A QUANTITATIVE METHOD FOR CONTINUOUS RECORDING OF SPONTANEOUS ACTIVITY IN SMALL ANIMALS

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As one of the routine screening tests for evaluating drug effects on the central nervous system, the effects on spontaneous movements of mice and rats have often been examined. Various devices have been employed so far for measuring spontaneous activity of small animals by using the jiggle cage (1-4), rotating cage and photoelectric cage methods (5-6). The jiggle cage method records general bodily movements while the rotating cage and photoelectric cage methods measure relatively purposeful motor activity such as walking and running movements. Akiyama (7) developed a kymographic method to record both general bodily movements and walking or running movements simultaneously. In all these methods, various recording devices, e.g. for counting the number of revolution of the rotating cage or the frequency of interrupting the light beam to the photoelectric cell, have also been developed in order to accomplish a more quantitative measurement of motor activity. However, much laborious works are required if frequent counting is needed at shorter intervals of time in order to follow up the temporal pattern of drug effects on spontaneous activity.

Movements of the jiggle cage were also integrated and thence recorded quantitatively by the use of work adder (1-2), or an elaborate electronic device (4).

The authors have developed a simple device for quantitative as well as continuous recording of the spontaneous activity of mice and rats by using the photoelectric cell method. In the present paper, the authors' method is introduced and the effects of some CNS stimulants on the spontaneous movements of the mouse are demonstrated.

METHODS

This apparatus records the frequency of the light beam blocked by the mouse or rat moving around in the cage, and its diagram is illustrated in Fig. 1.

The cage for the mouse is a polyethylene cylinder of about 20 cm in diameter and about 20 cm in height. On the floor of the cage, two fixed light beams from 6 volts lamps (A, A) are thrown crosswise onto two photoelectric cells (CdS cell, 2PK64, National, Japan) (Fig. 1. B, B). When the light beam is interrupted by the mouse, the photoelectric current is changed. This current change is amplified (C) to actuate the
electromagnet (D), which in turn runs a toothed wheel (E). Another wheel (F) is pressed against the wheel E by a wire spring, and a long tape is pinched inbetween these two wheels. The tape (G) is extended horizontally across a pair of pulleys (H, H) and is provided with a load (J) at its end, as seen in Fig. 1. As the wheel E is turned notch by notch with every action of the magnet D, the tape is pulled down and a recording pen (I), attached to the tape, is displaced stepwise by a constant length. When the pen moves at some fixed length from its original position, a hook (L) provided on the tape lifts up the arm of the switch (K) and activates a plunger relay (M) to release the pressing wheel F, hence the pen is returned to its original position by the load J, and repeats its stepwise movement. The movement of the pen is continuously recorded on a paper (N) running at a fixed speed.

The distance of the pen's position from the baseline where the pen started its first movement, i.e. the number of times interrupting the light beam necessary to reach the top of the tracing, can freely be adjusted to fit each experiment by shifting the position of either L or K.

From the records thus obtained, the number of times blocking the light beam is counted at time intervals of any length (e.g. in 10 minutes or even in hours) and is plotted on a graph, thus spontaneous activity is quantitatively and continuously measured.

In the present experiment, the cage itself is suspended by wire springs and its movements are also recorded simultaneously by the use of tambours, in order to compare them with the records obtained by the photoelectric cell method described above.

The drugs used in this experiments are d-amphetamine HCl, methamphetamine HCl, morphine sulfate, ephedrine HCl, caffeine sodium benzoate, pentylentetrazol, strychnine nitrate and nikethamid.
RESULTS

The mouse usually walked around actively in the beginning after it was placed in the cage, but the movements decreased gradually as the mouse became accustomed to the cage, and it moved only intermittently and capriciously afterwards.

![Graph showing the effect of methamphetamine.](image)

**FIG. 2.** The effect of methamphetamine.

V: movements of the cage, LB: recording of the number of time the light beam is interrupted by the mouse (each wave represents 20 interruptions of the light beam), T: time in minutes.

A: control activity, immediately after the mouse was placed in the cage, B: soon after subcutaneous injection of methamphetamine 5 mg/kg. C, D, E, F and G: one, 2.5, 4, 6 and 7 hours after the injection respectively.

The graph in the lower part of the figure illustrates the temporal pattern of the effect of methamphetamine calculated from the real tracing LB.
Effect of methamphetamine and d-amphetamine

The effect of methamphetamine on spontaneous activity of the mouse was shown in Fig. 2. Methamphetamine was injected subcutaneously in a dose of 5 mg/kg after spontaneous motor activity markedly decreased. The motor activity gradually increased in 5 to 10 minutes and reached its maximum hyperactivity in 30 to 40 minutes after the injection. This drug-induced hyperactivity continued for about 5 to 6 hours and the mouse became quiet 6 to 7 hours after the drug administration. Both movements of the cage (Fig. 2, V) and the number of times of light blocking (Fig. 2, LB) precisely showed the occurrence of these changes. The frequency of light beam interruptions in every 10-minute period was calculated from the real tracing LB, and plotted on the graph as shown in the lowest part of Fig. 2. The temporal pattern of the effect of methamphetamine on the spontaneous activity was thus quantitatively shown in this graph.

