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Reliability of Two Activity Boxes Commonly Used to Assess Drug Induced Behavioural Changes

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LJUNGBERG, T. *Reliability of two activity boxes commonly used to assess drug induced behavioural changes.* PHARMAC. BIOCHEM. BEHAV. 8(2) 191–195, 1978. – Rats were given different drug treatments known to affect central catecholamine neurotransmission and to induce different types of behavioural changes which were recorded simultaneously by two different types of conventional activity boxes: an Animex activity meter and a photocell cage. All animals were also visually observed simultaneously with the automatic recordings. It was found that the two activity boxes reflected the behavioural changes differently and that the results from the two boxes were not correlated. When comparing the observations with the automatic recordings it was found that some clearly observable changes in behaviour were not reflected as changes in the automatic recordings and conversely that increases or decreases in the recorded motor activity were not always related to any particular changes in behaviour. The results show that motor activity can not be regarded as a simple or homogeneous behaviour that is reliably measured in conventional activity boxes. It is an undescriptive measure consisting of an artificial summation of those components of behaviour that affect the movement-detecting device in the particular box which is used. Practical and theoretical implications of this are discussed.

Automatic registration of behaviour Activity boxes Locomotor activity Stereotyped behaviour Catecholamine transmission

AUTOMATIC registration of the level of general activity is commonly used to assess the effects of various experimental manipulations on the behaviour of rats and mice. Despite the many technically different devices that are used only the revolving drum technique is, however, generally regarded as giving distinctly different results from the other techniques (for reviews see [7, 9, 12]).

In psychopharmacological research there is a vast literature describing the effects of drugs and lesions on behavioural measurements referred to as activity, locomotor activity, locomotion or motility, while suprisingly little attention is paid to the question of what these measurements actually reflect. Many of these manipulations induce involuntary movements, stereotypies and other complex patterns of behaviour never found in the behavioural repertoire of the normal animal. In such experiments the design of the movement detecting device may be of crucial importance for how the recording reflects the actual behavioural changes and it may prove virtually impossible to compare the results obtained with one box with those obtained with other types of boxes.

In the present study rats were given a number of drug treatments known to induce various types of behavioural responses which were recorded with two commonly used activity boxes of different designs. The aim was to investigate how well the results from the boxes compared and how accurately the actual behavioural changes were reflected in the automatic recordings.

METHOD

Animals

The experiments were performed on 60 male Sprague-Dawley rats (Anticimex, Stockholm, body weight 160–210 g). The animals arrived in the animal colony at least 3 days prior to the experiments and were kept 5/cage under constant temperature and humidity conditions on a 12-hr light, 12-hr dark schedule with food and water ad lib.

Apparatus

The size of the test cage was 21 x 34 cm with 15 cm high walls and it was covered with a wire net. It was symmetrically fitted on the long side with three parallel photobeams using visible light 4.5 cm above the floor of the cage. The total number of photobeams interruptions and the interruptions of only the midpositioned photobeam were counted separately on two different counters and will be referred to as photocell cage motor-activity. The test cage with the photobeam was placed on top of an Animex activity meter type DO (FARAD AB; 14). The Animex activity meter involves a tuned resonance circuit. Movements of the animal will off-tune this circuit and if the off-tuning is large enough it will be detected and counted. The Animex type DO is equipped with two channels and two different sensitivities for the detection of the off-tuning (= movements) can be used. In the present study

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a 40 μA sensitivity was used for detection of very fine movements and was defined as Animex high sens. motor-activity and a 10 μA sensitivity was used for detection of coarse changes (= fast or large movements) and was defined as Animex low sens. motor-activity. The set up was placed in a ventilated sound-reducing box in a weakly illuminated room.

Procedure

Four hr before the experiments the animal was placed alone in a separate cage and moved to the weakly illuminated experimental room. After this 4-hr settling down period the animal was carefully transferred to the test box (see above) and the activity of the animal was recorded for 10 min. All experiments were performed during the 12-hr light period and the animals were used only once.

