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A Simple Technique for Quantifying Intoxication-Induced by Low Doses of Ethanol

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LÊ, A. D. AND Y. ISRAEL. *A simple technique for quantifying intoxication-induced by low doses of ethanol.* PHARMACOL BIOCHEM BEHAV **48**(1) 229–234, 1994.—A simple technique for the measurement of intoxication induced by low doses of alcohol in the rat was developed. Rats are required to maintained their balance on a rectangular wooden bar that oscillates in a 120° angle in an arch-like fashion. A steady baseline can be obtained for each animal with approximately 10 min of training time. Ethanol, in a dose range from 0.5–1.5 g/kg, given orally or by IP route, impairs animal's performance in a dose-related manner. At the same blood ethanol concentration, a higher degree of impairment is observed at higher oscillating frequency. Significant impairment of performance can be detected at ethanol dose of 0.5 g/kg given IP or orally. Pentobarbital and chlordiazepoxide, in doses of comparable potencies to those of ethanol doses also produce a dose-related impairment of performance. The oscillating bar test is a simple but sensitive test that can qualitatively assess intoxication induced by low doses of ethanol or other sedative hypnotic drugs.

Ethanol Intoxication Sensitivity Oscillating bar

ETHANOL produced a wide range of physiological and behavioral effects on human and experimental animals. Some of the intoxicating effects of ethanol, such as hypothermia or narcosis, can be elicited spontaneously in experimentally naive animals (20,27,28). Ethanol intoxication can also be assessed and quantified by examining its ability to impair the performance of the animals on a particular task in which they were previously trained (19). For example, the effects of ethanol on motor coordination and avoidance responses can be measured and quantified with the moving belt (7), the jumping test (29), or shuttle box avoidance tests (17).

At present, a variety of tests are available to assess and quantify ethanol intoxication in experimental rodents (19). There are, however, a number of limitations associated with these tests. In test systems where ethanol intoxication is spontaneously elicited, the required dose ranges are generally high. The hypothermic and loss of righting reflex induced by ethanol are usually assessed in dose range from 2.0–4.0 g/kg or 3.0–4.5 g/kg of ethanol, respectively (19). Intoxication induced by medium or low doses of ethanol, however, are usually assessed by examining their ability to impair the performance of animals on a previously learned task. For example, ethanol induced impairment of animal's performance on maze running for food reward (7,22) or motor coordination on the moving belt test (7) can be measured and quantify within the dose range of 0.5–1.0 g/kg or 1.4–2.0 g/kg, respectively. Similarly, impairment of operant performance can also be detected with ethanol doses ranging from 0.5 to 1.5 g/kg IP (11). The major problems with these tests are that they are time demanding and labor intensive, as prior training of the animals to criterion performance on a particular task is required. As a consequence, the number of animals that can be run is quite limited and thereby restricting the experimental questions.

The effects of ethanol on the transmission of various neurotransmitters in different brain areas have been shown to be dependent on ethanol concentration. For example, the release of brain dopamine in the nucleus accumbens but not in the striatum is sensitive to low doses of ethanol (12). In the studies of ethanol tolerance, it has been shown that the test doses, treatment doses, and their interaction are critical variables in determining the manifestation of tolerance (13,15,16). The absence of a simple test that can quantify intoxication induced

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by low doses of ethanol has restricted much research into the nature as well as the neurochemical mechanisms underlying tolerance to low doses of ethanol.

The purpose of the present study was to design a test system that is sensitive to the intoxication induced by low doses of ethanol and requires minimal prior training such that a large number of animals can be tested. The applicability of this test system to detect intoxication induced by other sedative hypnotic drugs was also examined.

METHOD

Animals

Thirty male Wistar rats weighing approximately 200 g were obtained from Charles River Laboratories (Montreal, Quebec). They were housed singly and fed a standard rat chow diet. Food and water were available ad lib. Ambient temperature was maintained at 21 ± 1°C, and lights were on from 0700 to 1900 h daily throughout the whole experiment.

