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Tube Running Activity in Mice: A Method to Evaluate the Behavioural Effects of Drugs

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PERSSON, S.-Å. AND G. WAHLSTRÖM. *Tube running activity in mice: A method to evaluate the behavioural effects of drugs*. PHARMAC. BIOCHEM. BEHAV. 12(2) 259–264, 1980.—A new method to study locomotor activity in mice has been developed. The time it takes for a mouse to run 100 cm in a narrow tube is measured. In the present study the effects of various environmental factors on this tube running activity have been examined. The most important factor was found to be the frequency of testing. With repeated testing the run time was increased, which probably is due to a decrease in exploratory activity. However, tube running seems not to be a specific measure of exploratory activity. Administration of nialamide alone (50–200 mg/kg IP) as well as combined with 5-hydroxy-DL-tryptophan (6.25–25 mg/kg IP) gave a dose dependent decrease in run time. The result of the present drug studies suggests that tube running activity may be useful for measuring 5-hydroxy-tryptamine mediated behaviour.

Tube running activity Exploratory activity Mice Nialamide Monoamine oxidase inhibition 5-Hydroxy-tryptamine

ADMINISTRATION of 5-hydroxy-tryptophan (5-HTP) to mice pretreated with inhibitors of monoamine oxidase (MAO) produces excitation with increased motor activity, tremor in the head and the forelegs as well as hyperextension and abduction of the hind legs [5,7]. The movements of the hind legs are ineffective for forward locomotion on a flat surface. However, preliminary experiments have shown that mice pretreated in a similar manner will pass through a narrow Plexiglas tube faster than untreated controls [10,11]. We now show that this behaviour can be quantified and used as a test. Various factors which influence this test are examined. The possible involvement of central 5-hydroxy-tryptaminergic mechanisms are considered.

METHOD

Subjects

Male N.M.R.I. mice weighing 20 g were purchased from Anticimex, Sollentuna, Sweden. Batches of 20 mice were kept in makrolon cages (550×330×200 mm). The animals were fed on Anticimex commercial type pelleted diet 213-3. They had access to food and water ad lib except during the experiments, when only water was available. The artificial light in the animal room was turned on at 08:00 and off at 20:00 hr.

The temperature varied between 22–27°C and the humidity between 20–80%. An adaptation period of one week was allowed before any experiments were performed. The animals were used in only one experiment, except when otherwise stated.

Apparatus

Figure 1 shows the experimental equipment. The runway consisted of a Plexiglas tube with an inside diameter of 34 mm. The start box was made of the same tube and was separated from the rest of the tube by a guillotine door. The start box was connected to a source of compressed air via a stop-cock and an air flow meter, TG 300 (Kebo-Grave, Sweden). In the present experiments two identical equipments were used and placed so that they were not mutually visible for the mice.

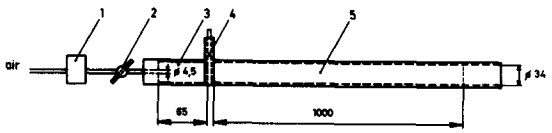

FIG. 1. The experimental equipment. (1) Air flow meter, TG 300; (2) Stop-cock NS 14.5, 2.5 mm; (3) Start box; (4) Guillotine door; (5) Plexiglas tube. All measurements are given in mm.

Drugs

5-Hydroxy-DL-tryptophan (5-HTP) and nialamide were purchased from Sigma Chemical Comp., St. Louis, MO. Nialamide was dissolved in acid saline solution and was neutralized with NaOH to pH 3–5. All other drugs were dissolved in 0.9% saline. The solutions were made just before injection. All drugs were administered intraperitoneally in a volume of 33 ml/kg. If no drug was administered an equal volume of 0.9% saline was given.