All the animals were visually observed during the automatic recordings and notes were taken on their behaviour. The pattern of behaviour displayed by the animals was also labelled according to a scoring system for stereotyped behaviour, slightly modified from that previously described [5, 6, 10]: 0 = No stereotyped behaviour, 1 = Discontinuous sniffing and/or repretitive head and limb movements, 2 = Continuous sniffing and/or repetitive head and limb movements, 3 = Discontinuous biting, gnawing or licking, 4 = Continuous biting, gnawing and licking. The scoring system was not used as a quantitative measure but as a qualitative description of the behaviour.

Drug Treatments

D-amphetamine-sulfate and phenoxybenzamine-HCl were dissolved in isotonic saline and apomorphine hydrochloride was dissolved by heating in isotonic saline with added ascorbic acid (0.2 mg/ml). The doses refer to the bases. Clozapine (Sandoz) was dissolved in a minimum quantity of 1 M hydrochloric acid and made up to volume with isotonic saline and reserpine (Serpasil, Ciba) was used as the commercially available injection solution. The doses of these drugs refer to the above-mentioned forms. The injection volume was 5 ml/kg body weight except for reserpine where the injection volume was 4 ml/kg. All the injections were given IP. The ten different pharmacological treatments and the injection schedule is shown in Table 1. Each group consists of six rats.

Statistics

All the data are presented as medians. For calculations of the degree of significance the Kruskal-Wallis one-way analysis of variance followed by the Mann-Whitney U test was used. The Spearman's rank-correlation was used to calculate the degree of correlation between the methods [13].

Each group of six rats was also assigned one of the scores from the scoring system. In the few cases when not all rats in the group was assigned the same score, the score that occurred with the highest frequency was given to the group.

RESULTS

Behavioural Results

Pretreatment times are given within parenthesis in the text below (see also Table 1).

TABLE 1

DRUG TREATMENTS: EACH RECORDED VALUE IS THE MEDIAN OF 6 ANIMALS. FOR DETAILS OF THE STEREOTYPY SCORE, SEE METHOD SECTION.

Treatments	Animex high sensitivity counts/10 min	Animex low sensitivity counts/10 min	photocell-cage counts/10 min	stereotypy-score
1. NaCl 5 min	1088.5	525.5	161.5	
2. Apomorphine 0.2 mg/kg 5 min	680	260.5	71.5	0
3. Apomorphine 1.0 mg/kg 5 min	1367	482	98	
4. Apomorphine 5.0 mg/kg 5 min	1335	713	166.5	2
5. Reserpine 10 mg/kg 24 h + Apomorphine 1 mg/kg 10 min	1515.5	788	148.5	4
6. Phenoxybenzamine 10 mg/kg 35 min + Apomorphine 5 mg/kg 5 min	1408.5	736	121	3
7. Clozapine 5 mg/kg 35 min + Apomorphine 5 mg/kg 5 min	1524	549	134	3
8. d-Amphetamine 2 mg/kg 50 min	1198	557	214.5	2
9. Phenoxybenzamine 10 mg/kg 80 min + d-Amphetamine 2 mg/kg 50 min	992	381.5	179	2
10. Clozapine 5 mg/kg 80 min + d-Amphetamine 2 mg/kg 50 min	1102	510	225.5	2

NaCl (5 min). The control animals showed high exploratory activity during the whole 10-min test period. It consisted of locomotion with sniffing and some rearing.

Apomorphine 0.2 mg/kg (5 min). This dose of apomorphine caused a reduction of the exploration as compared to the NaCl and the animals sat inactive for long periods of time in the corners. No repetitive sniffing was observed. There was a significant decrease ($p<0.01$) in motor activity in all motor activity measurements.

Apomorphine 1 mg/kg (5 min). This dose of apomorphine induced a behaviour characterized by slow forward locomotion with continuous sniffing on the floor. No licking or biting was observed. This behaviour was recorded as a significant increase ($p<0.05$) in the Animex high sens., no significant change in the Animex low sens. and a significant decrease ($p<0.01$) in the photocell cage motor activity as compared to the NaCl injected animals.