Apparatus

Basically, the apparatus (Fig. 1) consists of a 3 feet long rectangular wood bar (1-5/8″ by 0.75″) suspended 3′ above a grid floor of a partially enclosed Plexiglas box with a dimension of 3′ × 1.5′ × 3′2″ (L × W × H). The movement of the bar is controlled by an electrical motor (D-C Motor Speed Control, Series 200, Bodine Electric Company, Chicago, IL). A cam system connects the bar and the motor in such a manner that it permit the bar to oscillate in a 120° angle in an arch-like fashion instead of a complete rotation of 360° angle. The dimensions and construction of the cam system are shown in Fig. 2. The speed of oscillation can be controlled by varying the speed control on the motor power source. During the development phase of this test, it was found that some rats would jump to the grid floor rather than maintaining their balance on the oscillating bar. To prevent such behavior, the grid floor is wired to an electrical power supplied that can deliver different intensity of electrical shock. The shock intensity was set at 0.5 mA, as this current has been shown to be aversive to the rats and was used effectively in training rats for performance on the moving-belt test (7).

Training Procedure

The rats are placed in the middle of the bar and required to maintain their balance on the oscillating bar to avoid falling to the electrified grid floor (0.5 mA). The motor that controls the bar is activated immediately following the placement of the rat on the bar. The duration that the rat can remain on

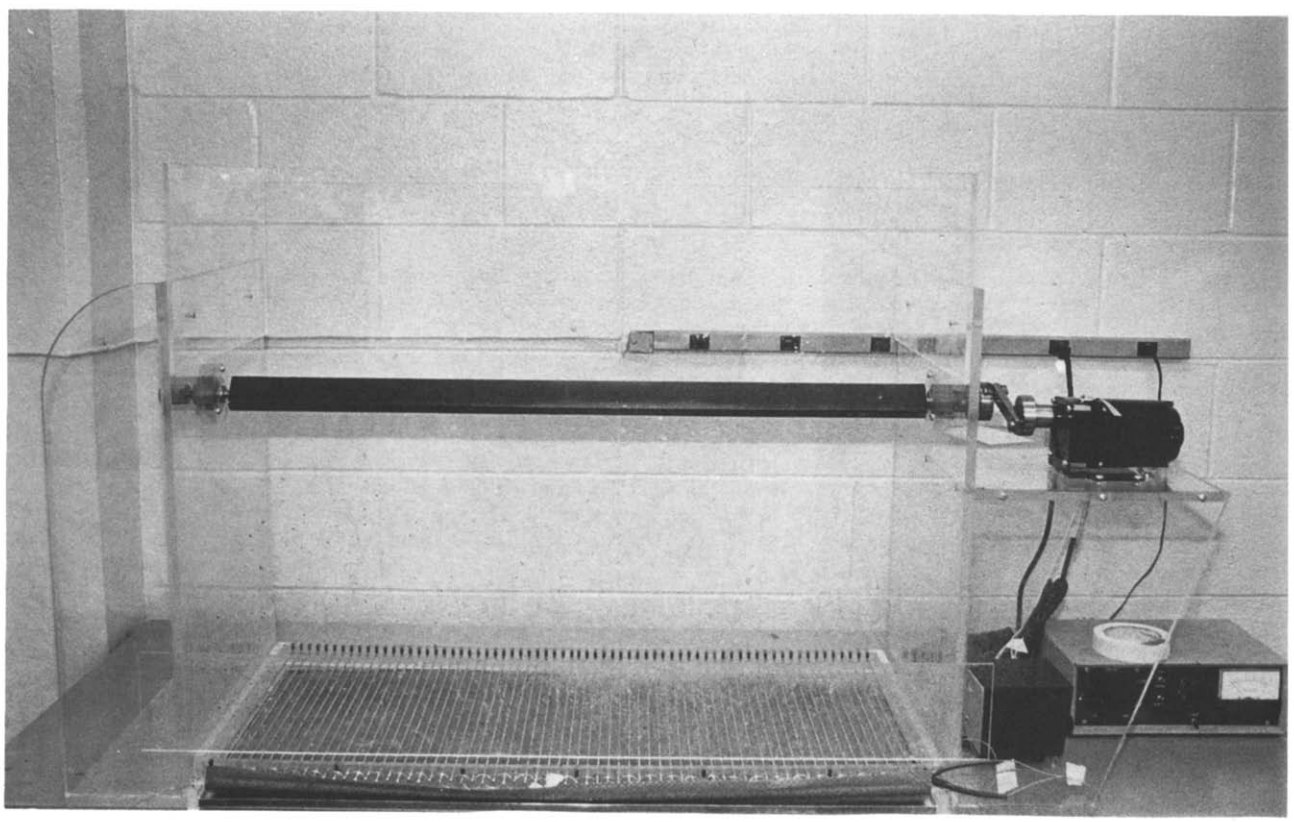

FIG. 1. A frontal view of the oscillating bar system. The rats are placed on the middle of the bar and have to maintain their balance on the oscillating bar to avoid falling onto an electrical platform. A power box (bottom left) controls the speed with which the bar oscillates.

