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A Method for Simultaneous Recording of Eight Behavioral Parameters Related to Monoamine Neurotransmission

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LJUNGBERG, T. AND U. UNGERSTEDT. *A method for simultaneous recording of eight behavioural parameters related to monoamine neurotransmission.* PHARMAC. BIOCHEM. BEHAV. 8(4) 483–489, 1978. – In response to the increasing demand for refined techniques to record drug induced changes in motor activity we have designed and evaluated against observations an automatic test box that quantifies eight defined components of behaviour in rats. Activity, corresponding to the recordings from the commonly used photocell activity boxes. Total, and forward locomotion, expressing the actual distance the rat walks. Corner count, and corner time, reflecting the position of the animal in the box. Hole count, and hole time, expressing the reaction of the rat to an environmental stimulus i.e. holes in the bottom of the test box. Gnawing, which is a direct counting of the number of gnaws made by the animal. The recording parameters relate to our own interest in behaviour influenced by monoamine neurotransmission and the result shows that the selected parameters are recorded with high reliability.

Automatic registration of behaviour   Locomotion   Stereotyped behaviour   Open field activity
Gnawing   Monoaminergic neurotransmission

CHANGES in monoamine neurotransmission are known, both from experiments with animals and from the clinic, to cause profound changes in motor behaviours. In animal experiments, the automatic techniques most commonly used to assess these changes are different types of activity boxes [8,15]. However, with the increasing knowledge in this field it is evident that the non-descriptive motor activity recordings are too crude measurements to reliably reflect these complex changes of behaviour [10,11]. Instead, more refined recording techniques, designed for the automatic quantification of defined components of behaviour, are needed to reflect these changes. During the last years new such techniques have been developed which record more defined components of drug induced behaviour e.g. locomotion [3,14] or gnawing [9].

As related to our own interest in drugs interfering with monoamine neurotransmission we have tried to design a test box which separately records several of those behavioural components that we can identify and that seems relevant to monoamine neurotransmission on the basis of our observations. The selection of these components will, of course, be subject to our own bias and interest. However, the fact that the components are defined and recorded separately will make it easier to recognize changes in behaviour as well as communicate the result. This paper is an account of the reliability of our test system.

METHOD

Animals

All experiments were performed on male Sprague-Dawley rats (Anticimex, Stockholm). They arrived to 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/dark schedule (6 a.m.–6 p.m.) with ordinary lab chow and tap water ad lib. Unless otherwise stated in the text, all the animals had a body weight of 160–210 g when tested and were used only once.

Apparatus

The behaviour of an animal was recorded in a test box designed for the automatic recording of eight components of behaviour. It consisted of a modified open field area (69 x 69 cm, with 25 cm high walls) where the animal could move around freely in the periphery but was unable to cross the middle part of the area due to a centrally placed cube of the same height as the walls (see Fig. 1). The movements of the animal were detected by interruptions of ten photobeams using white light symmetrically covering the open field area. Activity was defined as the number of interruptions of these ten photobeams. By feeding the photobeam interruptions into a digital logic, locomotion of the animal in the open field area could

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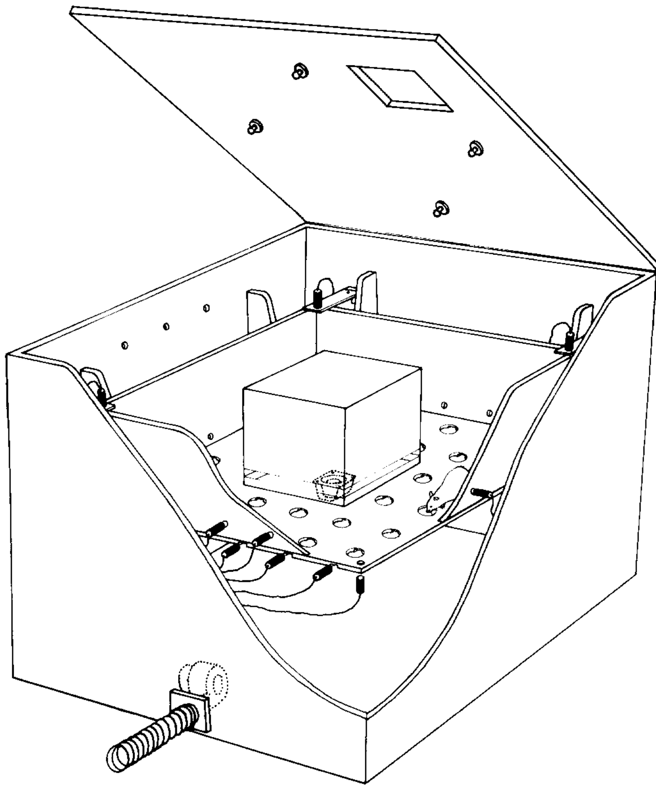

