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# THE ANALYSIS OF DRUG-INDUCED TREMOR IN MICE

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An improved method of recording tremor in mice has been developed using a gramophone pick-up and a cathode ray oscillograph. Permanent records are made on 35 mm. film from which it is possible to determine qualitative differences in the tremor caused by various drugs. In addition, the severity of the tremor may be estimated quantitatively by a tremor index. The rate and variations in the rhythm and amplitude and in the tremor index have been determined for Tremorine (1,4-dipyrrolidin-1'-ylbut-2-yne), harmine, harmaline, 3-amino-1,1,3-triphenylpropan-1-ol and lysergic acid diethylamide. The incidence of side effects has also been noted.

Conventional methods of recording gross movements in small animals by means of a jiggle cage or work adder are not sufficiently precise to permit a critical analysis of tremor. The record obtained depends greatly on the type of apparatus used and, in addition, is likely to show movements arising from the resonance of the apparatus.

An improvement in technique was introduced by Moore, Sigg, and Schneider (1957), who implanted small permanent magnets subcutaneously in mice. The mouse was then surrounded by a wire coil and the changes in the induced current caused by tremor-producing drugs were recorded. The main disadvantage of the method is that the animals require preliminary operation. In our experience the animals are liable to die from sepsis, even if the magnets are first coated with Perspex and carefully sterilized by ultra-violet light.

The fidelity with which a gramophone pick-up will follow movement applied to the stylus has been exploited not only in recording motor activity (Larsen, 1955), shivering (Boyarsky and Stewart, 1957) and epileptic seizures (Essig and Flanary, 1957) in animals, but also tremor in man (Rohracher, 1946). In the experiments reported here we have attempted to characterize the tremor induced in mice by a variety of drugs and to express the severity of the degree of tremor by means of a tremor index.

## METHOD

The mouse cage consisted of a transparent plastic soap box weighing about 30 g. (F. W. Woolworth Ltd.), the lid being perforated with ¼ in. holes to allow free access of air. The two halves were closed by two elastic bands. The box was attached to a gramophone pick-up (Model G.P. 31, Cosmocord Ltd.) by a short length of thin brass rod, one end of which was threaded and fixed to the lid by two nuts, the other end being inserted into the needle holder of the pick-up (Fig. 1). The output of the pick-up was fed directly into a cathode ray oscillograph (Cossor Model 1049) and the tracing photographed on 35 mm. film (Kodak R 55) with an oscillograph camera (Mk. II A, Cossor Instruments Ltd.). In some experiments, the output of the cathode ray oscillograph was capacitance-coupled to an amplifier driving a single channel direct writing pen recorder (Mk. 5, Henry Hughes and Sons Ltd.) and the record obtained on a continuous strip of

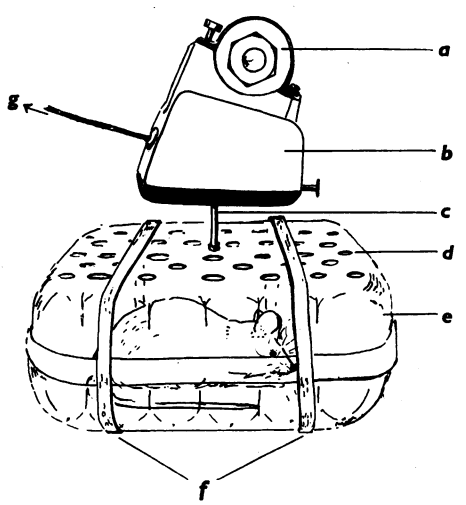


FIG. 1.—Soap-box pick-up assembly. *a*, supporting arm (viewed end on); *b*, pick-up; *c*, stylus; *d*, air holes; *e*, soap-box; *f*, elastic bands; *g*, screened lead to cathode-ray oscilloscope.

"Teledeltos" paper. The pick-up and soap box were mounted on a rigid slotted angle stand which was cushioned on thick sponge rubber to prevent vibrations being transmitted from the bench. Since the apparatus was microphonic, the cage and stand were placed under a telephone booth (Burgess "Accousti Booth") and the experiments conducted away from noise. The apparatus responded only to bodily movements of the mouse; movements attributable to respiration or heart beat did not appear. The frequency, wave-form and tremor index (see below) were obtained from the photographic records; the duration of tremor and the effect of anti-tremor drugs were obtained from the paper strip.

