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A Rapid Method to Evaluate Acute Ethanol Intoxication in Mice

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BLUM, K., S. F. ELSTON, H. SCHWERTNER, L. DELALLO AND A. H. BRIGGS. *A rapid method to evaluate acute ethanol intoxication in mice.* PHARMAC. BIOCHEM. BEHAV. **14**(6) 835–838, 1981.—A simple technique for the evaluation of ethanol intoxication based on the ability of mice to remain on a bar suspended above an electrified grid is reported. The characteristics that make this model useful to measure ethanol induced intoxication include: (a) low variability; (b) high sensitivity; (c) rapidity; (d) requires no previous training of animals to be tested; (e) objective scoring which can be quantified; and (f) dose-dependent correlation between brain and blood ethanol levels and bar holding response.

Alcohol Ethanol Intoxication Motor impairment

SEVERAL behavioral methods have been utilized to evaluate acute ethanol intoxication in laboratory animals and humans [3, 5, 6, 8, 9]. Each one has its own advantages and disadvantages. The tilted plane technique has a wide dose range (0–6.3 g/kg) but is insensitive to small dose changes, is sensitive to the animal's weight and has a high variability. The moving belt method has high sensitivity in the 1.0–2.5 g/kg (rats) dose range, but requires extensive training (14 days) and elaborate equipment. The rotorod is sensitive, but doesn't measure graded responses. For a more complete review, the interested reader is referred to the review by Alkana and Malcolm [1]. However, these methods for the most part do not meet all of the following minimal criteria: (a) low variability between tests; (b) sensitivity; (c) rapidity of test; (d) negligible training of the subjects; (e) objectivity of the measure of intoxication; and (f) correlation of brain and blood ethanol levels. In our search for an intoxication model which would decrease variability, increase sensitivity and improve objectivity, we were intrigued by the simple "pencil test" of Belknap *et al.* [4]. We report here the description of a rapid technique to evaluate acute ethanol intoxication in mice which has been developed in our laboratory as a modified and improved version of the "pencil test" [4].

METHOD

The apparatus consisted of a metal bar (6.4 mm diameter) fixed between two electrified copper plates suspended 27 cm above an electrified grid (Fig. 1) with a potential of 25 volts for the plates and grid. Careful evaluation revealed that although we utilized an electrified grid, it is probably not necessary. The electrified plates, however, appear to be more useful in terms of maintaining a consistant bar holding response. Male ICR Swiss male (Simonsen) weighing between 20 and 30 grams were housed in large standard laboratory cages (192 sq. inches) usually 16 mice per cage, with a light (0800–2000)—dark (2000–0800) cycle. The bedding consisted of pine shavings. The experiments were performed between 1000 and 1700 hr.

In our initial experiments, we utilized ten mice per group and four doses of ethanol ranging from 1.2 to 2.1 g/kg. Each mouse was first screened for bar holding response. The mice were carefully placed on the bar for three consecutive trials. Animals which did not remain on the bar for ten seconds in at least one of the three trials were eliminated. In our experience, animals which initially remain on the bar for ten seconds, maintain this behavior with very few exceptions. These bar-holding mice received intraperitoneal injections of 30%, v/v, ethanol in 0.9% saline solution. Five minutes after ethanol administration, each subject was replaced on the bar for three additional consecutive trials. Other mice were trained for 5 hours consisting of three consecutive trials every 30 minutes and each mouse performed at an individualized shock level ranging from 15–35 volts. These mice received intraperitoneal injections of ethanol in 0.9% NaCl at a dosage of 0.9 to 1.8 g/kg. The volume of injection was 26.4 μl/g.

Ten seconds was designated as the cut-off for the bar-holding response [4]. Intoxication scores were obtained by subtracting the number of seconds each mouse remained on the bar (up to ten) from the number ten. For example, an animal remaining on the bar for four seconds would therefore receive an intoxication score of six. Intoxication scores reported herein were the lowest score for the three trials recorded for each animal [4].

