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An Automated Device for Screening the Effects of Psychotropic Drugs on Aggression and Motor Activity in Mice

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PUGLISI-ALLEGRA, S. AND P. RENZI. *An automated device for screening the effects of psychotropic drugs on aggression and motor activity in mice.* PHARMAC. BIOCHEM. BEHAV. 13(2) 287–290, 1980.—An automated technique for measuring simultaneously aggressive behavior and motor activity in mice is described. This technique allows measure of aggressive behavior and motor activity continuously throughout long-lasting periods without disturbing the animals except for feeding, watering and cleaning the cages. The effects of n-D-propylacetate, a drug which affects aggression without affecting motor activity, were also tested.

Aggression Motor activity Psychotropic drugs Mice Method n-D-propylacetate

A DEVICE to detect and quantify fighting episodes and, at the same time—but in independent fashion—, general activity in groups of two or more mice is described. The apparatus was designed starting from the observation that general activity and fighting episodes of animals living in a breeding cage produce vibrations of different intensities on the floor of the cage. In particular, the collisions determined by the bodies, the legs and the tails against the floor during fighting produce vibrations that are the most intense among those produced by animals living in breeding conditions. Thus, such an apparatus should detect and discriminate different kinds of activity corresponding either to aggressive behavior or to non-aggressive motor activity.

APPARATUS

Components

The major components of the apparatus are the experimental cage and the processing unit. The experimental cage is a modified breeding cage in which the floor is replaced by a zincate mesh (weighing 1200 g/m^2 and with squares of 8 mm side) fixed to the walls at 4 cm from the tray which collects the excrements. The mesh is fixed by means of screws to four Plexiglas blocks glued to the walls of the cage. The distance between the lid and the floor of the cage is 6.5 cm. The mesh collects the vibrations produced by the animals' activity and transmits them to 4 dynamic microphones placed at the corners of the cage. Each microphone is fitted in a rubber capsule and plugged to the mesh (Fig. 1). This plugging allows removal of the microphones in order to clean the cage. The microphones are connected in series to obtain an improvement of sensibility throughout the mesh.

Microphone signals are processed by the circuit shown in Fig. 2. The processing unit consists of two amplifiers so that one channel will detect both low and high intensity noises (referred to as "total motor activity") independently of the other channel which will detect only high intensity noises corresponding to fighting behavior (referred to as "fighting motor activity"). The evaluation of motor activity without fighting activity (referred to as "general motor activity") is obtained by subtracting activity scores concerning "fighting motor activity" from activity scores concerning "total motor activity".

Related Equipment

A Grason Stadler Programmer was used for more detailed information on different aggressive patterns such as latency to the first fight, number and time of fighting episodes.

Feeding behavior is a kind of activity that will be recorded only if the lid—where the food container is located—is fixed to the cage; therefore it is possible to avoid recording of this activity by fixing the lid to supports that are not in contact with the experimental cage.

Furthermore, it must be pointed out that activity and fighting produce through the mesh noises differing only in intensity, not in frequency distribution. Thus, differences in the type of mesh or in the connections of the mesh to the cage do not affect discrimination between the two levels of noise. Finally, to avoid the interference of external noise each microphone can be covered with polyurethane foam and the entire apparatus can be placed in a sound-insulated cubicle.

SUPPORTING DATA

Animals

Male mice of the DBA/2 strain were used. The mice were randomly assigned to two housing conditions, either individually housed (isolated) for 8 weeks or in groups of 6 animals

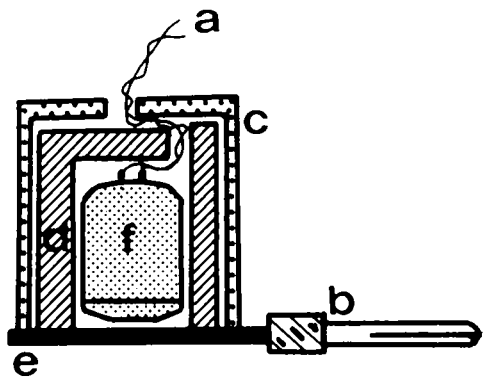

FIG. 1. Microphone arrangement. The microphone, previously covered with polyurethane sponge, is fitted in a rubber capsule. The border of the capsule is glued to an iron slab (3×3×0.2 cm) assuring that the surface of the microphone and the surface of the slab are in contact. The slab is soldered to a male plug which, through a hole in the cage wall, is inserted in the respective female socked to the mesh. (a) Connection with the processing unit; (b) Mechanical connection with the mesh; (c) Capsule; (d) Polyurethane sponge; (e) Metal slab; (f) Dynamic microphone.