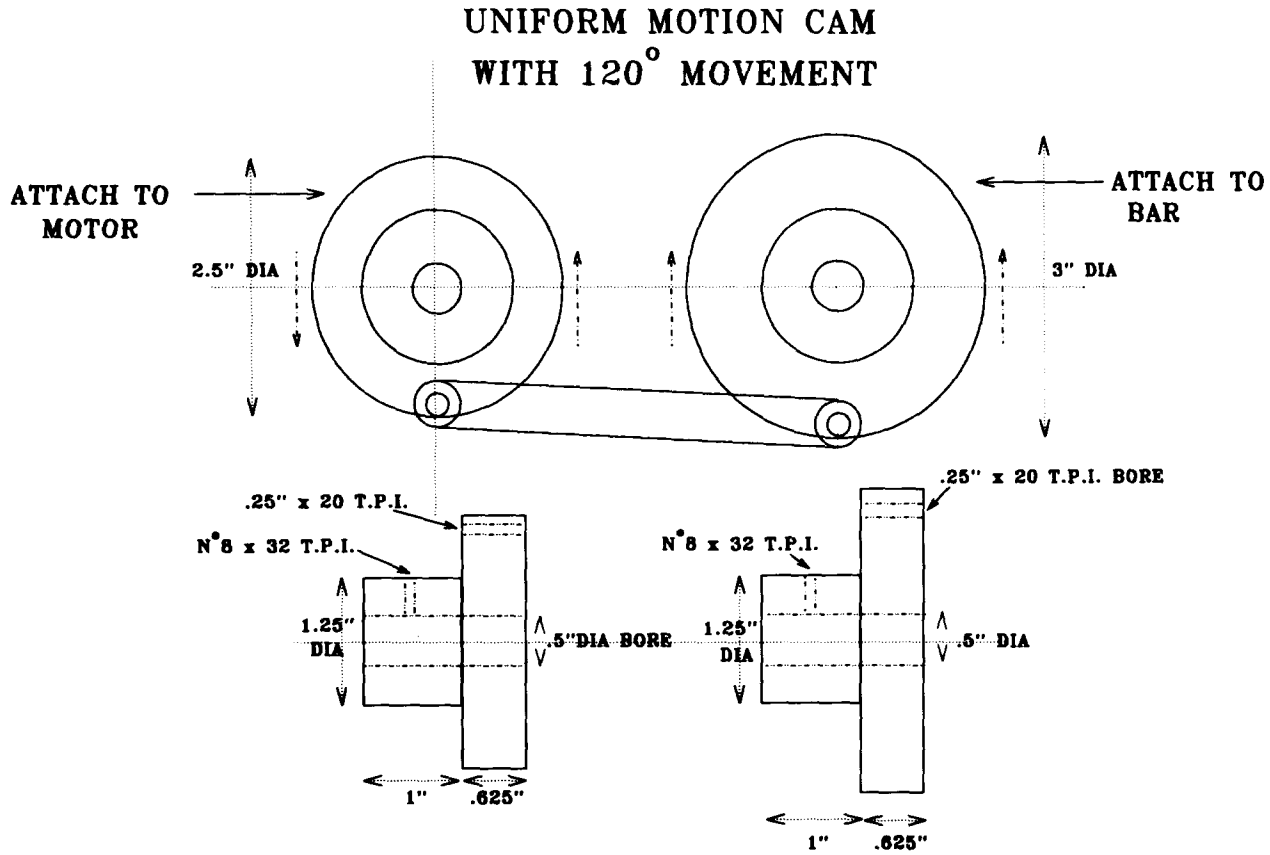

FIG. 2. End (upper) and sectional (lower) views of the uniform motion can system. DIA: diameter and T.B.I.: Threads per inch.

the bar is defined as the elapse time between the placement of the rat on and the time at which it falls off the bar. If the rat can remain on the bar for 2 min they will simply be removed and a score of 120 s is assigned. At the oscillating frequency of 60 times/min, the average duration that the rat can maintains on on the bar is 42 s.

