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Comparison of Two Methods for Measuring Drug-Induced Neurotoxicity

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Abstract □ A series of experiments is described in which a rotating and a stationary rod procedure were used to determine the neurotoxic effect of various depressant and antidepressant agents in mice and rats. The results of these studies revealed that the rotating rod technique is more sensitive in detecting drug-induced changes in performance. Observed differences between the two methods were more striking in mice than in rats.

Keyphrases □ Neurotoxicity, drug-induced—measurement □ Rotating, stationary rod—neurotoxicity measurements □ Drug-induced neurotoxicity—measurement method comparison

The neurotoxic effect of psychoactive compounds in both mice and rats has been commonly measured utilizing a rotating rod (rotarod) described by Dunham and Miya (1). Since then several investigators (2–10) have attempted modification of this method to establish the optimum parameters for its standardization. Recently Wright *et al.* (11), introduced a method to measure drug-induced neurotoxicity based on the ability of an animal to traverse a stationary, horizontal rod. Studies reported in this paper were conducted to compare the sensitivity of each procedure in two species of rodents, *i.e.*, mice and rats, as methods for measuring the neurotoxicity of various psychoactive drugs.

EXPERIMENTAL

Rotarod Test—The rotarod apparatus used [a modification of the method described by Dunham and Miya (1)] to test mice (male Swiss-Webster strain, 16–26 g.) consisted of a 2.54-cm. wooden dowel divided into 10 equal spaces of 11.43 cm. each by metal disks of 15.24 cm. in diameter. The rod used to test rats (male hooded Long Evans strain, 180–220 g.) was also a 2.54-cm. wooden dowel but was divided into six equal spaces of 20.32 cm. each by metal disks of 30.48 cm. in diameter. The rotarod speed in both instances was 5 r.p.m. Animals were trained to maintain themselves on the rotating rod for at least 1 min.

Stationary Rod Test—The stationary rod apparatus [a modification of the one described by Wright and others (11)] for mice consisted of a 2.54 cm. diameter metal rod 60.96 cm. in length with a platform at either end of the rod. For rats the metal rod used was also 2.54 cm. in diameter but 101.60 cm. long, again with a platform at either end. Training of animals to walk across the horizontal rod required an initial "nip" or "pinch" of their hindquarters as a stimulus to move after being placed on the platform. Additional pinches were not necessary since the animals learned to move along the rod until training criterion was achieved. In this procedure animals were trained to successfully walk the length of the rod twice within a 1-min. trial.

Procedure—In these studies test drugs were administered intraperitoneally. Mice were tested 15 and 30 min. after injection, while rats were tested 15, 30, and 60 min. postinjection. The additional testing interval given to rats was to insure that the time of peak effect for the drug would not be missed since it is accepted that the rate of metabolism in rats is slower than in mice. A trial, in the rotarod procedure, was considered unsuccessful when an animal fell from the rod more than once in a 1-min. period. In the stationary rod test, failure to traverse the rod in at least two of three trials in the 4-min. period was considered an unsuccessful trial. All animals judged unsuccessful at any one testing interval were said to have displayed neurotoxicity.

Drugs—The drugs studied included: chlorpromazine HCl, chlordiazepoxide HCl, tetrabenazine methanesulfonate, benzquinamide, meprobamate, trifluperidol, sodium pentobarbital, imipramine HCl, and thiazesim HCl. All drugs were either suspended or solubilized in 0.25% methylcellulose and dosed in a volume of 0.1 ml./10 g. mice and 0.2 ml./100 g. rats.

Statistics—The median effective dose (ED_{50}) for neurotoxicity with 95% confidence limits and potency ratios were calculated by the method of Litchfield and Wilcoxon (12) at the time of maximum effect. Ten mice or six rats per dose of test drug and a minimum of three dose levels were used for these calculations.

RESULTS AND DISCUSSION

Table I summarizes the results of this study. The approximate time of peak effect in mice for all the drugs studied was 15 min. while in rats this occurred uniformly at 30 min. Therefore, ED_{50} values in each species were dependent on the time between drug administration and testing. Of the drugs tested in mice, only chlordiazepoxide, meprobamate, and thiazesim do not differ significantly by the two methods. Only for pentobarbital was the stationary rod method in mice significantly more sensitive; however, the observed difference between the methods was small. In rats results obtained with the two methods did not vary significantly for chlorpromazine, chlordiazepoxide, tetrabenazine, benzquinamide, meprobamate, and pentobarbital. In this same species neurotoxic effects of trifluperidol were more sensitive to the stationary rod procedure than to the rotarod test. The observed differences seen frequently in mice and not in rats may be attributable to a species difference. High doses of drugs used in this study when given to mice caused marked depression which resulted in their falling off the rotating rod. On the other hand, when rats were given high doses of the drugs listed in Table I, a rigid catatonic-type depression (excluding chlorproma-