Experimental

The experiments were performed between 10:30 and

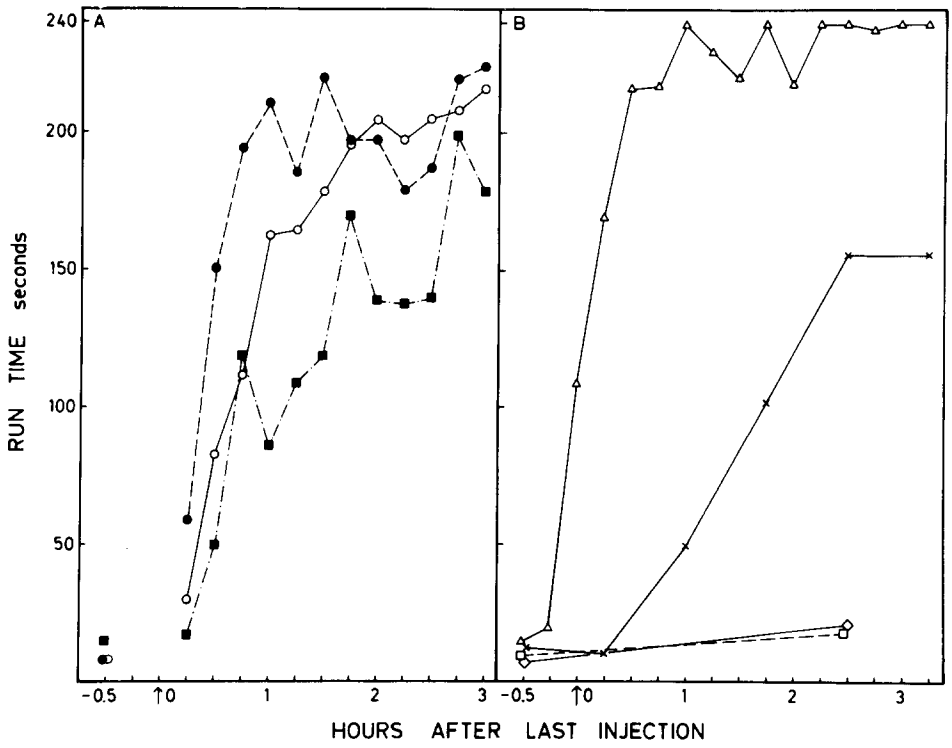

FIG. 2. (A) Tube running activity of saline treated mice adapted to the experimental equipment and exposed to the gentle air stream (○——○; n=37–38); not adapted to the experimental equipment, but exposed to the gentle air stream (■–·–·–■; n=10–11); adapted to the experimental equipment but not exposed to the air stream (●– – –●; n=9–10). Each point represents the mean of n recordings. Saline injections were given at −2 hr and at 0 hr (indicated by an arrow). (B) The effect of the frequency of testing on the run time of saline treated mice tested every 15 min (△——△; n=9–10); every 45 min (X——X; n=17–18); every 180 min (◇——◇; n=10) and every 180 min, and, in addition put into and taken out of the start box every 15 min (□——□; n=10). Each point represents the mean of n recordings. Saline injections were given at −2 hr and at 0 hr (indicated by an arrow). The animals tested every 15 min did not get the last saline injection.

16:45 hr in a laboratory where external light was excluded. Normal laboratory artificial light was used. If not otherwise stated each mouse was put in a Plexiglas tube with the same dimensions as the tubes of the experimental equipment for the first hour of each experiment. This procedure served as an adaptation to the equipment. At the time of testing the mouse was put into the start box. When it was oriented towards the guillotine door, the door was raised manually. At the same time a gentle stream of air (0.6 l/sec) was started. It blew in the direction of the run. The time it took for the mouse to run 100 cm, the run time, was recorded. The run time was usually measured every fifteenth minute. For practical reasons the maximal run time was set to 240 sec.

Statistical Analysis

Statistical significances were evaluated with the Mann-Whitney U-test. The two-tailed test was used with appropriate correction for ties.