Rats (n = 30) were trained to remain on the bar at oscillating frequency of 60 cycles/min. Each rat was given three training trials per day. A stable baseline for each rat can be obtained after 3 days of such training. The total training time required to achieve a stable performance for each animal is approximately 10 min.

Experimental Procedure

To evaluate the effects of drug on performance, on each test day rats that achieved a stable performance on the oscillating bar were divided in a stratified manner into three groups (n = 10 each) and were designated to receive different doses of the drug. Prior to drug administration, the performance on the oscillating bar was evaluated twice for each rat, and the average duration of the two performance was defined as the baseline or 100% performance. The effects of ethanol, pentobarbital, or chlordiazepoxide on motor performance were then assessed at 15 and 30 min after its administration. At each time, the rat was tested twice in a consecutive manner and the average of the two performances was employed. The maximum impairment occurred at either time is expressed as percentage of the baseline performance and employed to quantify the data.

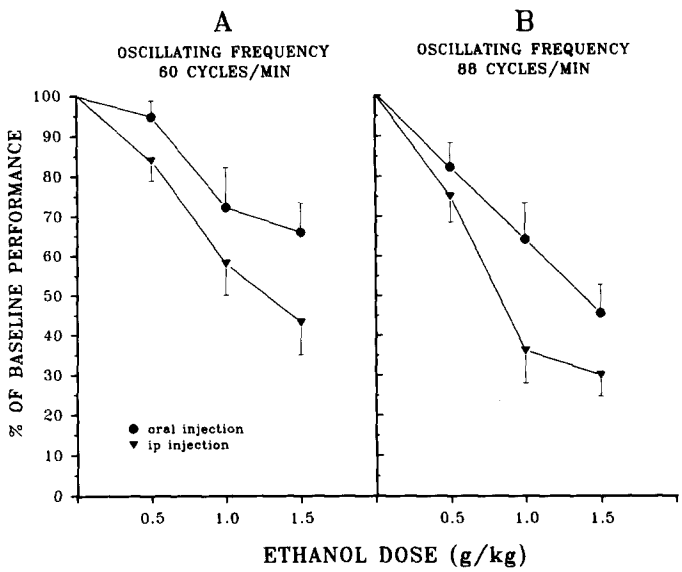

FIG. 3. Impairment of motor performance on the oscillating bar (expressed as percentage of baseline performance) induced by various doses of ethanol following oral and IP administration of various doses of ethanol as measured with oscillating speeds of 60 (left panel) and 88 rev/min (right panel). n = 10 animals per group. Values are means + positive or negative SEM.

TABLE 1

BLOOD ETHANOL LEVELS (mg/dl) IN VARIOUS GROUPS OF RATS TAKEN IMMEDIATELY AFTER THE COMPLETION OF TESTING (APPROXIMATELY 32 MIN) AFTER ORAL AND IP ADMINISTRATION OF VARIOUS DOSES OF ETHANOL ON VARIOUS TEST DAYS

		Dose		
Speed	Route	0.5	1.0	1.5
60 rpm	PO	20.6 ± 4.5	52.7 ± 7.3	91.8 ± 6.2
	IP	29.5 ± 1.4	102.6 ± 5.4	156.3 ± 5.8
80 rpm	PO	20.4 ± 3.3	59.8 ± 5.0	90.3 ± 6.5
	IP	26.1 ± 3.2	89.3 ± 4.2	136.6 ± 10.0

Values are means ± SEM.
n = 10 animals per group

The impairment of performance induced by IP and oral administration of various doses of ethanol (0.5, 1, and 1.5 g/kg) at the oscillating frequency of 60 cycles/min was examined on test day 1 and test day 2, respectively. Following three training sessions on the bar at the oscillating frequency of 88 cycles/min, the effects of similar doses of ethanol administered by IP and intragastric routes on performance were evaluated on test days 3 and 4. Ethanol was given as a 10% w/v saline or water solution for IP and intragastric administration, respectively. On test days 5 and 6, the impairment of performance induced by pentobarbital (5, 10, and 15 mg/kg) and chlordiazepoxide (15, 20, and 25 mg/kg) at an oscillating frequency of 88 cycles/min were examined. Pentobarbital and chlordiazepoxide were dissolved in saline and administered by IP route in a volume of 10 ml/kg.