PARTS LIST

IC 1, IC 3 – Integrated circuits μA 741
IC 2, IC 4 – Integrated circuits μA 709
C1, C2, C3, C4 – Condensers 0.1 μF, 20 V
D1, D2, D3, D4 = Diodes IN 4001
P1, P2, P3, P4 = Logarithmic potentiometers 10 kΩ 1/4 W
TR 1, TR 2, TR 3, TR 4 – Transistors BC 119
RE 1, RE 2 = RELAYS 12 V, 260 Ω
R3, R5, R6, R9, R10, R14, R17, R18 = Resistors 1 kΩ 1/4 W
R12, R1 = Resistors 2.2 kΩ 1/4 W
R7, R15 = Resistors 10 kΩ 1/4 W
R13, R4 = Resistors 100 kΩ 1/4 W
R2, R8, R11, R16 = Resistors 1 MΩ 1/4 W
M1, M2 · Dynamic microphones
Impedence 200 Ω
Freq. Resp. 50–17,000 HZ

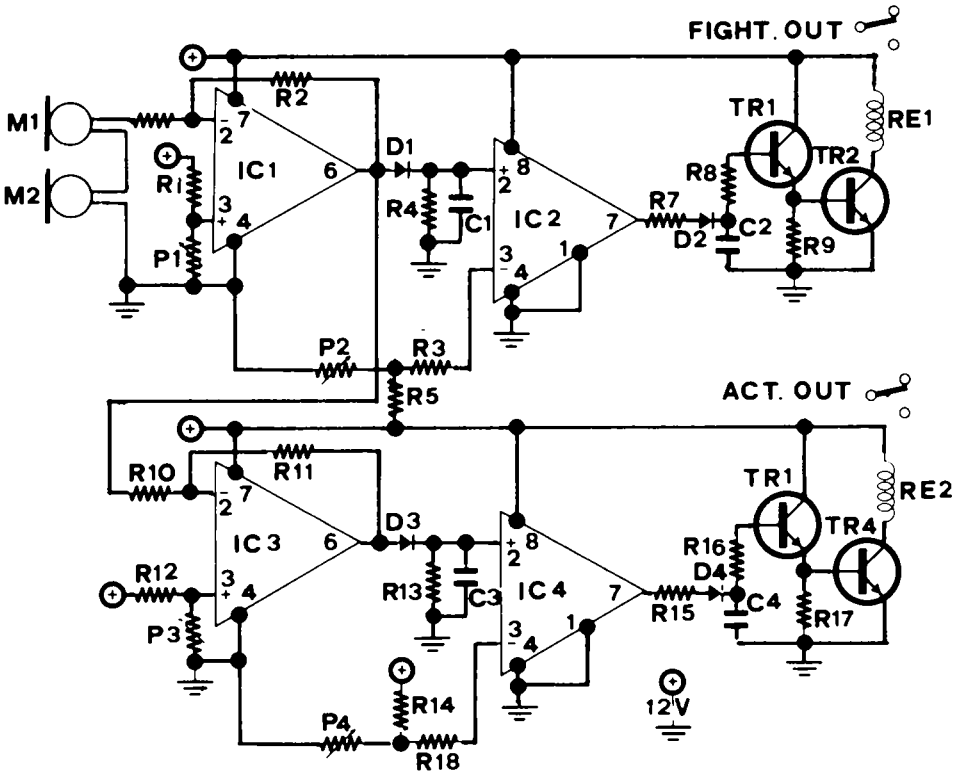

FIG. 2. Processing unit. Schematic diagram (for values see part list). The signal picked up by the dynamic microphones is fed into IC 1, which functions as an amplification stage, common to the two lines. IC 3 is an additional amplification stage for the measurement of activity. R4 C1 and R3 C3 integrate the signals with the same temporal constant of 220 msec. This output is fed respectively into IC 2 or IC 4 which function as detectors. The detector compares the voltage of the amplified-integrated input signal, with a reference voltage supplied through a voltage divider consisting respectively of R 5 and P 2 or R 14 and P 4, which controls the trigger point of "fighting output" and "activity output".

