Pharmacology Biochemistry & Behavior, Vol. 4, pp. 721–723.

BRIEF COMMUNICATION

A Simple Method for Quantifying Tremor in Rodents [1]

GARY REMINGTON AND HYMIE ANISMAN

Carleton University, Ottawa, Ontario K1S 5B6, Canada

(Received 21 July 1975)

REMINGTON, G. AND H. ANISMAN. *A simple method for quantifying tremor in rodents* PHARMAC BIOCHEM BEHAV. 4(6) 721–723, 1976. – A simple and accurate device to evaluate frequency and intensity of involuntary tremor is described. Discrete and quantifiable measures of tremor could be obtained in terms of vertical (i.e., absolute effects at a given time) and horizontal (i.e., temporal) changes. The technique was evaluated employing dosages of physostigmine 0.1–0.7 mg/kg. Applications to other behavioral indices, such as locomotor activity and wet-dog shakes, are discussed

Tremor Tremor quantification Physostigmine Locomotor activity

IT is well documented that drugs which increase cholinergic activity or decrease dopaminergic activity elicit body tremor together with muscle weakness and salivation [1, 2, 3]. Typically, the degree of tremor elicited by pharmacological treatments has been evaluated using a likert-rating scale [1]. While this method of evaluation may result in good inter-rater reliability, there is little doubt that the finer measurements or indices of tremor are lost. For example, small differences in the intensity of tremor cannot be quantified readily, and in addition, variations in tremor at various times after drug treatments are obtained with difficulty. In the present report a simple apparatus is described which allows for the quantification of the intensity, frequency and temporal pattern of tremor, as well as other behaviors which are often difficult to quantify.

METHOD

An 8 watt speaker measuring 20 cm in dia. was covered by a 0.012 cm mylar sheath. The mylar was sealed between two O rings attached to the metal frame of the speaker. As a result a constant air pressure was formed between the speaker and the mylar. Consequently, even a small displacement of the mylar sheath produced fluctuation in air pressure and this induced deflection in the speaker cone and varied the electromagnetic flux. The speaker was shielded by copper screening and was connected directly to a Beckman type RM dynograph (A-C coupler, type 9806A, Amplifier, type 482). The Polygraph was calibrated such that a 30 g object resting on the mylar did not produce movements of the pens. Tremor or other movements, however, resulted in pen deflections corresponding directly with the intensity and frequency of the animal's movements. Variations in weight seen in mice, typically in the magnitude of only a few g do not affect sensitivity of the device. However, sensitivity is reduced in heavier rodents, such as rats, and a voltage transformer is needed to obtain sensitive recording.

Sixty-four male Swiss-Webster mice, 90–120 days of age, procured from the Bio-breeding laboratories served as subjects. Mice were housed communally, 5/cage, and permitted ad lib access to food and water. Mice were randomly assigned to 8 groups (n = 8/cell) and injected intraperitoneally with physostigmine salicylate in doses of 0.1–0.7 mg/kg in 0.1 mg/kg increments. One min after injection mice were placed on the mylar surface, and recording commenced 30 sec afterwards. Tremor was recorded for 15 sec of each min for a 20 min period.

Throughout, the experimenter observed the animal and recorded periods of locomotor or head movements on the stripcharts. In addition, a rating of salivation, tremor and muscle tone was made on a 4 point scale.

RESULTS AND DISCUSSION

The frequency of tremor (tremor/15 sec) and the intensity of tremor, as defined by pen deflection of either 2–10 or greater than 10 mm were calculated. Deflections less than 2 mm were discounted as being artifactual due to respiration. As seen in Fig. 1, it was observed that the intensity and frequency of tremor increased with dosage ($F(5,47) = 6.01$ and 2.48, p's<0.01, 0.05 for the < 10 and > 10 deflections, respectively), in addition tremor occurred

