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BRIEF COMMUNICATION

Hind Limb Extensor Response: A Method for Assessing Motor Dysfunction in Rats[1,2]

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CABE, P. A. AND H. A. TILSON. *Hind limb extensor response: A method for assessing motor dysfunction in rats.* PHARMAC. BIOCHEM. BEHAV. 9(1) 133–136, 1978.—A cost-effective, time-efficient technique for measuring neuromuscular dysfunction localized in the hind limbs of rats is described.

Muscular dysfunction Hind limb strength

HIND (or lower) limb muscular weakness has been reported in both human and animal subjects as a toxic consequence of exposure to compounds from a variety of disparate chemical classes. Acrylamide, a monomer used in the plastics industry, is one example of such a compound [5,6]. Organic solvents, such as methyl-n-butylketone and n-hexane, also produce hind limb weakness [4,7], and therapeutic agents—nitrofurantoin, for instance [3]—may produce lower limb weakness as a side effect.

The fact that many of these compounds may be of economic importance, and therefore may pose health hazards in industrial, environmental, or clinical contexts, indicates the need for methods that can be used in screening agents for such a neuromuscular toxic effect.

A number of techniques for the assessment of neuromuscular strength and control have been described, ranging from extremely simple devices such as the inclined screen [8] to sophisticated electronic force transducer systems [2]. Most suffer from one disadvantage or another: expense, expertise required on the part of the user, relatively extensive training required of the subjects, lack of sensitivity due to the level of measurement (eg., present/normal vs. absent/depressed, or similar categorical ratings), or other problems.

We have attempted to develop methods for the assessment of neuromuscular dysfunction that are both cost-effective and time-efficient for use in screening neurotoxic agents. The criteria for such methods are that they be: (a) sensitive to mild dysfunction relative to existing techniques such as the inclined screen test; (b) capable of producing a continuous level of measurement; (c) usable without extensive training for either the subjects or the experimenter; and (d) usable in repeated measures designs across days or weeks. Localization of dysfunction has also been a major concern. Finally, the cost of the equipment should be reasonable.

Recently, we described an apparatus for evaluating forelimb grip strength that meets all these criteria [1]. We here describe a parallel technique suitable for assessing hind limb strength in rats. Our procedure makes use of the fact that an abrupt air puff delivered to the rump produces a startle response, one major component of which is a rapid hind limb extension, a jump. This is termed the hind limb extensor response (HLER).

METHOD

Animals

To date, the HLER technique has been used only with rats, ranging in weight from about 250 g to over 500 g.

Apparatus

The apparatus is pictured in Fig. 1. The central component is a push-pull recording strain gauge (Chatillon Model DPP; J. A. King and Co., Box 21225, Greensboro, NC 27420), to which has been attached a T-bar constructed by inserting a 5″×1/8″ dia. (12.7×0.3 cm) brass rod through a hole near one end of a 3″ (7.6 cm) hexagonal threaded aluminum standoff. The strain gauge is rigidly mounted at a 45°

[1]Mention of components by brand name should not be taken to imply endorsement by the National Institute of Environmental Health Sciences, the National Institutes of Health, or the U.S. Department of Health, Education, and Welfare.

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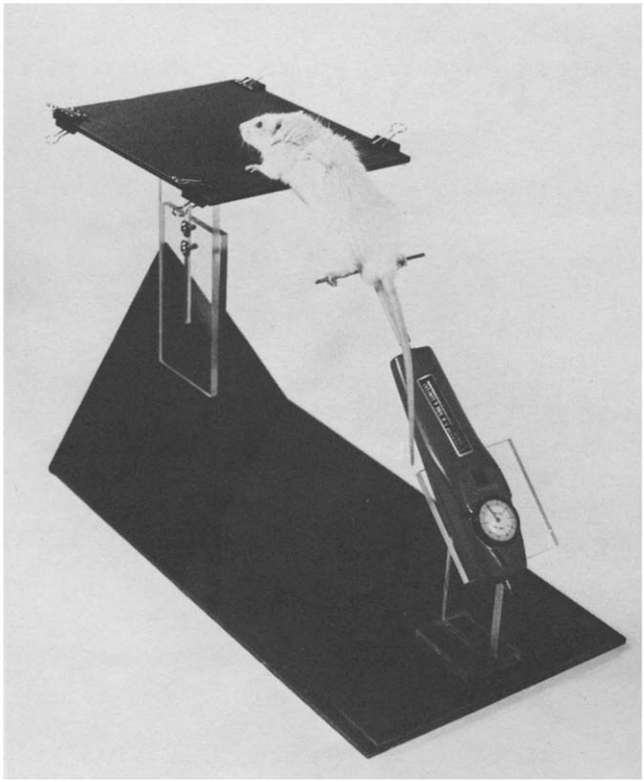