Albino mice weighing 18 to 30 g. of both sexes were used. Tremor was induced by intraperitoneal administration of the following drugs: Tremorine (1, 4-dipyrrolidin-1′-ylbut-2-yne) (30 mg./kg.), an amino alcohol (3-amino-1,1,3-triphenylpropan-1-ol) (30 mg./kg.), harmine (15 mg./kg.), harmaline (15 mg./kg.), lysergic acid diethylamide (4 mg./kg.), eserine (5 mg./kg.), and nicotine (5 mg./kg.).

*Recording of the Tremor.*—A mouse was placed in the soap box which was then fixed in position on the pick-up and the animal allowed to settle down for 10 to 20 min. During this time the spontaneous activity subsided almost completely and, when the mouse was quiet, control records were taken. The mouse was then removed, given an effective dose of one of the tremor producing drugs and replaced in the box. Records were then made for 10 sec. at intervals of 5 min. Some mice were treated with anti-tremor drugs and the results recorded in the same way.

*Interpretation of the Records.*—After processing, lengths of the film were bound between 16 in. strips of perspex and the tracing was examined under a five-fold magnification in a photographic enlarger. From the image cast on the screen, the frequency could be determined and an impression gained of the regularity of the rate and amplitude. The severity of the tremor was estimated by relating the distance traversed by the spot of light as a multiple of the straight line which the spot would have traced had no tremor taken place at all. This was done as follows: two parallel pencil lines were drawn on a piece of white cardboard 18 in. apart and the card was placed on the enlarger board so that the lines were intersected perpendicularly by the image of the light track. The length of the light track between these two lines was then measured to the nearest 0.1 in. by a sensitive opisometer. The length of the light track in inches divided by 18 gave the *Tremor Index*. For example, if the image of the light track as measured by the opisometer was 54 in., this divided by 18 would give a tremor index of 3.0.

## Results

### *Untreated Mice*

If an untreated mouse, or one given an injection of saline, was put in the box it showed the usual exploratory activity, cleaning movements and frequent alterations in position. Such spontaneous activity caused very irregular waves of small amplitude (Fig. 2, upper record). A few larger waves appeared in groups here and there and these were associated with cleaning movements. The frequency of the spontaneous movements was 22 to 25 cycles/sec. Left undisturbed, the mouse would settle down in about 10 to 20 min. The mice were allowed food and water *ad libitum* before the experiment since it was found that hungry mice took much longer to settle down. The tremor index of spontaneous activity varied between 1.30 and 1.51 when the mouse was first placed in the box and this decreased to 1.00 during the settling-down period.

### *Tremorine*

Mice receiving Tremorine, 20 to 30 mg./kg. intraperitoneally, showed severe tremor, diminished normal motor activity, and profuse salivation, lachrymation and diarrhoea. The tremor was regular and continuous, and had a frequency of 14 to 18 cycles/sec. (Fig. 2, middle record). There was no marked variation in the amplitude of the tremor during the peak of the effect. The tremor index varied from 2.06 to 3.90 (Table I).

There was a latency of 2 to 5 min. before tremor appeared. The onset was gradual, fine tremor appearing first in the head and spreading later to the body and tail. In most animals the peak of the tremor occurred between 10 and 20 min. after giving the drug. The tremor persisted at rest and during movement. After about 30 to 45 min. the tremor became coarse and intermittent, resembling shivering. Some animals showed this coarse episodic tremor for as long as 4 hr.

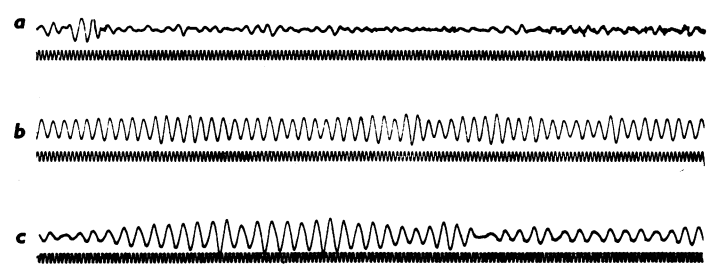


FIG. 2.—Cathode-ray oscillograph recording of (*a*) normal movement in the mouse, (*b*) tremor caused by Tremorine (30 mg./kg.) and (*c*) harmaline (15 mg./kg.). Time, 50 cycles/sec.