[1]The research was supported by Grant A9845 from the National Research Council of Canada to Hymie Anisman. Requests for reprints should be addressed to the first author, Department of Psychology, Carleton University, Ottawa, Ontario K1S 5B6. The assistance of D Weening in the evaluation and construction of an earlier model of the recording device is gratefully acknowledged

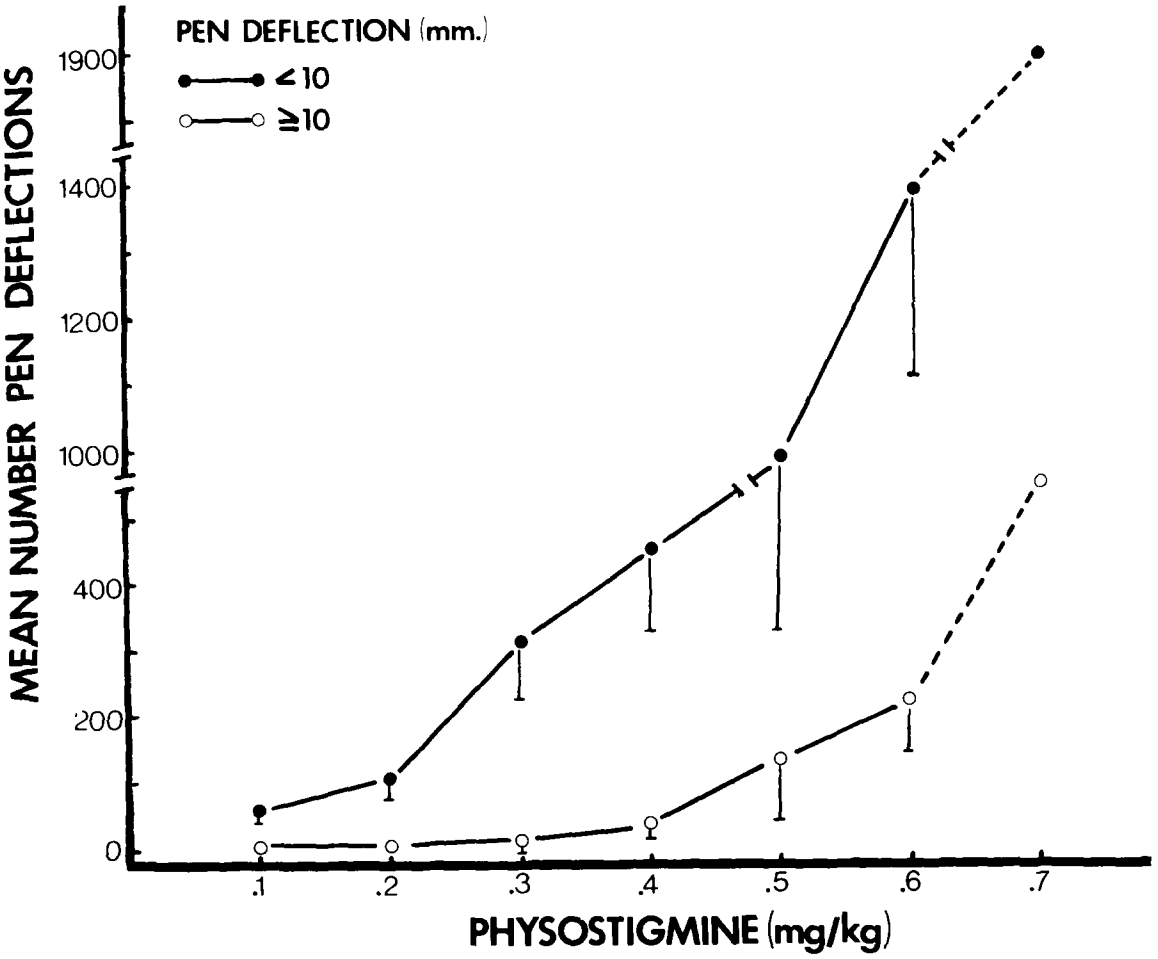

FIG. 1. Mean number of pen deflections recorded for 15 sec of each min over a 20 min period in physostigmine treated mice High and low intensity tremor were designated as pen deflections of < 10 mm and > 10 mm, respectively Broken lines are used to indicate tremor induced by 0.7 mg/kg. Only 3 of 8 animals survived this dosage, and thus this point probably is not an adequate assessment of tremor