Apomorphine 5 mg/kg (5 min). This dose of apomorphine induced a behaviour with high locomotion, intense sniffing on the floor and in the corners and periods with licking and biting in 2 of 6 animals. In both the Animex high sens. and low low sens. this behaviour was recorded as a significant increase ($p<0.05$ and $p<0.05$) in activity but in the photocell cage it was recorded as no significant change from the NaCl injected controls.

Reserpine 10 mg/kg (24 hr) + apomorphine 1 mg/kg (10 min). The pretreatment with reserpine changed the apomorphine 1 mg/kg induced behaviour into a behaviour characterized by continuous biting and gnawing in the cage with low locomotion. This great change in behaviour was recorded as a nonsignificant increase in the Animex high sens., a significant increase ($p<0.01$) in the Animex low sens. and as a borderline significant increase ($p<0.1$) in the photocell cage as compared with only apomorphine 1 mg/kg.

Phenoxybenzamine 10 mg/kg (35 min) + apomorphine 5 mg/kg (5 min). The pretreatment with phenoxybenzamine changed the apomorphine 5 mg/kg induced behaviour towards a stereotyped behaviour characterized by more biting, licking and gnawing. This was not recorded as a significant change in any of the three motor activity measurements as compared with only apomorphine 5 mg/kg.

Clozapine 5 mg/kg (35 min) + apomorphine 5 mg/kg (5 min). The animals pretreated with clozapine showed a decrease in locomotion and an increase in the biting, licking and gnawing behaviour. These behavioural changes were more pronounced than after the phenoxybenzamine pretreatment but it was still not recorded as a significant change in any of the three motor activity measurements as compared with only apomorphine 5 mg/kg.

d-Amphetamine 2 mg/kg (50 min). This dose of amphetamine induced a hyperactivity with continuous sniffing and locomotion. It was recorded as no significant change in the Animex high sens. or low sens. and as a significant increase ($p<0.02$) in the photocell cage as compared with the NaCl injected controls.

Phenoxybenzamine 10 mg/kg (80 min) + d-Amphetamine 2 mg/kg (50 min). The phenoxybenzamine pretreatment changed the d-amphetamine hyperactivity into a more automatic behaviour with sniffing on the floor and with slow forward locomotion. This change was recorded as a significant decrease ($p<0.05$) in the Animex high sens. but caused no significant change in the Animex low sens. or in the photocell cage as compared with only d-amphetamine 2 mg/kg.

Clozapine 5 mg/kg (80 min) + d-amphetamine 2 mg/kg (50 min). The clozapine pretreatment partially counteracted the d-amphetamine induced behaviour, i.e., the stereotypy and locomotion seemed less intense but the animals still showed locomotion, sniffing and some repetitive head movements. There was no significant change in any of the three motor activity measurements as compared with only d-amphetamine 2 mg/kg.

Statistical Comparison Between the Methods

The one-way analysis of variance showed that there was a highly significant variation between the different pharmacological treatments: Animex high sens. $\chi^2 = 35.3$, $f = 9$, $p<0.001$; Animex low sens. $\chi^2 = 31.6$, $f = 9$, $p<0.001$ and photocell cage $\chi^2 = 38.0$, $f = 9$, $p<0.001$.

The intra method correlation showed that there was a high positive correlation ($r_S = 0.92$; $p<0.001$) in the photocell cage between the counts obtained from all three photobeams and the counts obtained from only the midpositioned photobeam. Because of this high correlation only the results from the total photobeam interruptions are included. There was only a weak positive correlation ($r_S = 0.72$; $p<0.05$, see Fig. 1) between the two different sensitivities used in the Animex activity meter and therefore the results from both the sensitivities are included.

The inter method correlations showed that there were no significant correlations between the two different systems used to record motor activity (see Fig. 1).

There was a relation between the rated stereotypy score and the motor activity counts recorded with the Animex high sens. in that the treatments that were manually labelled with score 3 and 4 also produced many counts in the recording (see Fig. 1). This relation was much less clear for Animex low sens., and there was no such relation between the stereotypy score and the motor activity counts recorded with the photocell cage (see Fig. 1).