RESULTS

Basic Studies on Tube Running in Untreated and Saline Treated Mice

An untreated inexperienced mouse placed in the start box will run through the tube when the guillotine door is opened. An inside diameter of about 35 mm was found suitable. If the diameter was decreased to 25 mm the inexperienced mice sometimes hesitated and did not run through the tube spontaneously. The reason for this is unclear, since there was also ample room for them in the tube with this smaller diameter. Most experiments were performed over a period of a year. No seasonal variation in tube running was observed. Within the range 21.5–31.5 g the body weight of the mice did not influence their tube running.

Figure 2A shows the effect of adaptation to the equipment and exposure to the gentle air stream. If the saline treated mice had adapted to the experimental equipment (Fig. 2A, unfilled circles, unbroken line), there was a continuous increase in the run time when the animals were tested every 15 min. During the last hour most animals reached the maximal value for the run time (240 sec). If the saline treated mice had not been adapted to the experimental equipment (Fig. 2A, filled squares, broken and dotted line) the curve was similar but the animals had a tendency to run somewhat faster through the tube. However, a significant difference ($p<0.05$) between the two conditions was reached only at two points. Omitting the gentle air stream when testing animals adapted to the equipment slightly increased the run time during the first hour of the experiment (Fig. 2A, filled circles, broken

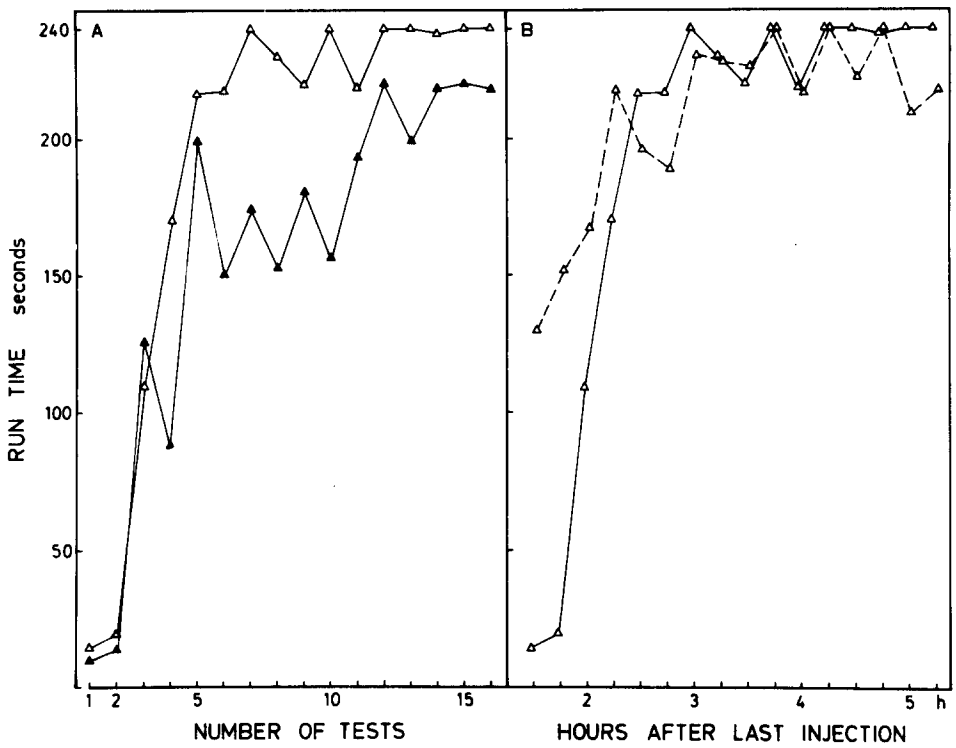

FIG. 3. (A) The effect of the number of runs on the run time of saline treated mice, tested every 15 min (△——△; n=9–10), and similarly treated mice tested with the shortest possible interval (▲——▲; n=10). The duration of the last experiment was 70 min. Each point represents the mean of n recordings. (B) The run time of saline treated mice with no experience of tube running and tested every 15 min (△——△; n=10) and the same animals tested every 15 min in a new experiment 24 hr after the first one (△- - - -△; n=10). Each point represents the mean of n recordings. Saline injection were given at 0 hr in the first experiment. No further injection was given in the second experiment, and for the results obtained in the second experiment 24 hr should be added to the time given on the abscissa.

line). In comparison with the corresponding controls a significant difference ($p<0.05$) was obtained only at one point. A tendency of the gentle airstream to decrease the run time was also seen in mice treated with nialamide or nialamide together with 5-hydroxy-tryptophan. In addition both the gentle airstream and the adaptation to the equipment tended to reduce the variability. Therefore both measures were retained in the permanent design of the test.