In order to correlate bar-holding behavior with ethanol intoxication, we determined ethanol concentrations in the blood and brain in animals that had been scored for bar holding. These mice received injections of ethanol equaling doses of 1.2, 1.5, 1.8 or 2.1 g/kg and were scored for bar holding 5 minutes post-injection. Immediately following the bar holding test, the animals were decapitated and blood was col-

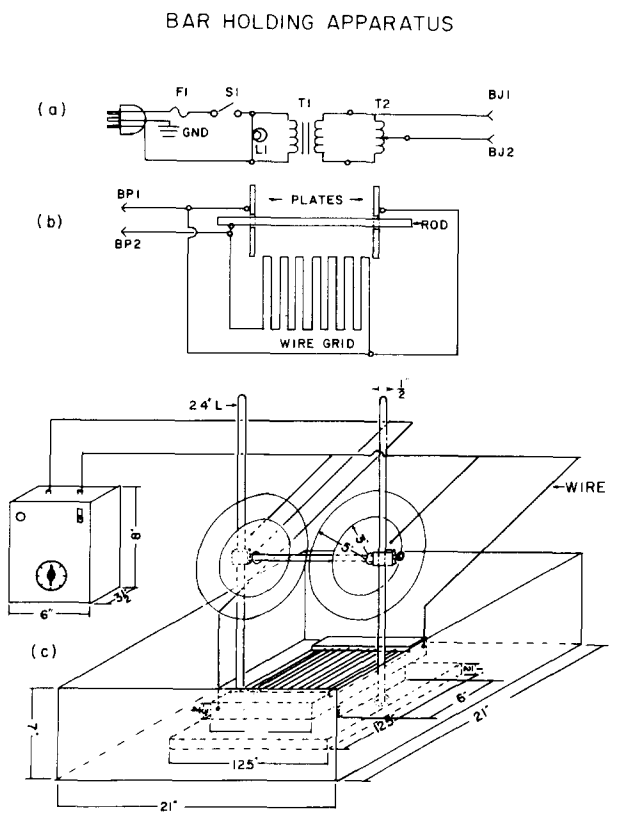

FIG. 1. Diagram of bar-holding apparatus. (a) schematic of transformer. F1: fuse 0.3 amps, GND: ground, S1: switch, L1: pilot light 125 V, T1: transformer 36 V, T2: ohmitran #VT02, BJ1 and BJ2: banana jacks, (b) schematic of grid, bar and plates. BP1 and BP2: banana plugs, (c) sketch of apparatus placed in open metal box.

lected in ice cold heparinized tubes which contained an internal standard. The brain was then removed and added to a solution of 1.5 ml chilled 5% zinc sulfate (w/v) and 50 μl of an isopropanol standard. After the tissue was homogenized, 0.25 ml of 0.3 N barium hydroxide was added and then the sample was brought up to a 3.0 ml volume. Fifty μl of blood and 1.0 ml of brain homogenate were then analyzed by gas chromatography by a method described by Tabakoff *et al.* [10].

For blood analysis, approximately 0.8 g of crystalline sodium fluoride was added instead of sodium fluoride impregnated filter paper discs. For brain analysis, approximately 2.4 g of sodium chloride per ml of brain homogenate was used to enhance transfer of ethanol into the vapor phase.

RESULTS

Figure 2 illustrates time-action effects of various doses of ethanol from 0.9 to 1.8 g/kg administered IP in ICR Swiss mice on the bar holding response. It is evident from these results, for each dose utilized, peak response occurred within 5 minutes post injection and the magnitude of the impairment response was dose-dependent except for the 1.2 g/kg ethanol dose. It is important to also note that saline injections did not alter the bar-holding response in ICR Swiss mice.

Figure 3a illustrates a full dose response curve for ethanol at a dosage ranging from 1.2 to 2.1 g/kg of body weight in ICR Swiss mice.

The data in Fig. 3a reveals a dose-dependent decrease of bar-holding scores on untrained animals induced by ethanol administration. Estimation of the intoxication score 5 (IS_5) for ethanol in this experimental condition was low at 1.5 g/kg.