TABLE I
COMPARISON OF PATTERNS OF TREMOR INDUCED BY VARIOUS DRUGS IN MICE
n.r. indicates not recorded.

| | Drugs | | | | | | |
|---|---|---|---|---|---|---|---|
| | Tremorine | Harmine | Harmaline | Lysergic Acid Diethylamide | 3-Amino-1,1,3-triphenyl-propan-1-ol | Eserine | Nicotine |
| Delay in onset (min.) .. | 3–6 | 3–5 | 3–5 | 7–10 | 6–15 | 1–2 | 1–2 |
| Duration (min.) .. .. | 30–180+ | 15–40 | 20–40 | 15–30 | 30–120+ | 1–3 | 5–8 |
| Cycles/sec. .. .. | 14–18 | 14–16 | 14–16 | 18–20 | 15–17 | n.r. | n.r. |
| Variability in amplitude | Slight | Slight | Slight | Moderate | Marked | ,, | ,, |
| Tremor index .. .. | 2·06–3·90 | 2·20–2·38 | 2·1–2·5 | 1·97–2·3 | 2·25–3·4 | ,, | ,, |

*Antagonism of Tremorine Tremor.*—Blockus and Everett (1957) reported that atropine, hyoscine and other anti-parkinsonian drugs antagonized tremor induced by Tremorine. The effect of hyoscine on the tremor index was obtained as follows. A mouse was given Tremorine (30 mg./kg.) intraperitoneally and, during the peak of the tremor, received hyoscine hydrobromide (10 mg./kg.) intraperitoneally. The tremor ceased within 5 min. and normal behaviour returned in 15 to 30 min. The tremor index was lowered from 3.2 to 1.2, which is within normal limits.

*Harmine and Harmaline*

Harmine and harmaline are two alkaloids from the seeds of *Peganum harmala.* As early as 1895, Neuner and Tappeiner reported that these alkaloids could produce tremor in laboratory animals. The pharmacological properties of the two substances have been described by Gunn (1911, 1913).

Harmine produced tremor in mice after an injection of 10 to 20 mg./kg., intraperitoneally. The tremor appeared within 4 to 6 min. and lasted for 15 to 20 min.; the peak effect came between 8 and 15 min., at which time the tremor was continuous. The tremor was rather fine and generalized and was present both at rest and during movement. The animals showed the Straub tail phenomenon and hunched-back attitude but could walk with normal gait. The mice responded in an almost normal manner when handled. Reflexes were not exaggerated, there was no muscular weakness or lethargy and parasympathomimetic effects did not occur.

The pattern of tremor and the changes in behaviour induced by both harmine and harmaline were alike except that the intensity and duration of tremor were slightly greater with harmaline. Harmaline tremor appeared soon after the injection, lasted for 30 to 40 min. and was accompanied by slight piloerection. The tremor was regular and continuous although the amplitude waxed and waned (Fig. 2). The frequency of the tremor induced by both drugs lay in the range 14 to 18 cycles/sec. which is very near that given for harmine (15 to 20 cycles/sec.) by Hara and Kawamori (1954).

*Antagonism of Harmine Tremor.*—The results of Hara and Kawamori (1954) and Zetler (1957) indicate that harmine tremor can be effectively controlled by a variety of drugs, including anti-parkinsonian drugs, ganglion blocking agents, sedatives, ataraxics, anti-epileptics, 5-hydroxytryptamine and its antagonists. It is of interest to note that lysergic acid diethylamide, which resembles harmine in its chemical structure and which itself produces tremor, antagonizes the harmine tremor in very small doses (Zetler, 1957).

*Lysergic Acid Diethylamide*

Woolley (1956) has described the abnormal type of behaviour and tremor exhibited by mice after receiving lysergic acid diethylamide. After an initial moderate restlessness, the mice made backward movements with the hindlimbs outstretched and toes spread wide apart. The animals showed marked piloerection and changed their positions frequently on the floor of the cage, using their forelimbs only. They could not walk as could those treated with harmaline. After about 20 to 30 min., the mice remained quietly in a corner of the cage with hunched backs. Tremor appeared 5 to 10 min. after the intraperitoneal injection of lysergic acid diethylamide (4 mg./kg.) and it was fine, intermittent and interspersed with normal bodily movement (Fig. 3, lower record). The peak effect occurred between 8 and 15 min. after injection. The amplitude varied markedly, the frequency lying between 22 and 25 cycles/sec. The tremor index (Table I) indicates the severity of the tremor was much less than that following Tremorine or harmine.

*Antagonism of Lysergic Acid Diethylamide.*—Lysergic acid diethylamide tremor is not antagonized by 5-hydroxytryptamine or parasympathomimetic drugs (Woolley, 1956).