DISCUSSION

There were two aims with the present study: Firstly to investigate how well the results from different types of activity boxes compared with each other and secondly to investigate how well the automatic recordings reflected different types of drug induced behavioural changes. Rats were treated with drugs that are known from the literature to induce different types of behavioural responses and the locomotor activity was simultaneously recorded automatically with an Animex activity box (Farrad AB; 14) and with a photocell cage. All the animals were also observed visually and notes were taken on their behaviour. The ten treatments (see Table 1) included normal exploring animals, animals pretreated with different doses of the dopamine receptor stimulating drug apomorphine [2,6], the DA and NA releasing drug d-amphetamine [4,8], the NA α-receptor blocker phenoxybenzamine [1], the atypical neuroleptic drug clozapine [15,16] and the monoamine depleting drug reserpine [3].

When evaluating the results obtained from the photocell cage, it was found that there was a high positive correlation between the counts obtained with only the midpositioned photobeam and the counts obtained with all three photobeams indicating that in this study the density of photobeams did not qualitatively change the results. The Animex used in this study was equipped with two channels and two different sensitivities for the detection of movements can be used. The sensitivities were set very high and very low,

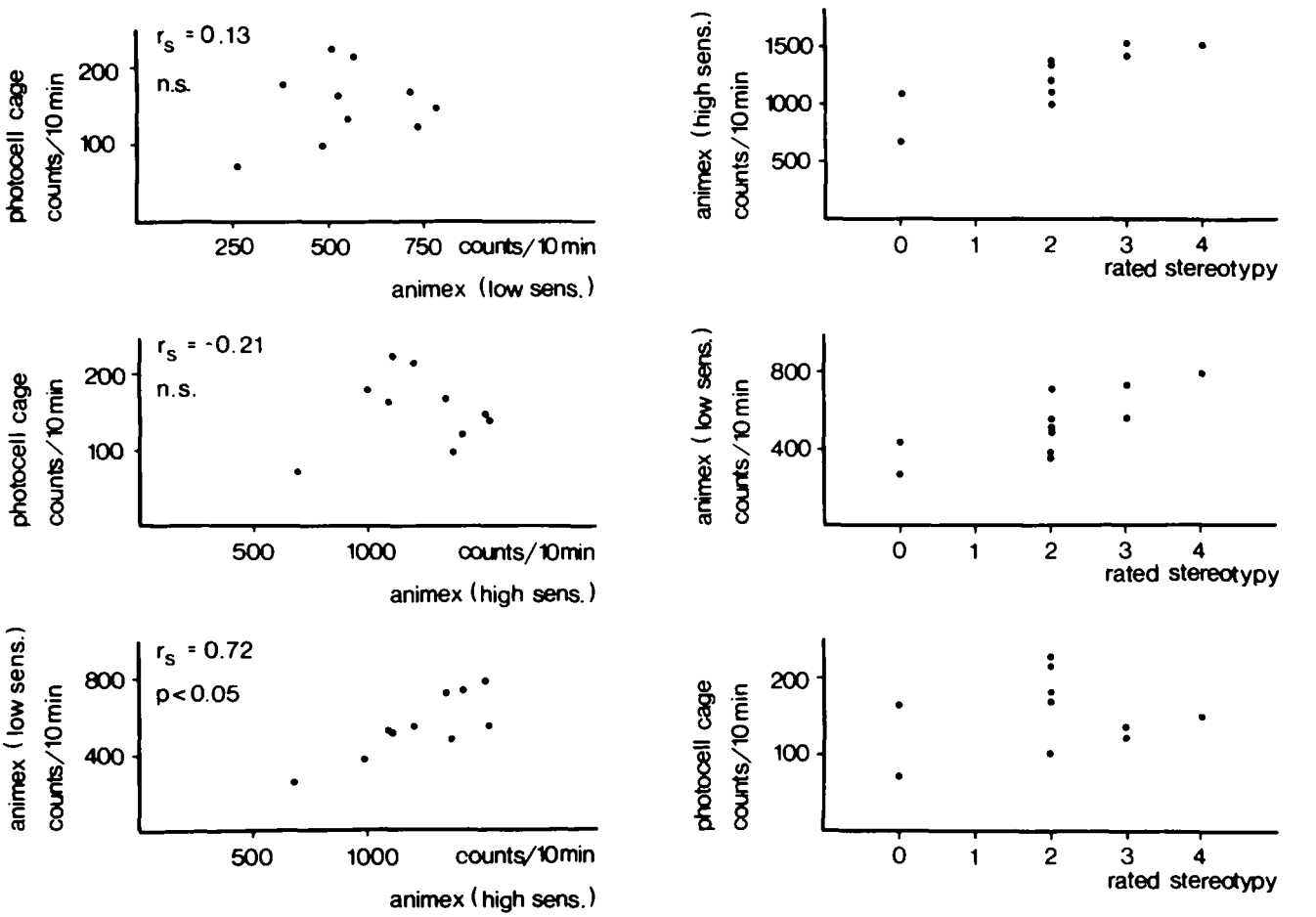

FIG. 1. The left-hand part of the figure shows the inter-measurement correlations. There is a weak positive correlation between the two channels of the Animex (lower left) but there is no significant correlation between the Animex and the photocell cage (middle and upper left). The right-hand part of the figure shows the relation between the activity counts and the stereotypy scores: behavioural patterns that were labelled with score 3 and 4 produced high counts in the Animex (upper and middle right) while these patterns were not associated with high counts in the photocell cage (lower right).

respectively, but there was still a significant positive correlation between the channels. The intermethod correlation showed that there was no significant correlation between the results obtained with the Animex and the results obtained with the photocell cage (see Fig. 1) showing that the motor activity recorded with the Animex is something else than the motor activity recorded with the photocell cage and that the results are not equivalent. This can be illustrated by the behavioural change caused by apomorphine 1 mg/kg which causes an increase, no change or a decrease in the recorded motor activity dependent upon which of the motor activity records that is considered.