FIG. 1. The hind limb extensor response test apparatus. The meter, T-bar, sandpaper-covered platform, height-adjustment bolts, and foot placement can all be seen in this illustration.

angle to vertical by means of pre-tapped holes in the body of the instrument, such that the T-bar is parallel to the edge of a 9″×12″ (12.9×30.5 cm) platform covered by a sheet of coarse sandpaper. The sandpaper sheet is attached with spring clips, so it can be replaced when it becomes soiled. The bar is (arbitrarily) 13″ (33 cm) above the bench top and the platform is typically positioned 1 1/2″ (3.8 cm) higher than and 3″ (7.6 cm) laterally displaced from the T-bar. This bar-platform gap size was derived through pilot testing.

The apparatus shown is constructed of Plexiglas, with the platform horizontally and vertically adjustable by means of slots and screws. The particular support arrangement can be varied; a workable early version of the apparatus was put together with ring stands and burette clamps. However, the relation of the T-bar to the platform, displaced vertically as well as horizontally, seems to be important.

A study over any appreciable period of time must take into account the fact that both the body length and strength of the rat will increase. The bar-platform gap should therefore be adjusted as necessary to accommodate different sized rats. It has been our experience that, for animals in the 250–350 g range, the gap size described above is appropriate. The gap should be altered, with the restriction that the ratio of horizontal and vertical displacements (2:1) be maintained, for larger or smaller rats. The criterion should be that the rat's body be neither stretched across the gap nor flexed to such a degree that the hind limbs cannot respond in some midrange of their extension.

Since the muscle mass, and consequently the strength, of the hind limb muscle groups is relatively great, the range of the meter chosen should be considered carefully in relation to the animal's size. It is not unprecedented to record HLER forces that are as much as four times the weight of the rat. The Chatillon DPP strain gauge comes in several load range models; at least a one kilogram meter should be used and the 2.5 kg device is recommended. It would be desirable to have several load ranges at hand.

Procedure

As a precautionary first step, the strain gauge should be calibrated by recording the meter deflection as the instrument is loaded with weights covering the range of the meter. The deflection, in our experience, is invariably a linear function of weight, but, since the meter is a mechanical device, friction, wear, possible corrosion or other variables may result in a meter reading which does not accurately reflect the force applied.

In use, the strain gauge is set to record meter deflections produced by pushing forces. The rat is held in the experimenter's right hand around the shoulder girdle. The rat's tail is taken in the left hand and the plantar surfaces of the rat's hind feet placed symmetrically on the bar. The antebrachia are then placed on the sandpaper-covered platform, and the animal is released from the experimenter's grip.

At this point, rats quite typically step across to the platform. It has been our practice to repeat the placement procedure until the rat sits still in position long enough to zero the meter with the animal's weight on the T-bar and to administer a sharp air puff to the rat's rump, by mouth from about 15 cm to the rear. Other methods of delivering the air puff have been tried (eg., rubber bulb, aerosol can), but have not been found to be as effective as the human source.

Frequently, animals will attempt to back off the platform. To avoid this, the experimenter should release the rat slowly; if the animal attempts to back off the platform, it may then be restrained, replaced, and released again until backing extinguishes. This backing tendency is a major factor in selecting the gap size between the T-bar and platform. A larger gap tends to promote backing.

By releasing the body first, the left hand maintaining a light grip on the tail until the meter is zeroed, the placement of the animal on the HLER device can be controlled. Rats which persist in stepping across may be momentarily blocked with one hand until the air puff can be applied.

RESULTS AND DISCUSSION

Delivery of the air puff produces a startle response, in which the hind legs are rapidly extended, registered as a meter deflection read in grams of force. It has been our practice to average three readings or to take five readings and average the highest three.

Derived measures have been given some consideration, for example, the force exerted as a proportion of body weight or a ratio of maximal force to average (typical) force. Neither of these seems to have any clear advantage over the average force reading per se.

Table 1 shows the results of several experiments with compounds known to produce neuromuscular deficits. Values for the control groups suggest typical HLER scores. In each of the cases listed, the dosed group's HLER scores were significantly less than the control group's scores.