Figure 2B shows the influence of the frequency of testing on the run time. With tests performed every 15 min the maximal run time (240 sec) was reached 1.0 hr after the start (Fig. 2B, unfilled triangles, unbroken line). With an interval of 45 min between the tests, this level was never attained (Fig. 2B, X, unbroken line). If the test interval was 180 min only a small but significant ($p<0.002$) change in the run time could be established between the two tests (Fig. 2B, unfilled diamonds, unbroken line). A similar increase ($p<0.05$) was obtained if the animals were tested with an interval of 180 min but put into the start box every 15 min during that interval (Fig. 2B, unfilled squares, broken line). The experience of the run as such is thus the most important factor to get the increase in run time seen in the tests performed with a 15 min interval.

To study further the importance of the frequency of testing, the mice were tested with an interval as short as possible. The mouse had to perform a new test immediately after one finished test. The whole experiment lasted about 70 min. Figure 3A shows that the curve obtained after testing with this short interval (filled triangles, unbroken line) is roughly similar to the curve obtained after testing with a 15 min interval (unfilled triangles, unbroken line). No significant difference between the two groups was observed at any test point.

Figure 3B shows the effect of previous experience of tube running. The same groups of animals participated in two experiments with an interval of 24 hr. There was during the second experiment (unfilled triangles, broken line) a significant increase in run time during the first two tests in comparison with the same tests during the first experiment, when the mice had no experience of tube running (unfilled triangles, unbroken line). The two p-values were <0.02 and <0.002.

Effect of Nialamide

Nialamide was administered after one hour of adaptation to the equipment. Mice treated with nialamide 50 mg/kg had a gross behaviour which did not differ from that of saline treated controls. Mice treated with nialamide 200–400 mg/kg showed an increased forward locomotion on a flat surface. This activity gradually became more uncoordinated and aimless. The animals also exhibited tremor, especially of the head and forelegs, and a tendency toward extension and abduction of the hindlegs. These symptoms developed gradually and were pronounced four to five hours after nialamide

