted for approximately 18 hr before being dosed orally with the test drugs or vehicle (0.05% methocel).

The protocol for the rotarod test was as follows: the mice were given three training sessions on the day before the test and one training session on the day of the test. Mice that fell from the 2.9 cm diameter rod, which was rotated at a constant 15 rpm, in 110 sec or less were scored as having failed the test. Mice which failed the test during the fourth training session were not used in the drug experiments.

In the horizontal screen test, untrained mice are placed individually on top of a square (13 cm × 13 cm) wire screen (No. 4 mesh) which is mounted horizontally on a metal rod. Six such screens are mounted on a single rod so six mice can be tested simultaneously (Fig. 1). The rod is then rotated 180 degrees so that the mice are on the bottom of the screens. The majority of mice not treated with drugs either climb to the top of the screen or cling to the bottom during the 60 sec test period. Two values are recorded: (1) the number of mice that fall from the screens, and (2) the number of mice that fail to climb to the top of the screens (i.e. the sum of those that remain clinging to the bottom of the screens and those that fall from the screens).

Experiments were designed such that the same mice

[1] This research was carried out with animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

[2] A preliminary report of this work was presented as a poster demonstration at the Neuroscience Meetings in Toronto, 1976 (*Neurosci Abstracts*, II: 865, 1976).

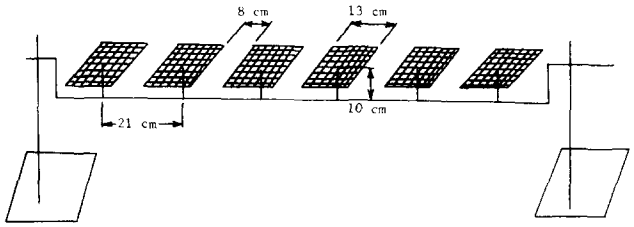

FIG. 1. Schematic drawing of the horizontal screen apparatus. The drawing shows the apparatus as it would appear at the start of an experiment; i.e. before the rod is rotated 180 degrees.

were used in both the rotarod and screen tests, and mice were tested 30, 60, 90, and 120 min after being dosed. In a given experiment, the mice at each dose were divided into two groups. At the first test time (30 min after dosing) half the mice were tested first on the rotarod, then on the screen test; the other half were tested in the reverse order. At the second test time those mice that were initially tested first on the rotarod were tested first on the screen, and vice versa. This alternating pattern was used throughout the experiment in an effort to balance out any influence due to fatigue or the order of testing. Data were analyzed with the nonlinear regression technique developed by Waud [7] for quantal data.

The following drugs were used: chlordiazepoxide hydrochloride, chlorpromazine hydrochloride, diazepam, haloperidol, sodium phenobarbital, and thioridazine hydrochloride. All compounds were administered orally with 0.05% methocel used as the vehicle. Compounds which did not go into solution were sonicated to produce suspensions. All doses are given in terms of the free base.

RESULTS

Results of the experiments designed to compare the two tests are presented in Table 1. Although tests were done at 30, 60, 90, and 120 min after dosing, only the results of the 60 min test are given in the table. Similar results, particularly in regard to the comparison between tests, were obtained at the other times, so for the sake of simplicity we chose to present data from only one test period. One can see that the ED_{50} values obtained with the rotarod test agree quite well, in general, with those based on the failure of mice to climb to the top of the inverted horizontal screen. ED_{50} doses calculated on the basis of the number of mice which fall off the screen are higher in all cases. This reflects the fact that it is easier for mice simply to cling to the inverted screen than it is to climb over the edge of the screen to the top. In one instance (chlorpromazine) the ED_{50} for failure to reach the top of the screen is slightly (not statistically significant ($p>0.05$)) higher than the ED_{50} for falling off the screen. This apparent discrepancy in the data is due to the fact that there happened to be two control mice which failed to reach the top of the screen whereas none of the control mice fell off the screen in this experiment. This difference in the control data for the two parts of the test accounts for the difference in the reported ED_{50} values.

With both measures of impaired motor function derived from the screen test, there were fewer control failures than with the rotarod procedure. The pooled values from all experiments summarized in Table 1 show that for failure to reach the top of the screen, 3 out of 67 control mice tested failed, and for failure to cling to the screen, only 1 out of 67 control mice failed. With the rotarod, 13 out of 67 control mice failed.