This lack of correlation between the methods is in disagreement with the assumption that different types of activity boxes give essentially the same results (see [7, 9, 12]). The present findings instead support the finding of Tapp *et al.* [17] who showed, in studies of normal animals, that different types of activity recording devices give results that are essentially independent of one another.

When the visual observations are compared with the automatic recordings it is found that observable changes in behaviour are not reflected in the automatic recordings (see e.g., the clozapine + apomorphine and apomorphine alone) and that increases or decreases in the recorded motor activity are not related to particular changes in the behaviour (see e.g., the reserpine + apomorphine and apomorphine alone).

It therefore seems that motor activity can not be considered a simple or homogeneous behaviour but instead a multiple phenomenon. The behaviour underlying the motor activity recorded in the present study consisted of many different components of behaviour such as walking, rearing, sniffing, licking and gnawing. Every treatment was characterized by a certain combination of behavioural components, i.e., a pattern of behaviour. Because of different movement detecting principles, the two activity boxes used in this study were not equally sensitive to all components of behaviour, i.e., some components of behaviour that were included in one recording were not causing any counts in the other. This is illustrated by the right hand part of Fig. 1: Patterns of behaviour that were manually labelled 3 or 4 also produced high counts in the motor activity recorded by the Animex high sens. while they were not associated with high counts in the photocell cage. These findings are in agreement with those of Krśiak *et al.*

[11] who in a study of another type of photocell cage concluded that only some components of behaviour are related to the recordings and "complex changes of behaviour are masked by the relatively crude photocell counts."

It thus seems that if the behaviour consists of a well defined component or a well defined pattern of behaviour and the behavioural changes to be analysed consists of intensity changes in this component/pattern then motor activity recordings might be a reliable technique to detect these changes. If, however, the behavioural changes consists of qualitative changes in the pattern of behaviour, it seems that the non-descriptive motor activity recording is a too crude measurement to reliably reflect these changes.

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