Figure 3b shows a similar dose response curve for ethanol with trained animals. Estimation of the IS_5 for ethanol was

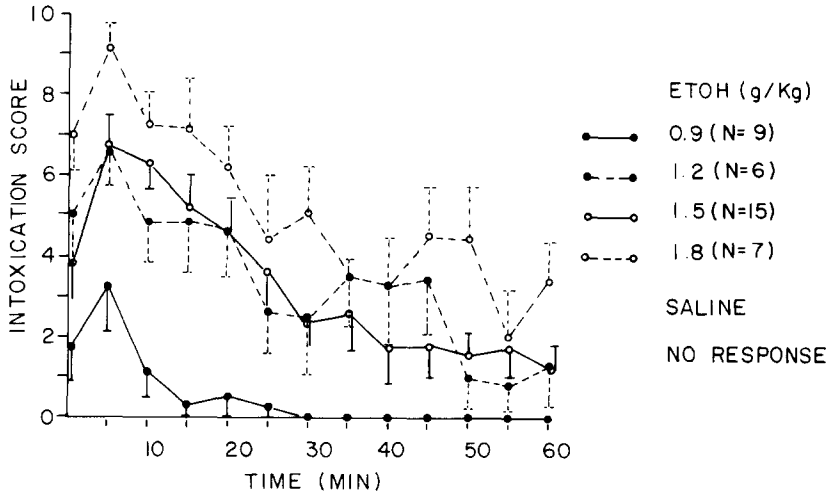

FIG. 2. Bar holding response of ethanol at different doses over a 60 min period. Each line represents a different dose of ethanol (0.9–1.8 g/kg). The mice were scored every five minutes except at the 1.0 min point. (N) represents the number of animals for each line plot and the vertical bars represent SEM.

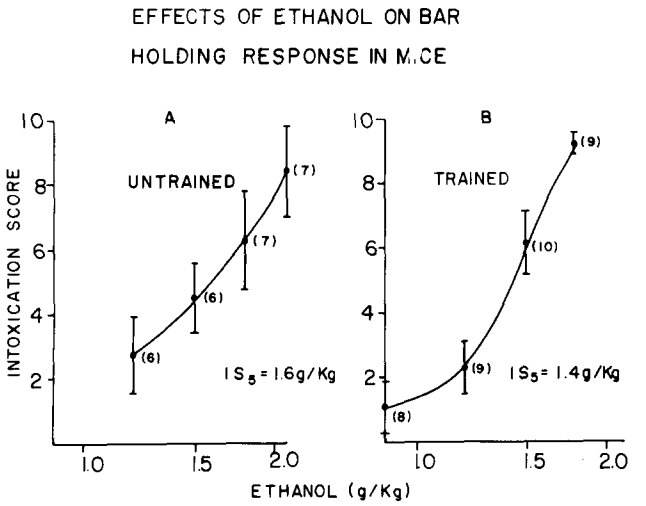

FIG. 3. Effects of ethanol on bar-holding response. (a) Dose response curve for untrained mice, using only animals which demonstrated ability to remain on the bar when first placed there. (b) Dose response curve for mice which have been trained to remain on the bar. (N) equals number of mice per point. Vertical bars indicate ± mean standard errors.

1.4 g/kg which did not significantly differ from the dose response curve observed in Fig. 3a.

As can be seen in Fig. 4, there was a significant correlation between both blood and brain levels of ethanol and bar holding scores. The correlation coefficient between brain or blood ethanol concentration and bar holding scores was 0.65 ($p<0.001$). Saline-injected animals had a bar holding score of 2.8 ± 1.5.

DISCUSSION

We believe that this model meets criteria previously stated in the introduction, since (a) it is less variable than other techniques (for example, loss of righting reflex [7] as evidenced by rather small standard deviations observed per dose group tested); (b) is sensitive to low doses of ethanol (intoxication observed at 1.2 g/kg of ethanol); (c) is rapid compared to other techniques; (d) required no previous training of the animals; (e) is objective since the scoring can be quantified; and (f) there is a dose-dependent correlation between brain and blood ethanol levels to bar-holding response.