FIG. 1. Schematic drawing of the test apparatus. The walls of the outer sound protecting box and the inner test box are cut open to demonstrate the design. Activity, total locomotion and forward locomotion are detected by horizontal photobeams localized above the floor while hole count and hole time are registered by horizontal beams running in grooves, milled on the underside of the box across each row of holes. Corner count and corner time are registered by the vertical photobeams in each corner. Gnawing is sensed by the small loudspeaker screwed to the underside of the box. All lamps and photocells are fixed to the outer box. The test box can thus be lifted up to facilitate cleaning and slides in place on supporting consoles ascertaining a proper line up of the photobeams with the transparent Plexiglas disks in the test box walls and the Plexiglas band in the cube. The lamps in the lid provides a weak illumination. Air enters through small holes at the top of the outer walls and leaves through the electrical fan at the bottom which is connected to the exhaust of the laboratory. Slightly more than half of the test box can be observed during the experiment through the small window in the lid.

also be recorded and separated from repetitive interruptions of one photobeam. Total locomotion was defined as the number of times the animal walked a fixed distance which was slightly less than the length of the side of the box and defined by the digital programming of the photocells. Forward locomotion was defined as the number of times the animal walked the same distance but continued from one arm into the next arm (see Fig. 2).

The digital circuitry was designed according to the following principles:

Foward locomotion. Photobeams A and B are leading beams, if A is interrupted a forward locomotion count is produced and a photobeam A forward locomotion memory is set high. When this memory is set high further interruptions will not produce new forward locomotion counts. The animal then has to interrupt photobeam B in order to produce a new forward locomotion count. This sets the photobeam B memory high and the photobeam A memory low. The animal then has to interrupt photobeam A in order to get a new count and so on (see Fig. 2, dotted arrow).

Total locomotion. Photobeams A and B are leading beams. However, the photobeams A and B total locomotion memories are not only set low by interrupting photobeams B and A respectively but also, in case of photobeam A, set low by interruption of one of the photobeams C and in case of photobeam B set low by interruption of one of the photobeams D. Therefore the animal does not only receive total locomotion counts when passing the distance illustrated by the solid arrow parallel to the dotted arrow in Fig. 2 but also when passing the distance illustrated by the other solid arrows.

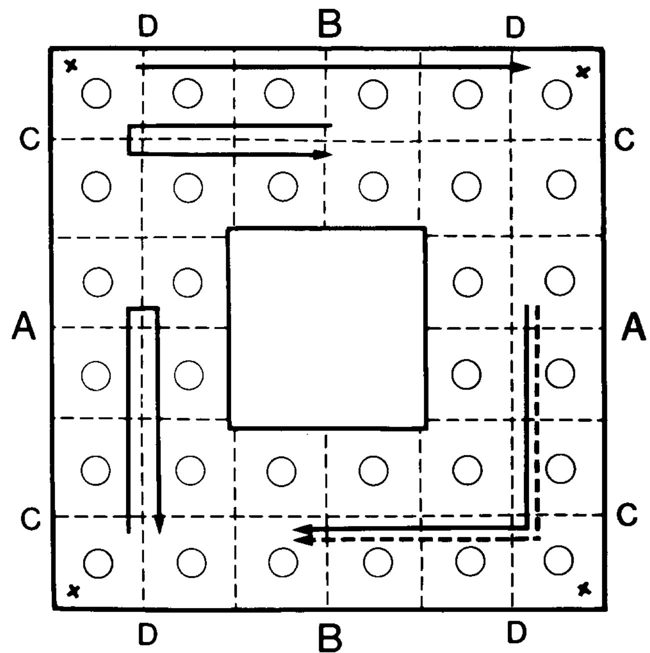

FIG. 2. The test box is symmetrically covered by ten photobeams (thin dotted lines). Activity is defined as the number of interruptions of these photobeams. A total locomotion count corresponds to the distance walked by the animal as shown by the solid arrows and a forward locomotion count corresponds to the distance shown by the dotted arrow. These conditions are set by the programming of the digital electronic circuitry. The X in the corners shows the position of the vertical photobeam. Corner count is defined as the number of interruptions of these photobeams and corner time is the accumulated time of interruption.