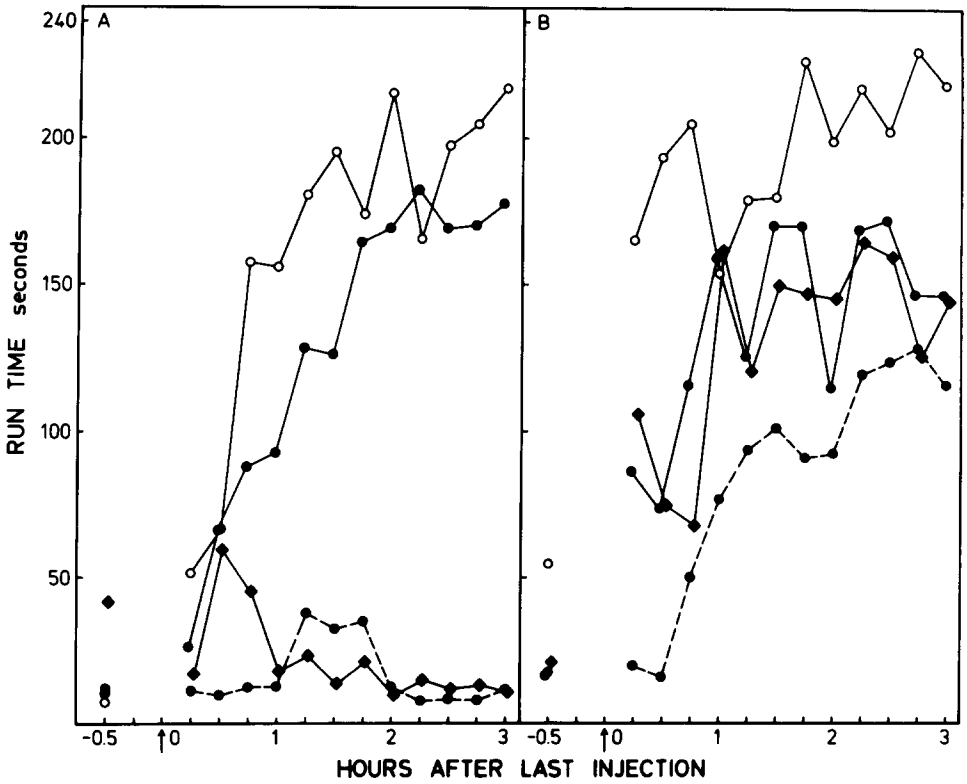

FIG. 4. The effect of nialamide treatment on the run time. Nialamide and saline respectively were administered 2 hr before the last injection. Saline was always given as the last injection. Each point represents the mean of n recordings. (A) The run time of mice treated with saline (○——○; n=9–10), nialamide 50 mg/kg (●——●; n=8–10); nialamide 200 mg/kg (●– – –●; n=9–10) and nialamide 400 mg/kg (◆——◆; n=10–11). (B) The run time of the same animals as shown in Fig. 4A during a new complete experiment performed 24 hr after the first one. No further injections were given. Twenty-four hr should thus be added to the time given on the abscissa.

administration. At this time the forward locomotion on a flat surface was impaired. The symptoms described above did not persist 24 hr after nialamide administration. Ten percent of the mice treated with nialamide 400 mg/kg were dead within 24 hr.

Figure 4A and 4B shows the run time of animals treated with nialamide 50–400 mg/kg and tested in two complete experiments with an interval of 24 hr. In the first experiment (Fig. 4A) there was a dose dependent decrease in run time after administration of nialamide 50–200 mg/kg. Nialamide 400 mg/kg (filled diamond, unbroken line) did not increase this effect as compared with nialamide 200 mg/kg (filled circle, dotted line). Thus, the decrease in run time seemed to be already maximal after nialamide 200 mg/kg.

In the experiments performed 24 hr later (Fig. 4B) only the animals treated with nialamide 200 and 400 mg/kg showed a decrease in run time as compared with saline treated controls. Significant differences ($p<0.002$–0.05) were reached at several test points throughout the experiment between mice treated with nialamide (200–400 mg/kg) and controls.

Effect of Treatment with Nialamide and DL-5-HTP

In mice pretreated with nialamide 50 mg/kg administration of DL-5-HTP 50 mg/kg two hr after the nialamide administration resulted in tremor in the head and forelegs, and abduction together with hyperextension of the hindlegs. The positions of the hindlegs greatly impaired forward locomotion on a flat surface. These symptoms started after about 15 min and were fully developed 30 min after the DL-5-HTP administration. After DL-5-HTP, 25 mg/kg, the same but slightly decreased symptoms appeared. At a DL-5-HTP dose of 12.5 mg/kg these symptoms appeared only occasionally.

Figure 5 shows the effect of different doses of DL-5-HTP administered to mice pretreated with nialamide 50 mg/kg in comparison with controls treated with the same dose of nialamide and saline. No significant difference in run time was observed between mice treated with DL-5-HTP 6.25 mg/kg (filled squares, unbroken line) and the nialamide treated controls (filled circles, unbroken line). The main effect of DL-5-HTP 12.5 mg/kg (unfilled squares, unbroken line) was a decrease in the run time. This decrease was first seen approximately 1 hr after the last injection. The decrease was significantly different from the nialamide treated controls during the last part of the test period starting 1 hr 15 min after the last injection ($p<0.05$–0.0001). If the DL-5-HTP dose was increased to 25 mg/kg the mice ran still faster through the tube (filled squares, broken line). Statistical significance ($p<0.01$–0.0001) was reached during the same test period as after the administration of DL-5-HTP 12.5 mg/kg. Further increase of the 5-HTP dose to 50 mg/kg (unfilled squares, broken line) did not result in a further decrease of the run time.