To minimize any carry-over effects of testing, the test days are separated by at least 1 week. In between test days, rats received three training sessions on the oscillating bar to maintain a stable performance.

Determination of Blood Alcohol Levels

In all test days that involved ethanol administration, a blood sample of 50 μl was taken from the tip of the tail of the animal immediately after assessing motor performance for the determination of blood ethanol concentration. Blood ethanol levels were determined by gas-liquid chromatography technique with n-butanol as an internal standard (21).

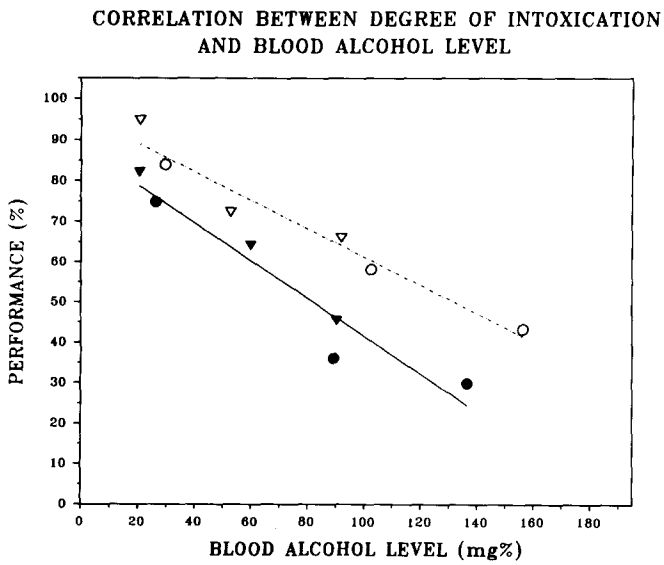

FIG. 4. Relationship between blood ethanol concentrations produced either by IP or oral administration of various doses of ethanol and performance on the oscillating bar at osciallating frequencies of 60 (dashed line) and 88 (solid line) cycles/min. n = 10 animals per point.

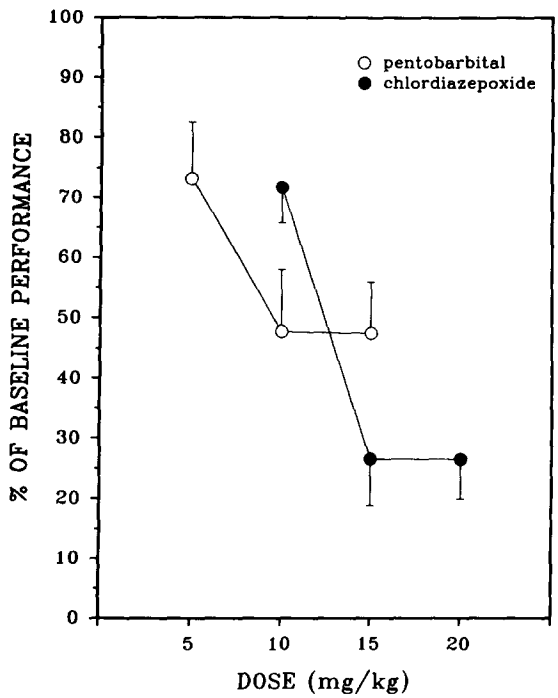

FIG. 5. Impariment of performacne on the oscillating bar (exposed as percentage of baseline performance) induced by various doses of pentobarbital and chlordiazepoxide as measured at the oscillating speed of 88 cycles/minute. n = 10 animals per group. Values are means + positive or negative SEM.

RESULTS

The baseline performance on the oscillating bar is related to the speed of oscillation. At the oscillating frequency of 60 cycles/min, the average time that the rats can remain on to the bar was 42 ± 4 s, which was significantly higher than an average mean of 25.1 ± 2 s observed at the frequency of 88 cycles/min. The effects of IP and oral administration of various doses of ethanol on the rat's performance on the bar at the oscillating frequencies of 60 and 88 cycles/min are shown in panels A and B of Fig. 3, respectively. An overall analysis of variance shows a significant effect of dose, $F(2, 26) = 22.1$, $p < 0.001$, speed, $F(1, 26) = 7.3$, $p < 0.01$, and route of administration, $F(1, 26) = 15.5$, $p < 0.001$. The same analysis, however, reveals no significant two-way or three-way interaction among these variables. These results indicate that ethanol produces a dose-dependent impairment of performance on the oscillating bar and that the degree of impairment is more pronounced with IP route than oral route of administration. Furthermore, these results also showed that for the same dose of ethanol and the same route of administration, the impairment is more pronounced at higher oscillating frequency. At the oscillating frequency of 88 cycles/min, paired Student t-tests revealed that the dose of 0.5 g/kg given either orally, $t(18) = 2.05$, $p = 0.05$, or by IP, $t(18) = 3.1$, $p < 0.02$, significantly impairs the performance of the animals.