earlier with the higher dosages. The L. D. 50 in this strain of mouse was 0.7 mg/kg. Since only a small portion of mice were represented at the L. D. 50 dose, these animals were not included in the analysis of variance. However, the mean of 3 animals is shown in Fig. 1. The important point here is that the apparatus was sensitive in detecting tremor produced by even small dose differences, and this was the case for both high and low intensity tremor. Subjective ratings of tremor also revealed that tremor increased as a function of drug dosage. However, the use of a 4 point rating scale did not permit precise assessment of between-subject variance within a group. In contrast, employing the quantitative measure of tremor, between-subject differences were readily discernible. As a case in point, in Fig. 2, subjective rating of A and C would not indicate differences, whereas inspection of the recordings reveals substantial differences between the degree of tremor in the 2 instances.

Figure 2 shows sample recordings obtained. Panel A shows tremor at low intensity-low frequency, while Panel B shows high intensity-high frequency tremor. Panel C shows low intensity-low frequency tremor interpolated with voluntary head/body movements.

In the present investigation locomotor activity was recorded on the strip charts by an observer. Pen deflections produced during locomotion were not included in the analysis of tremor. The degree of locomotor activity was calculated in terms of the total time spent walking. Locomotor activity decreased with increasing drug dosages ($F(5,42) = 13.17$, $p<0.01$, $\bar{x}$ sec locomoting = 211.19, 113.75, 49.81, 34.31, 32.5, 34.06, 26.33, for 0.1–0.7 dosages, respectively). In the dosages of 0.4 mg/kg and higher, locomotor activity was almost entirely absent after 5 min. In fact, the mean time spent walking during the last 15 min of the test session was 4.29 sec for these groups. Thus, as locomotor activity decreased, tremor increased. It is noteworthy that continuous rating of tremor is not necessary, and a time sampling procedure can be employed instead. Using a time sampling procedure occasions during which locomotor activity occurs can be noted by the observer without excessive effort. Although locomotor activity was recorded on the strip chart by an observer in the present study, gross locomotor activity also can be determined via an array of photoelectric cells, or alternatively by a band-pass filter, since tremor frequency differs from that of locomotor activity.