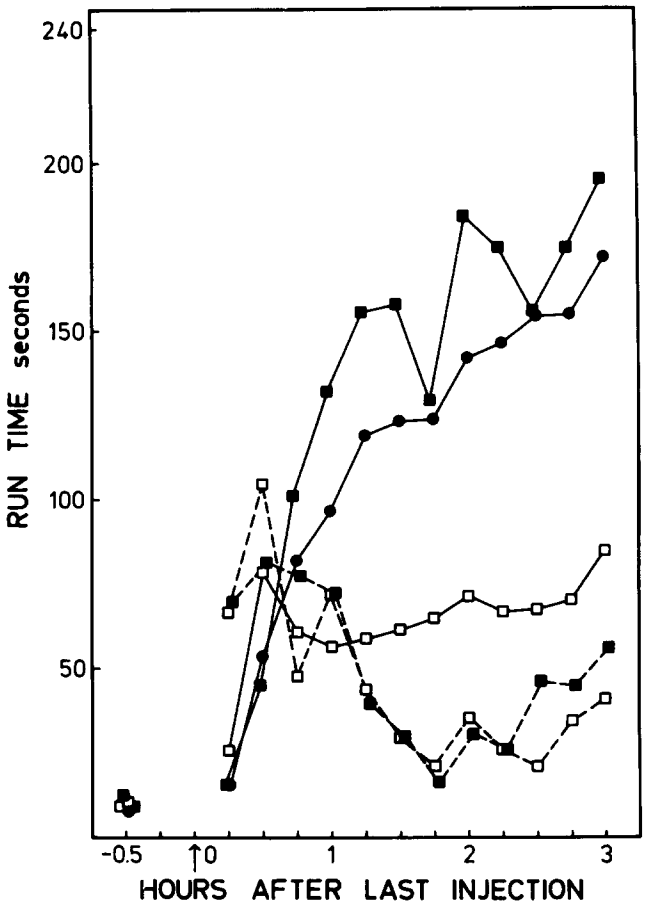

FIG. 5. The effect of DL-5-HTP in nialamide pretreated mice. Nialamide, 50 mg/kg, was always administered 2 hr before the last injection. DL-5-HTP was given as the last injection and is indicated by an arrow. The control mice received saline instead of DL-5-HTP. Each point represents the mean of n recordings. The run time of nialamide pretreated mice treated with saline (●——●); n=64–70), DL-5-HTP 6.25 mg/kg (■——■; n=17); DL-5-HTP 12.5 mg/kg (□——□; n=18–20); DL-5-HTP 25 mg/kg (■– – –■; n=19–20) and DL-5-HTP 50 mg/kg (□– – –□; n=18–23).

Even if the late decrease in run time was the most conspicuous finding after nialamide and DL-5-HTP administration, a short lasting increase in run time was also seen. This increase occurred soon after 5-HTP administration (Fig. 5). Fifteen min after DL-5-HTP administration a significant difference as compared with the nialamide treated controls was found at dose levels of 25 mg/kg ($p<0.0001$) and 50 mg/kg ($p<0.05$). Thirty min after the DL-5-HTP administration all doses over 6.25 mg/kg significantly increased the run time ($p<0.05$–0.01).

DISCUSSION

Studies of locomotor activity in naive animals always seem to reveal an exploratory activity regardless of the method used. However, it is difficult to define exploratory activity without using an operational definition. It may be broadly defined as activity of a subject in an unfamiliar environment (for review see [4]).