Although blood and brain levels were correlated with performance, the correlation was not perfect explaining only a portion of the variability. At present, we do not have an exact reason for this discrepancy; however, it is tempting to speculate that the impairment observed because of its rapidity and very short duration may be mediated in part by acetaldehyde, the ethanol by-product known to reach a peak in blood within ten minutes following ethanol imbibation in humans [2,11]. Alternatively, an explanation as to why the correlation was not higher may be simply due to a procedural problem, such as a truncated distribution versus a bivariate normal, which could cause some attenuation of the correlation solely because a score of 10 is the ceiling score while the other variate (ethanol concentration) is not similarly constrained. If this method were sensitive to degrees of intoxication greater than the ceiling, a higher correlation coefficient would probably have been seen. In addition, the data also reveals that the regression lines for both brain and blood intersect on the y-axis at about the exact point non-ethanol injected (saline) mice do. It is important to note that mice trained (5 hours) to hold on to the bar are resistant to any bar-holding deficits induced by saline injections.

It is our contention that this model offers improvements in existing techniques and thus is extremely useful in evalua-

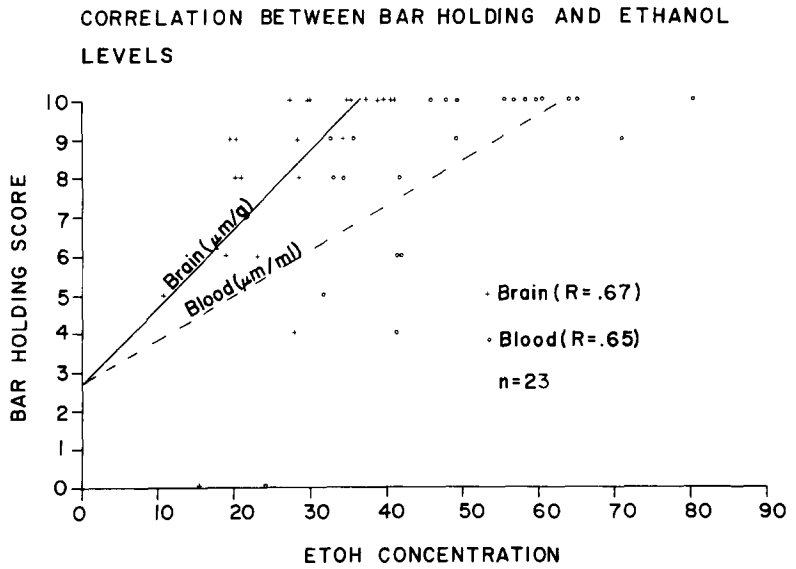

FIG. 4. Correlation between bar holding and ethanol levels in the blood and brain. Blood ethanol levels: ○, brain ethanol levels: +. Ethanol concentrations reported in micromoles per milliliter of blood (μm/ml) or micromoles per gram of brain (μm/g). R=correlation coefficient. N=number of mice for the experiment.

tion of not only ethanol intoxication, but also interactions between ethanol and other pharmacological manipulations important in central nervous system mechanism studies.

It is noteworthy that comparative point to point significance ($p<0.05$) utilizing t-tests were performed on the data in Fig. 3a and b. In Fig. 3a, in untrained animals, there was no statistical significance between each higher dose from its antecedent dose and in addition, significance ($p<0.02$) was found only between the lowest (1.2 g/kg) and the highest (2.1 g/kg) points. This may indicate either a lack of sufficient animals or a "floor effect." In contrast, the trained animals showed consistent significance between each point, except between 0.9 g/kg and 1.2 g/kg.

Although it might be possible to have greater significance in the untrained group by increasing the number of animals, thereby minimizing the effect of a few idiosyncratic subjects, we feel a day of prior training also eliminates those few animals unable to perform the required task and provides less individual variability.

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