The apparatus described was exquisitely sensitive to

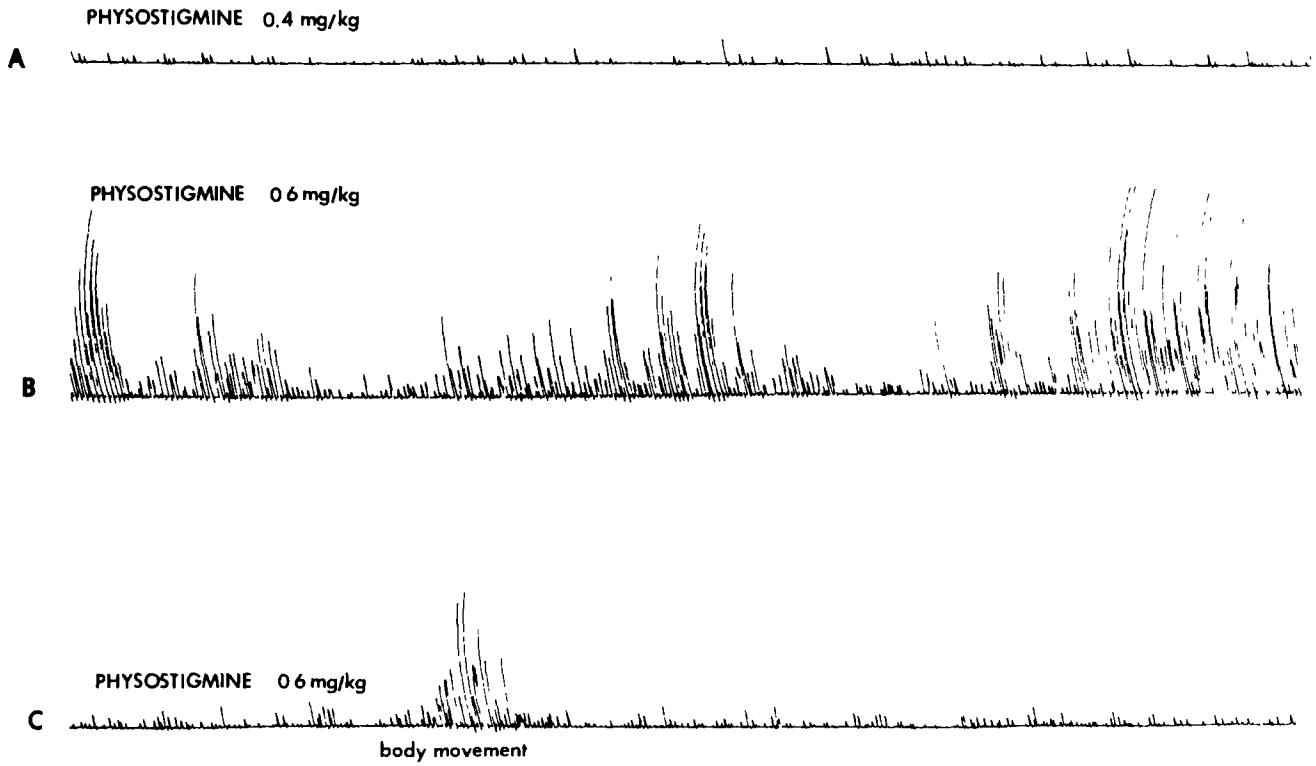

FIG. 2 Sample recordings of tremor activity. Panel A shows low intensity-low frequency tremor 12 min after injection of 0.4 mg/kg physostigmine Panel B shows high frequency-high intensity tremor 10 min after 0.6 mg/kg physostigmine Panel C represents low intensity tremor activity interpolated with body movement, 6 min after 0.6 mg/kg physostigmine

detect variations in frequency and intensity of tremorigenic activity. Unlike previously used methods, frequency and intensity of tremor could be quantified on ratio and interval scales, respectively. Availability of this sensitive technique permits quantification not only of discrete vertical variations in tremor, but also accurate assessment of horizontal changes in tremor. Moreover tremor could be divorced from other motoric factors, such as voluntary locomotion and head movement.

In addition to the measurement of tremor, the apparatus described was previously found to be a sensitive indicant of several other behaviors which are often difficult to quantify. In particular, because the apparatus is sensitive to grooming and gnawing behavior it may be used to measure stereotypic behaviors elicited by drugs such as amphetamine or apomorphine. Moreover, employing an earlier version of this apparatus (Anisman & Weening, unpublished report) in which frequency and intensity were integrated, a quantifiable measure of opiate withdrawal (i.e , wet-dog shakes, body or head shakes, wild running, and torso extensions) was obtained. Finally, employing an integrated index, a sensitive recording of a startle response can be obtained.

REFERENCES

1 Ambani, M L and M. H. Van Woert Modification of the tremorigenic activity of physostigmine *Br. J. Pharmac.* **46**: 344–347, 1972.

2. Frances, H. and J Jacob Comparison des effects de substances cholinergiques et anticholinergiques sur les taux cerbraux d-acetylcholine et sur la motilite chez la souris. *Psychopharmacologia* **21**: 338–352, 1971.

3 Pradhan, S. N and S. N. Dutta Central cholinergic mechanisms and behavior. In *International Review of Neurobiology*, edited by C. C. Pfeiffer and J R. Smythies, New York. Academic Press, 1971.