The question of body weight influence on force readings was raised early on. Somewhat surprisingly, total body weight does not seem to be a major factor in the HLER,

TABLE 1

SUMMARY OF SOME CHEMICAL EFFECTS ON HIND LIMB EXTENSOR RESPONSE (HLER) SCORES AND RELATIONSHIP TO BODY WEIGHT IN FISCHER STRAIN RATS

Compound	Control Group Mean HLER Score	Control Group Standard Deviation	Control Group N	Control Group r* vs. Body Weight	Dosed Group Mean HLER Score	Dosed Group Standard Deviation	Dosed Group N	Dosed Group r vs. Body Weight	Dosing Regime†
Acrylamide									
Acute	425.40	112.49	20	0.08	223.18	14.80	9	0.30	1 dose (200 mg/kg)
Subchronic	559.70	170.73	10	0.25	406.00	81.56	10	0.12	2 weeks × 5 doses/ week (20 mg/kg)
Chronic	392.23	124.78	20	0.13	234.77	76.98	10	0.16	6 weeks × 3 doses/ week (20 mg/kg)
Carbon disulfide	361.17	149.17	20	0.05	203.83	126.53	20	0.23	6 weeks × 5 doses/ week × 4 hours/ day (2 mg/liter air, inhalation)
Polybrominated biphenyls (Firemaster FF-1)									
Males	321.20	168.94	15	0.19	203.77	48.71	13	0.25	6 months × 5 doses/ week (10 mg/kg)
Females‡	239.00	74.90	9	0.46	202.88	34.73	8	0.33	

*Pearson product-moment correlation

†Dosing indicated is that to the point at which the HLER scores in this table were recorded, i.e., to the point at which the first significant difference was found in HLER scores between dosed and control groups. Controls were either nondosed or vehicle dosed.

‡All other values are for male rats.

since we have seen significant changes in hind limb strength in the absence of significant body weight differences, nonsignificant hind limb strength differences where body weights among groups do differ significantly, and significant differences where body weights also differ significantly. Part of the reason for this is that in use the meter is zeroed with the animal's weight on it, a taring procedure, so that meter readings are force in excess of that contributed by body weight. Table 1 shows that body weight and HLER scores do not correlate highly. In no case is more than about 25% of the variance of the HLER score accounted for by body weight variance.

The effect of differences in placement, ie., the proportion of body weight supported by the hind limbs, across tests and dose groups has also been raised. In one experiment, the chronic acrylamide study listed in Table 1, the meter deflection due to the rat's weight was recorded and compared with the animal's weight. This proportion did not differ among dose groups even though HLER scores did differ from control values. Since experience on the apparatus is equivalent across all groups, the only factor that could account for differences in position on the apparatus (assuming a conscientious experimenter) is a chemical effect. Since HLER scores differ where proportion of body weight supported by the bar does not, our conclusion is that positional change or differences in proportion of weight on the bar are not serious sources of variability in the results.

Variability in the HLER technique, however, requires some comment. Clearly, the readings obtained will be a function of the animal's hind limb strength, modulated by other factors. Since the stimulus is produced by the experimenter, it can vary among experimenters and from trial to trial; the same experimenter should, therefore, test all animals in a given experiment and should be instructed to attempt to produce the same stimulus intensity at every trial. Further, the experiment should be run blind with dose groups tested in randomized blocks across the whole test session. The position of the rat on the apparatus will vary slightly depending on the exact size of the rat, since the apparatus cannot be adjusted for each animal. Further, since there is a lag between placement of the animal and delivery of the stimulus, the animal may change position slightly. Repeated trials with the same animal allows habituation to the stimulus, as well as affording the opportunity for central tendency effects (when the trial by trial readings are averaged) to minimize variability across trials in a given animal.

The advantage of a continuous level of measurement becomes apparent at this point: parametric statistics allow the experimenter to evaluate error variance from all these sources, relative to treatment variance. This is difficult to impossible with procedures giving ordinal or nominal levels of measurement. The HLER values, of course, can also be treated as ordinal measures if desired.

The device shown in Fig. 1 has been used in several studies in our laboratory and has been shown to be sensitive to group differences in hind limb strength following acute, subacute, and chronic exposure to acrylamide, subchronic exposure to inhaled carbon disulfide, and chronic exposure to polybrominated biphenyl compounds. The apparatus is inexpensive, relatively simple to use, requires little experience or training on the part of the experimenter, minimal training on the part of the animal, and it produces data at a continuous level of measurement. Compared to other apparatus intended to measure muscular dysfunction, it is sensi-

tive to mild dysfunction of insidious onset, dysfunction that is not discernible on gross observation, and localizes the dysfunction in the hind limbs. Therefore, we believe the device to be a cost-effective, time-efficient tool for the specific assessment of hind limb neuromuscular function.

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