The effect of methamphetamine was rather variable in individual mice. It was frequently observed that marked hyperactivity was first induced for an hour or two, but the activity was markedly reduced in the next period of 1 to 2 hours and markedly increased again for about 1 to 2 hours.

The effect of d-amphetamine was almost the same qualitatively and quantitatively as that of methamphetamine. The effects of both drugs varied with environmental conditions, particularly with temperature. In general, the higher the temperature, the more marked increase in motor activity was induced by these drugs. The result illustrated in Fig. 2 was obtained at room temperature of 24°C in June. Hardinge and Peterson (8) also observed that the toxicity of amphetamine much varied with different temperature.

Effect of caffeine

Caffeine, injected subcutaneously in doses of 100 to 200 mg/kg, caused no significant changes in spontaneous activity of mice. However, caffeine increased the activity in the majority of cases. One of the effects of caffeine in a dose of 500 mg/kg s.c. was shown in Fig. 3. The effect of caffeine was relatively rapid in onset and its duration was about an hour as seen in the graph illustrated in the lowest part of Fig. 3. Movements of the cage (V) generally paralleled in degree with the frequency of light blocking (LB), but occasionally only the former was augmented without an increase in the latter, as seen in the panel D of Fig. 3.

Effect of ephedrine

Spontaneous activity of mice was not significantly changed by ephedrine in subcutaneous doses less than 100 mg/kg, but was increased in larger doses. The effect of ephedrine in a dose of 200 mg/kg was shown in Fig. 4.

The frequency of light beam interruptions in each 10-minute period was graphed in the lower part of the figure. The onset of action was rather slow in this particular case, the peak effect was reached at about an hour after the subcutaneous injection, thereafter the activity once decreased and then increased again.
Effect of morphine

When morphine sulfate was injected subcutaneously in doses larger than 5 mg/kg, the mouse held the tail up and the spontaneous activity increased. The real tracings (V and LB) in Fig. 5 showed the effect of morphine 20 mg/kg injected subcutaneously. Spontaneous movements of the mouse were markedly increased in 20 to 30 minutes after the injection, and the effect lasted for about 4 hours. This effect was graphed together with the effects of morphine in doses of 10 and 50 mg/kg in the lower part of Fig. 5. The increase in spontaneous activity was more marked as the dosage of morphine was increased.

Fig. 3. The effect of caffeine.
A: control activity, B: 5 minutes after subcutaneous injection of caffeine 500 mg/kg, C and D: 50 minutes and 2 hours after the injection respectively.
The other abbreviations are the same as the previous figure.

Effect of morphine

When morphine sulfate was injected subcutaneously in doses larger than 5 mg/kg, the mouse held the tail up and the spontaneous activity increased. The real tracings (V and LB) in Fig. 5 showed the effect of morphine 20 mg/kg injected subcutaneously. Spontaneous movements of the mouse were markedly increased in 20 to 30 minutes after the injection, and the effect lasted for about 4 hours. This effect was graphed together with the effects of morphine in doses of 10 and 50 mg/kg in the lower part of Fig. 5. The increase in spontaneous activity was more marked as the dosage of morphine was increased.
Effect of pentylenetetrazol, nikethamid and strychnine

Pentylenetetrazol, nikethamid and strychnine had no significant increase in the spontaneous activity of the mouse in their subconvulsive doses.

When general convulsion was induced by these drugs, movements of the cage markedly increased, while the frequency of light beam interruptions did not increase.
DISCUSSION

The spontaneous activity of mice or rats was continuously recorded by this device, without the presence of experimenters, even for such a prolonged period as lasting over days. From the data thus obtained, changes in spontaneous activity could be calculated in any ways desired after the experiment was terminated. This is one of the greatest advantages of this device, and much tedious efforts of investigators were thus eliminated. Secondly, changes in the activity can be quantitatively determined at time intervals of any length in terms of the frequency of interruption of the light beam. This is
particularly effective in a follow-up observation of the drug effects on the spontaneous activity. A temporal pattern of drug effects can be graphed at any time intervals, such as in every 10 minutes or even in hours.

The present experiments with this method showed that the spontaneous activity of mice was not increased by pentylenetetrazol, nikethamid and strychnine, but was well increased by the psychomotor stimulants, like methamphetamine, d-amphetamine, caffeine, and ephedrine. These results well agreed with those observed by Dews (6).

In evaluating the effects of CNS stimulants, particularly of pentylenetetrazol-type drugs, on the spontaneous activity, the jiggle cage method which records well the bodily movements, should also be employed simultaneously, although Lal et al. (9) suggested the photoelectric cell method was more reliable, since the jiggle cage method produced a "cage effect" which interacted with drug effects.

SUMMARY

A simple apparatus was devised to record the spontaneous movements of either mice or rats, by the use of the photoelectric cage method. The spontaneous activity was measured quantitatively as well as continuously and the temporal pattern of drug effects was much effectively observed by this method. The activity of mice was not increased by pentylenetetrazol, nikethamid and strychnine in subconvulsive doses, but was increased by methamphetamine, d-amphetamine, caffeine, ephedrine and morphine.

REFERENCES

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