Thirty-two holes (2.5 cm dia.) were symmetrically distributed over the entire floor. Six photobeams, one for each row of holes, were mounted under the floor in such a way that a beam was interrupted as soon as the nose of the animal was lowered deeper than 0.5 cm into a hole as measured from the surface of the floor (see Fig. 1). Hole count was defined as the number of interruptions of these photobeams and hole time was the accumulated time these photobeams were interrupted. One photobeam

directed vertically was positioned in each corner. Corner count was defined as the number of interruptions of these photobeams and corner time was the accumulated time these photobeams were interrupted. The box was built out of black 1 cm thick PVC plastic and supported on rubber pads. The gnawing of the animal, which was nearly always performed on the edges of the holes, caused a characteristic sound/vibration in the box. This sound/ vibration was sensed by a miniature loud speaker screwed to the underside of the box (5 cm dia., 8 Ω). The signal was amplified 1000 times in an amplifier, filtered to give a maximum frequency response at 1000 Hz, fed into a Schmitt-Trigger and converted to TTL pulses. By a time-delay unit the system for recording gnawing was limited to maximum 5 counts/sec. The detection system was adjusted so that one gnaw gave rise to one digital pulse by careful observation of the animals. The test box itself was placed in an outer sound protecting box (1 × 1 × 1 m) with a small Plexiglas observation window at the top. The floor of the test box was positioned 40 cm above the bottom of the outer box. A rat looking down through a hole in the test box would thus see the bottom of the outer box 40 cm underneath. The test box and the outer box were weakly illuminated by the lamps of the photobeams and by four 2.5 W lamps positioned on the lid of the outer box (see Fig. 1). The average illumination was 50 photopic lux on the floor of the test box and 5 photopic lux on the floor of the outer box. The colour temperature was 2200 K. An electric fan ventilated the box at the same time as it provided a constant background noise. The assembly was placed in a weakly illuminated room (10 photopic lux).

The eight channels from the digital logic were fed into four bit buffer memories which were read into a WANG 2000 S minicomputer every 2 sec. The counts were added up during a preselected time period and printed out during the end of each such period to allow continuous monitoring of the experiment. At the end of the experiment each channel was plotted and the data stored on tape for later group comparisons and statistical analysis.

Observations

Concomitant with the automatic recordings the animals were observed and notes were taken with descriptions of the observed behaviour. The print out of the automatic recordings were continuously compared with these observations in order to detect any abnormalities in the recordings or if the observed behavioural patterns were not adequately recorded.

Drug Treatments

Ro 4-4602 (Roche, Basel) and d-amphetamine sulfate were dissolved in isotonic saline and injected IP in a volume of 5 ml/kg body weight. L-DOPA was dissolved in isotonic saline during heating and was injected IP in a volume of 10 ml/kg. Reserpine (Serpasil, Ciba) was used as commercially available injection ampoules and injected IP in a volume of 4 ml/kg body weight. The doses for Ro 4-4602 and reserpine refer to the above mentioned forms and the doses for L-DOPA and d-amphetamine refer to the bases. Apomorphine was dissolved in saline by quick heating and injected either IP in a volume of 5 ml/kg body weight or SC into the left flank in a volume of 1 ml/kg body weight. In one experiment (Experiment 3 B) apomorphine was obtained as commercially available 1 ml ampoules (Injectabile apomorphini 5 mg/ml, Apoteksbolaget, Sweden, with an injection vehicle containing 1 g $NaHSO_3$, 0.9 g 1 M HCl, 8.23 g NaCl, 2 g methylparaoxybenzoate, aq. dest. add 1000 ml), which was injected SC in a volume of 1 ml/kg body weight. The dose refers to the base.

Statistics

As it was found that all recorded data were not normally distributed, nonparametric statistics were used. All the data are presented as medians. The Kruskall-Wallis one-way analysis of variance was used to calculate the degree of significance between n independent samples, the Mann-Whitney U-test was used to test 2 independent samples and the Wilcoxon matched-pairs signed-ranks test was used to test 2 dependent samples. The Spearman rank correlation test was used to test the degree of correlation between two measures [13].