Blood ethanol levels taken approximately 32 min following ethanol administration on each test day are shown in Table 1. As can be seen from this table, independent of the route of administration, blood ethanol leveAls are related to the dose of ethanol administered with the higher the dose the higher the level, $F(2, 26) = 23.4$, $p < 0.001$, for IP route, $F(2, 26) = 25$, $p < 0.001$. For the same dose of ethanol, the blood ethanol level achieved, however, is much lower when given by oral route as compare to IP route of administration. Independent of the route of administration, a good relationship between blood ethanol level and impairment can be observed as shown in Fig. 4.

The impairment of performance induced by IP administration of various doses of pentobarbital and chlordiazepoxide is shown in Fig. 5. Analysis of variance reveals an effect of dose for pentobarbital, $F(2, 26) = 3.4$, $p < 0.05$, and for chlordiazepoxide, $F(2, 26) = 13.4$, $p < 0.01$, which indicated that the impairment of performance by these drugs is a dose-related phenomenon.

DISCUSSION

The ability of the rat to maintain its balance on the bar is related to the difficulty of the task imposed. The higher the frequency of oscillation, the shorter the duration that the rat can maintain its balance on the bar. Although the oscillating frequency of the bar can vary from 30 to 160 cycles/min, the frequency of 60–88 cycles/min was chosen for several reasons. At lower speed the animal can remain on the bar for a long time and physical exhaustion will, therefore, be the main limiting factor. At such frequency, the duration of the experiment will be much longer and higher doses of ethanol will be required to produce any impairment. On the other hand, at high oscillating frequency, the animal can hang on to the bar for a very short period of time (in matter of a few seconds) and, thereby, would compromise the accuracy of the testing. This problem is also amplified as the impairment is expressed as percentage change from baseline performance.

Independent of the route of administration, the impairment of motor performance on the oscillating bar is highly correlated with blood ethanol concentrations. Significant impairment can be detected at blood ethanol concentration of 29 mg% produced by oral gavage of 0.5 g/kg of ethanol. A higher blood ethanol level as well as a greater degree of impairment are attained when the same dose of ethanol is administered by IP route than by intragastric gavage. The frequency of oscillation at which testing is carried out is also a critical variable in determining the extent of ethanol effect. For any given dose of alcohol, the impairment was more pronounced when animals were tested at higher oscillating frequency. For example the degree of impairment produced by oral or IP administration of 1 g/kg at the oscillating frequency of 88 cycles/min is essentially similar to that induced by 1.5 g/kg at the oscillating frequency of 60 cycles/min, respectively.

As mentioned earlier, a variety of test systems are available for determination of motor impairment effects induced by ethanol or other sedative hypnotic drugs. The tilting plane test (1) and the rotarod test or Dowel Test (4) have been employed extensively to measure ethanol intoxication in experimental rodents over the last few decades. Little or no prior training is required for these tests. However, higher doses of ethanol (>1.5 g/kg) are generally required to produce significant impairment on these tests (1,2,5,6,10,18,26). Recently, Maier and Pohorecky (25) also described a Dowel test in which the rats are required to maintain their balance on a rotating rod at 9 rev/min. Although the amount of training for this test is comparable to the present one, a dose of 2.5 g/kg ethanol administered orally was used (25).

The sensitivity of the present test is at least comparable to other tests in which extensive training is required prior to testing for intoxication. This test system, however, offers several advantages for testing intoxication induced by ethanol or by other sedative-hypnotic drugs. The minimal demand of training time as well as its sensitivity in detecting impairment are the obvious advantages. In studies concerning with voluntary intake of alcohol, rats have been shown to consume an average of 0.7–1.0 g/kg during 10 to 60 min access to ethanol solution (8,23,24). This oscillating bar test would be useful to examine whether such voluntary consumption of alcohol would produce intoxication.

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