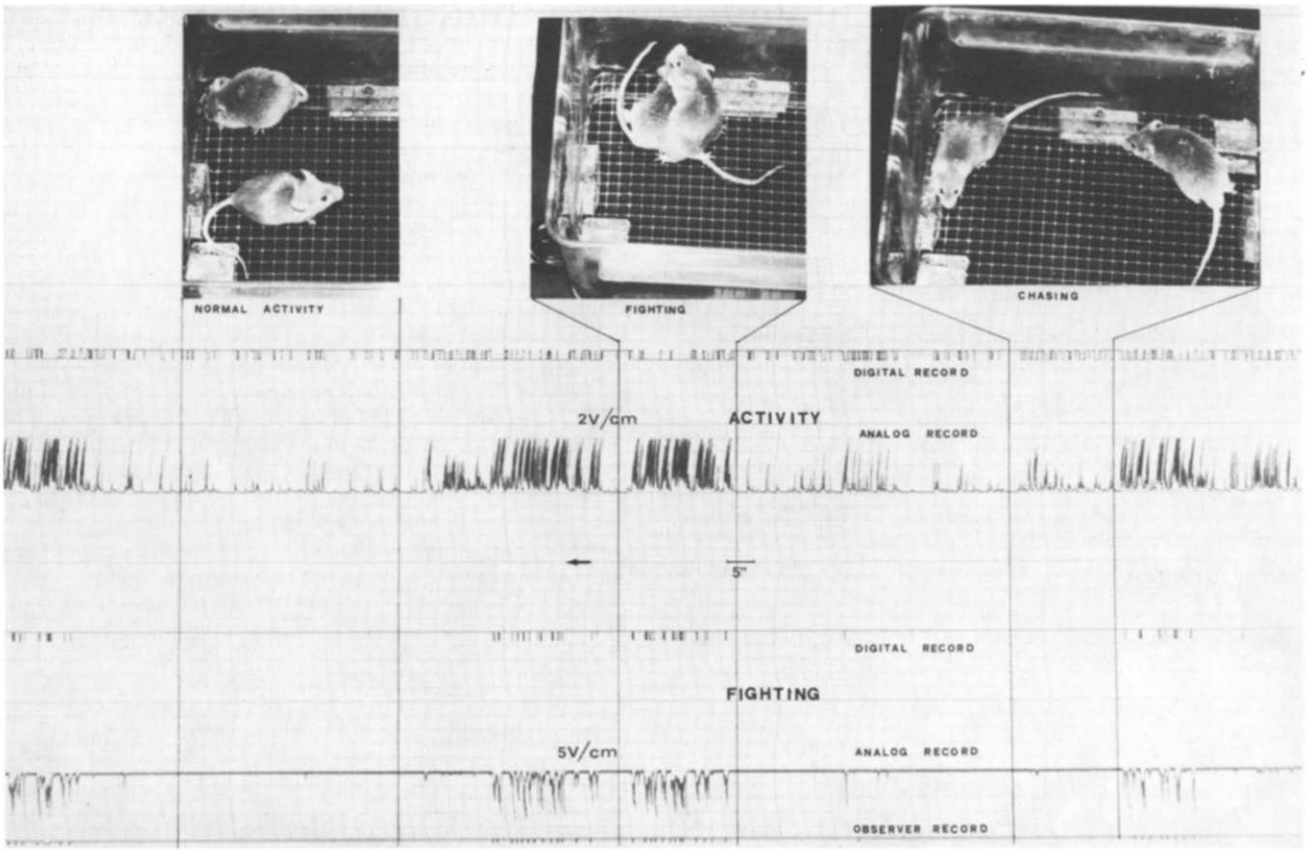

FIG. 3. Simultaneous recordings of motor activity and fighting behavior by automated apparatus and observer. The outputs from the observer, the aggression detector and the activity detector were simultaneously recorded by a recording device. In particular, the analogic outputs were picked up before the comparator that fixes the trigger point for aggression line (IC 1) and for activity line (IC 2) and fed into a Beckman Type RM Dynograph Recorder. As it can be seen in the figure, the comparison among apparatus and observer outputs shows that the apparatus detects selectively fighting behavior among other motor patterns including chasing behavior. The fighting threshold level of the apparatus was empirically selected by pairing two isolated mice and observing fighting behavior. This level was found to correspond to the level at which the apparatus detects the vibrations produced on the center of the mesh by a polyurethane sponge ball (weighing 10 g and having 7 cm diameter) dropped from a support placed at 8 ± 0.5 cm from the mesh. Similarly, the activity threshold level, which was previously empirically selected placing one or more grouped mice in the experimental cage for several min was fixed by placing the plate at 5–4 mm from the mesh. The polygraph amplifier gain was set at 2 V/cm for activity recordings and at 5 V/cm for fighting recordings.

(grouped). At the moment of the experiments animals of both housing conditions were 19–20 weeks old.

Aggressive behavior exhibited by paired mice was simultaneously recorded by our automated device and by two observers. Twenty four isolated mice were employed. Two observers recorded the fighting behavior of paired mice and pushed a switch during every fight. The outputs from the observers, the aggression detector and also the activity detector were simultaneously recorded by a recording device (Fig. 3).

Two mice were placed in opposite halves of the experimental cage separated by a guillotine door. Following a 60 sec adaptation period the recording equipment was activated and at the same time the guillotine door was raised allowing the mice to encounter for a period of 10 min. The observer then pressed the hand-held switch whenever a fight occurred, holding the switch down for as long as the fight lasted.