Table I—Neurotoxicity of Various Psychotopic Drugs in Mice and Rats as Measured by Two Different Procedures

Drug	Mice			Rats		
	Stationary Rod ED_{50} mg./kg. (CL)[a]	Rotarod ED_{50} mg./kg. (CL)	PR[b]	Stationary Rod ED_{50} mg./kg. (CL)	Rotarod ED_{50} mg./kg. (CL)	PR
Chlorpromazine	3.0 (2.4–3.7)	0.7 (0.4–1.1)	*	1.1 (0.6–2.0)	1.7 (1.0–2.8)	
Chlordiazepoxide	9.9 (6.7–14.7)	9.2 (8.6–9.8)		6.2 (4.3–8.9)	8.8 (5.9–13.1)	
Tetrabenazine	36.1 (23.2–56.3)	11.5 (9.5–13.9)	*	1.4 (1.0–2.1)	1.5 (1.0–2.1)	
Benzquinamide	120.6 (107.0–135.8)	29.0 (13.1–64.4)	*	8.7 (7.7–9.8)	6.8 (4.3–10.7)	
Meprobamate	83.4 (62.8–108.0)	94.5 (83.0–107.6)		97.6 (78.0–122.1)	85.0 (67.1–107.6)	
Trifluperidol	2.4 (2.1–2.8)	0.3 (0.2–0.4)	*	0.16 (0.12–0.21)	0.28 (0.26–0.33)	*
Pentobarbital	13.5 (12.5–14.5)	15.7 (14.4–17.2)	*	5.7 (3.2–9.9)	10.5 (7.1–15.2)	
Imipramine	42.6 (36.9–39.2)	22.1 (12.7–38.5)	*	41.2 (33.1–51.2)	23.0 (16.8–31.6)	*
Thiazesim	37.6 (28.8–49.2)	41.4 (39.9–42.9)		54.9 (46.7–64.6)	32.2 (23.4–44.2)	*

[a] 95% Confidence limits. [b] Potency ratio. Asterisk indicates two methods are significantly different in potency at the $p < 0.05$ level.

zine and chlordiazepoxide) occurred, and the animals when placed on the stationary rod failed to move. The animals did not actually fall from the stationary rod but they failed to walk its entire length. Animals displaying this inability to traverse the stationary rod had to be considered as unsuccessful and lower doses of the drug were tested. Since catatonia, a state of increased muscle tone at rest which is abolished during voluntary movement, must be a function of the drug and the animal, it is possible that the rotarod caused abolition of this phenomenon whereas the stationary rod did not. This may then account for the lack of differences between the two methods in the rat. In the rotarod procedure this type of catatonic behavior failed to materilize in both mice and rats administered the same drugs.

Because of its widespread success as an accurate and simple model the rotarod method appears to be one of the most popular techniques to measure neurotoxicity. Dunham and Miya (1) used it for detecting neurological deficit of psychotropic agents in mice and rats; Herr *et al.* (3) compared the effects of tranquilizers and antidepressants; and Plotnikoff *et al.* (6) studied the effects of stimulants on rotarod performance. Kinnard and Watzman (13) reported on a phenomenon which they called a "free ride." This was defined as "... one revolution in which the animal holds on without walking." This particular event was never observed in these studies. A possible explanation is that the rotarod speed was much slower compared to several earlier studies by other investigators. In addition, this slower speed resulted in a higher percentage of the animals that achieved training criterion.

The stationary rod method would appear to be a potential model for measurement of neurotoxicity because of simple construction and ease of training animals. Results of the present study, however, indicate that this method is less sensitive than the rotarod method, especially for mice. Furthermore, two exceptions must be noted. In mice the stationary rod was superior to the rotarod in revealing the neurotoxic effects of pentobarbital. Additionally, the stationary rod procedure in rats was more sensitive to the neurotoxic activity of trifluperidol. Further studies comparing the two methods described using several analogs of both pentobarbital and trifluperidol might reveal an understanding of these findings.

The question may then arise as to whether the two procedures for measuring neurotoxicity used in this study could represent two different behavior patterns. If this is the case then comparison of the techniques would be questionable. However, the validity of comparing the two methods as measurements for drug-induced neurotoxicity is conceivable since the ability to perform a working task is required of animals in both procedures; *i.e.*, maintaining themselves on the rotarod (5 r.p.m.) and walking across the stationary rod.

In conclusion, therefore, the results of this study suggest that since rodents are quite often used in testing the neurotoxic effects of experimental compounds, it would be more advantageous to use the rotarod procedure over the stationary rod test since the former appears to be much more sensitive.

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