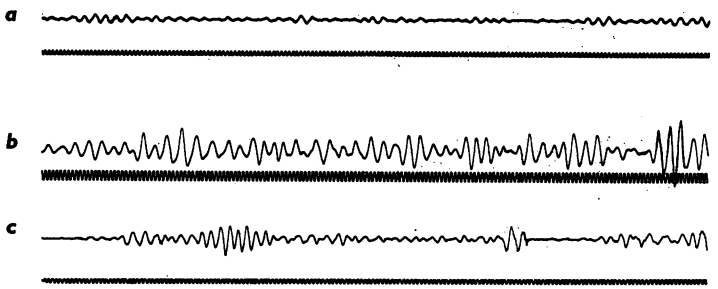


FIG. 3.—Cathode-ray oscillograph recording of (*a*) normal movement in the mouse, (*b*) tremor caused by 3-amino-1,1,3-triphenylpropan-1-ol (30 mg./kg.) and (*c*) lysergic acid diethylamide (4 mg./kg.). Time, 50 cycles/sec.

*Aminotriphenylpropanol*

Out of the twelve aminoalcohols synthesized in this laboratory, 3-amino-1,1,3-triphenylpropan-1-ol appeared to be the most potent tremor producing drug. The pharmacology of this aminoalcohol has been reported by Ahmed, Marshall, and Shepherd (1958).

In doses of 20 to 40 mg./kg. given intraperitoneally to mice, the drug produced the following effects: body extension, restlessness, hurried respiration, Straub tail phenomenon, and difficulty in walking forward. Tremor appeared within 8 to 15 min., occurring first in the head and then extending to the body and tail. The animal assumed an extended posture with outstretched limbs and the abdomen touching the floor. When tremor occurred there were continuous struggling movements to go forward, continuous extensions of the neck, body and forelimbs, but the hindlimbs failed to propel the body forward. The animal could, however, alter the position of the forepart of its body with its forelimbs and could make a circle lying in the same position. The animal frequently walked backward. The characteristic pattern of tremor associated with profound restlessness, struggling, ataxia and backward walking and circling movements were not seen with other tremor-producing drugs so far studied. Furthermore, one of the remarkable features of this compound was the production of severe hyperexcitability, an effect not seen with other drugs which produced tremor.

Tremor was present both at rest and during movement and was accentuated by external stimuli. The peak of the tremor occurred between 20 and 40 min. after the injection of the drug. The tremor was coarse, severe, and continuous, and lasted from 30 min. to 3 hr. Fig. 3*b* shows a tracing of aminoalcohol tremor, being characteristically irregular, with marked variations in rhythm and amplitude, sometimes distorted by struggling movements. The tremor was unlike that following harmine, harmaline, and Tremorine. With the tremor after lysergic acid diethylamide, such large amplitudes were not seen. The frequency of the aminoalcohol tremor ranged from 14 to 16 cycles/sec.

## DISCUSSION

Table I shows the pattern of tremor and Table II the changes in general behaviour induced in mice by different drugs which produce tremor. The onset, amplitude, frequency, duration of effect and incidence of side effects varied considerably between the drugs. The technique of recording used enabled one type of tremor to be distinguished from another in a way which was not possible by direct observation of the animal. Harmine and harmaline give rise to a sinusoidal type of tremor pattern which closely resembled that of Tremorine but was not accompanied by the central parasympathetic stimulation caused by Tremorine. The tremor due to the aminotriphenylpropanol was in marked contrast to that due to the previous three drugs since it was completely irregular in amplitude and frequency. The record in this instance resembled, in many ways, an

TABLE II

COMPARISON OF EFFECTS PRODUCED BY DIFFERENT DRUGS IN MICE

— indicates absent, + slight and ++ marked effect.

| Effect | Drugs | | | | | | |
|---|---|---|---|---|---|---|---|
| | Tremorine | Harmine | Harmaline | Lysergic Acid Diethylamide | 3-Amino-1,1,3-triphenyl-propan-1-ol | Eserine | Nicotine |
| Parasympathetic stimulation | ++ | — | — | — | — | + | + |
| Piloerection | — | — | + | ++ | — | — | — |
| Straub tail | + | + | + | — | + | + | + |
| Running movements | — | — | — | — | — | + | ++ |
| Walking backwards | — | — | — | + | + | — | — |
| Hyperexcitability | — | — | — | + | ++ | — | — |

exaggeration of the normal movement of the animal. The tremor records after lysergic acid diethylamide had a superficial resemblance to those after aminotriphenylpropanol, but the tremor was less severe and the tremor index lower.

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