The standard errors associated with the estimated ED_{50} values tend to be a smaller percentage of the mean with the screen test than with the rotarod test. It is also noteworthy that with three of the rotarod experiments the dose-response relationship was very poor and the ED_{50} doses had to be estimated by visual inspection of the data.

DISCUSSION

The information presented in Table 1 suggests that the screen test described in this paper provides two somewhat different indices of motor function impairment in mice. The ED_{50} doses for failure to climb to the top of the screen in general agree well with those obtained with the rotarod, although there is a tendency for the neuroleptics, especially chlorpromazine and haloperidol, to require higher doses to impair behavior on the inverted screen than the rotarod. The significance of this potential difference between the two procedures is not clear. The apparent discrepancy with haloperidol could be due at least in part to the fact that the dose-response relationship with haloper-

TABLE 1

ED_{50} VALUES OBTAINED FROM THE ROTAROD AND SCREEN TESTS

Compound	$ED_{50} \pm SE$ Rotarod*	Screen Test Fail to Reach Top†	Screen Test Fall off Screen‡
Chlordiazepoxide	46.0 ± 9.70	41.4 ± 10.0	57.4 ± 11.4
Chlorpromazine	4.67 ± 2.30	31.9 ± 10.8	27.8 ± 5.27
Diazepam	≅ 7§	6.19 ± 2.75	9.21 ± 4.52
Haloperidol	≅ 2§	15.1 ± 2.81	38.0 ± 5.59
Phenobarbital	66.2 ± 11.9	80.4 ± 10.3	84.6 ± 9.33
Thioridazine	≅ 32§	37.7 ± 5.45	102. ± 22.2

*Dose (mg/kg) required to make 50% of the mice fall off the rotarod in 110 seconds or less.
†Dose (mg/kg) required to make 50% of the mice fail to climb to the top of the inverted screen.
‡Dose (mg/kg) required to make 50% of the mice fall off the inverted screen.
§Poor dose-response; ED_{50} estimated by visual inspection of the data.

idol was very poor in the rotarod test. The discrepancy with chlorpromazine remains unexplained. Overall then, both failure to reach the top of the inverted screen and the rotarod appear to provide information on the same type of motor function impairment. Doses required to make 50% of the mice fall off the screen are higher than those required to induce 50% failure in climbing to the top of the screen, as one might expect. This part of the screen test seems to provide a quantitative measure of the grasping reflex rather than of the more complex motor coordination required in climbing movement.

The lower number of control failures in the screen test means that one is working with a more stable baseline and would make one expect to see a lower variance in the estimation of effective dose levels, an expectation which our results tend to fulfill. However, the very low control failure rate also means that one cannot measure improvement in performance, something which the accelerating rod procedure of Jones and Roberts [3] and Watzman and Barry [5] apparently can do.

The ED_{50} values for the rotarod test provided in Table 1 agree well with those estimated by other workers. Kršiak [4] reported ED_{50} values of 47, 8.8, and 4.1 mg/kg for chlordiazepoxide, chlorpromazine, and diazepam respectively (oral administration 30 min prior to the test). Based on estimates from illustrations in their papers, Christensen [1], Jones and Roberts [3] and Watzman and Barry [5] all appear to find a 50% decrement in performance on the rotarod with chlorpromazine in the dose range of 3 to 5 mg/kg.

In summary, the screen test introduced here appears to provide the same type of information as the rotarod procedure but with several advantages. Because untrained mice are used, one saves the time used to train mice as required in some of the rotarod protocols [1, 2, 4]. Fewer control failures and less variability in the test in general mean that fewer mice can be used to get the same information as provided by the rotarod. The fact that the apparatus is stationary and very simple in design means that one does not have to be concerned about the many parameters, such as diameter of the rod and rotation speed, which play a role in the rotarod test [6]. The experimenter must, as in most tests which involve animals, be alert to note instances in which results may be distorted due to effects of the test agents other than those of primary concern (e.g. a compound may cause immobilization of the mice along with an increased tendency of the mice to cling to the screen), but overall we have found the screen test to be easier to perform and more reliable than the rotarod test.

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