The latency of the first fight, the number of fights, the number and the time of fighting episodes were considered and analyzed in terms of correlation between the recordings of the observers and the recordings of the automated apparatus. A fighting episode was defined as including different separate fights. The end of a fighting episode was defined as the termination of a fight followed by a period of 5 sec in which no fight occurred. For each of the four parameters examined the following correlations were obtained: Latency to the first fight, $r(99)=0.9380$; number of fights, $r(99)=0.9540$; number of fighting episodes, $r(99)=1$; time of fighting episodes, $r(99)=0.9970$. For all four values were <0.001.

As concerns motor activity our apparatus was compared to an electronic activity meter (Selective Activity Meter (Model S), Columbus Instruments, Columbus, OH) with its threshold regulated at the mean levels of the scale. Six grouped mice were individually placed for a period of 10 min in the experimental cage which was directly placed on the activity meter platform. The activity scores of our apparatus were correlated with those of the activity meter. The correlation between the two sets of scores was: $r(49)=0.9510$; the p value was <0.001.

Finally, the effects of n-D-propylacetate (n-DPA), a drug

TABLE 1

EFFECTS OF N-DPA ON MOTOR AND AGGRESSIVE ACTIVITY OF ISOLATED DBA/2 MALE MICE

	Saline	n-DPA (300 mg/kg)
Total motor activity	458.75 ± 47.35	449.87 ± 46.57
Fighting motor activity	42.75 ± 5.91	11.50 ± 9.20*
General motor activity	416.00 ± 48.63	438.37 ± 42.07

Values are mean ± SEM. Data were statistically analyzed by ANOVA.

*Significantly different from saline group ($p<0.2$).

TABLE 2

EFFECTS OF N-DPA ON THE AGGRESSIVE BEHAVIOR OF ISOLATED DBA/2 MALE MICE

	Saline	n-DPA (300 mg/kg)
Latency to the first fight (sec)	41.12 ± 24.64	483.87 ± 78.54*
Number of fighting episodes	3.87 ± 0.54	0.50 ± 0.37*
Total time of fighting episodes (sec)	29.00 ± 3.93	4.62 ± 3.62*

Values are mean ± SEM. Data were statistically analyzed by ANOVA. Those couples of mice that failed to fight during a ten minute experimental session were assigned a maximum latency score of 600 sec.

*Significantly different from saline group ($p<0.001$).

that affects aggression [1, 6, 7], on aggressive behavior and motor activity were also investigated in order to check the reliability and selectivity of this method. Two groups of isolated mice were used. Thirty min before testing, mice of the experimental group were injected IP with 300 mg/kg of n-DPA while control animals were injected with saline solution (NaCl 0.9%). The procedure for the aggression test was the same as in Experiment 1. Mice encountered during a 10 min session. Tables 1 and 2 show that no difference in total motor activity and general motor activity was found between mice injected with saline and n-DPA, whereas they were different in fighting motor activity. In particular, with respect to aggressive behavior, data obtained by processing fighting motor activity scores (see page 287) showed that the two groups of mice were different in latency to the first fight, in number and in time of fighting episodes.

These results are consistent with those of a previous experiment [6] in which aggression was measured by an automated apparatus which allows recording of bites between paired mice [5] and motor activity by an activity meter.

DISCUSSION

These results show that this technique is very accurate in simultaneously detecting motor activity and fighting behavior. In the field of psychopharmacology drugs with possible effects on aggressive behavior need to be evaluated with respect to their side effects on locomotor and/or general activity. Although general motor activity levels can not be considered the only evidence of specific antiaggressive effects of drugs, it may be very useful for a number of psychopharmacological and psychobiological experiments to have an experimental technique that allows assessment of simultaneously aggressive responses and activity and to evaluate aggressive behavior continuously throughout long-lasting periods without disturbing animals except for feeding, watering and cleaning the cages.

Other techniques measuring motor activity are available such as Animex, Varimex and motility meters based on the Doppler principle [2, 4, 8, 9]. While all these techniques can detect variations of the speed of the animals, they do not allow to discriminate different behaviors consisting of movements at similar speeds such as chasing and fighting behaviors (see Fig. 3).

Another advantage of this technique lies in the fact that it allows evaluation of effects of antiaggressive treatments on motor activity in the same environment in which aggressive behavior is assessed, as recently stressed by Miczek and Krsiak [3]. Our technique does not intend to be an ethological approach to the quantification and the evaluation of the different components of fighting and aggression. However it offers a reliable, automated and unbiased tool for the study of fighting behavior and of the effects of drugs upon it.

Ongoing experimental studies will possibly suggest a way to discriminate more accurately among different behavioral patterns.

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