When comparing different methods used for measuring exploratory activity, it is apparent that the time interval used for recording is dependent on the method. In mice, Thompson [13] recorded the number of squares traversed or free exploration of a Y-maze. The time limit was 10 min. When a hole board is used to study exploratory activity a 5 min period is sufficient [2]. The duration of the exploration will probably also be different in experiments performed with a single animal or with a group of animals. Thus an increased activity is observed during the first 30–60 min after a group of rats or mice has been handled or placed in an unfamiliar cage [6].

A phenomenon similar to exploratory activity has also been recorded in the present tube running experiments. When saline treated mice were tested with a 15 min interval most animals reached the maximal run time (240 sec) after about 1.0 hr (Fig. 2B). Thus, there was an adaptation during the test procedure, which probably is due to a decrease in exploratory activity. The duration of this adaptation was long when compared to the duration of the exploratory activity in the studies which measured exploratory activity of a single animal, as discussed above. This is not surprising since it was clearly demonstrated in the present experiments that the frequency of testing was more important to the adaptation than the test interval (Figs. 2B and 3A). The interval testing as used in the present study might have divided the exploratory activity into smaller parts. Thus duration can not be used as a dependent variable since it can be controlled by the experimenter.

Methods have been developed to measure exploratory activity more specifically. Shillito [12] claimed that the number of tunnels entered by a mouse during each min of a 5 min observation period was a specific measure of exploratory activity. The test was said not to measure general locomotion. Minck *et al.* [8] using a hole board technique tried to separate exploration from locomotion. They considered the "hole-looking" as exploration and the traversing of the squares of the hole board as locomotion. If examination of tunnels or dipping the head into the holes of a hole board should be regarded as a specific measure of exploratory activity, then tube running could be an even more specific method since the mouse remains in the tube throughout the test. Exploratory activity certainly is involved in the present method as in all other methods used for measuring motor activity. However, a separation of exploratory and locomotor activities is not possible in the tube situation and tube running can probably not be used as a specific measure of exploration. If exploratory activity is a part of locomotor activity, as pointed out by Berlyne [1], such a separation is not possible.

Administration of DL-5-HTP to mice pretreated with nialamide resulted in hyperextension and abduction of the hindlegs. We initially suspected that these positions of the hindlegs were especially suitable for passing a narrow tube. However, treatment with only nialamide, 200 mg/kg, gave no hyperextension and abduction of the hindlegs for the first hours after the drug administration. Nevertheless, the run time was short. This result thus did not support the idea that the positions of the hindlegs were critical for the tube running.

An increased motor activity has been demonstrated after nialamide, 200 mg/kg [9]. The maximum of the recorded motor activity was reached 4 hr after the drug administration. After that the activity decreased, probably due to the tremor, hyperextension and abduction of the hindlegs, which appeared at that time. In the present investigation no decrease in the run time was recorded even 5 hr after the

nialamide administration (Fig. 4A) in spite of the development of the same syndrome, as described by Modigh and Svensson [9]. Thus, our method could still record effects of nialamide, when the behavioural syndrome invalidated a general motor activity recording.

The behavioural effects observed after nialamide administration are ascribed mainly to 5-HT [3,9]. Our findings of a dose dependent decrease in the run time after treatment with nialamide, 50–200 mg/kg (Fig. 4A) suggested a role for 5-HT also in the tube running activity of mice. 5-HTP is known to potentiate the behavioural effects of nialamide, probably through an increased 5-HT level (for review see [5] and [7]). The dose dependent decrease in the run time after administration of DL-5-HTP, 6.25–25 mg/kg (Fig. 5) provides further support for a role of 5-HT in tube running activity. Tube running activity in mice could be a useful method to measure behavioural effects mediated by 5-HT. However, only indirect evidence for the role of 5-HT in tube running activity has been presented in the present investigation.

The interval testing may be very useful to study the effects on tube running activity of drugs with different duration of action, since it has been demonstrated that the frequency of testing is more important for this adaptation than is the interval used between the tests. The length of the test interval can be changed so that it will be the most suitable one for a given drug. Thus it is always possible to follow the effect of the drug on the gradual adaptation of the mouse to the test situation.

ACKNOWLEDGEMENTS

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