RESULTS

Behaviour of Normal Animals

One hour before the start of the experiment the animal to be tested was placed alone into a clean cage and moved to the weakly illuminated experimental room. At the start of the experiment it was carefully transferred to the test box and allowed a 15 sec calming down period before the start of the registration. The data were accumulated during 15 min periods during the whole recording period.

The animals showed a high exploratory behaviour during 10–15 min after being placed in the test box which consisted of varied locomotion, mainly along the outer walls with short periods of inactivity in the corners. Relatively few attempts were made to explore the middle part of the open field area i.e. the area around the cube in the middle. The behaviour was accompanied by sniffing, head dips into the holes and some rearing.

As the experiments were started from 9:00 a.m. until 7:00 p.m. it was investigated whether the behaviour recorded during the initial 15 min in the test box was dependent upon the time of the day. Forty-four animals were therefore tested at different time points of the day and results were plotted against the time when the experiment was started. The results showed that the response was stable during the whole day apart from a slightly decreased activation after 6 p.m. In order to perform a statistical analysis of the results the day was divided into four periods (9:00–12:00 a.m., 12:00–3:00 p.m., 3:00–6:00 p.m., 6:00–9:00 p.m.) and the variation between the periods was tested by a one-way analysis of variance. No significant variations due to the time of the day was found in any of the channels.

It has previously been reported that rats display low activity and exploration in the early afternoon [5]. We have sought to minimize such variations by keeping the animal alone in a cage in the weakly illuminated experimental room 1 hr before it is transferred to the test box. Our results show that this procedure effectively decreased variations in activity related to the time of the day.

The stability of the population of animals received from the breeder was also tested. The animals received during a week were pooled and the variation between

weeks over a 7 week period was tested (n = 61). Corner time just reached the level of significance while the other channels did not. In order to test if variations observed between animals were dependent on biological factors or on random factors one group of 10 animals were tested for 10 min, brought back to the colony and tested again for 10 min one week later. There was no significant correlation in any of the channels between the two testings. The pattern of the exploratory behaviour was, however, greatly changed from the first to the second test. The animals were less active in the open field area and spent more time inactive in the corners as is seen in Fig. 3. This change in pattern was not caused by the extra one-week-stay in the colony or changes in body weight which was controlled for in separate experiments by comparing animals received 3–7 days before testing with animals received 12–14 days before (n = 13).

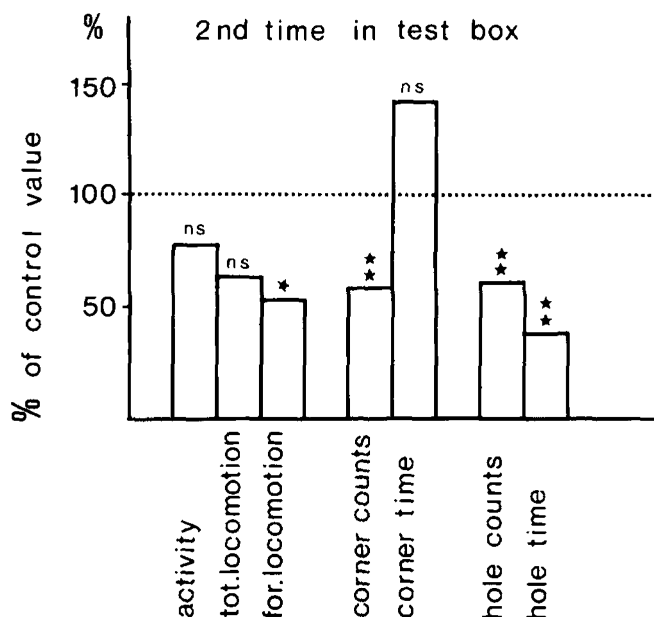

FIG. 3. One group of animals (n = 10) was tested for 10 min in the test box, brought back to the animal colony and tested again for 10 min one week later. The figure shows the results from the second testing in per cent of the results from the first testing. There was a decrease in the exploratory behaviour with an increased time spent in the corners when tested for the second time (* = $p<0.05$ and ** = $p<0.01$).

After the initial 10–15 min the activity of the animals decreased, they showed less walking around and stayed for longer periods in the corners. After 60 min the animals laid inactive in the corners and the only activity seen was when the animals occasionally left the corners for short periods of increased activity with some walking around in the cage.

In order to test the effect of an injection procedure 3 groups of 6 animals were used. One control group which was left for 90 + 60 min in the test box without receiving any injection and two experimental groups where one was injected IP (5 ml/kg) and the other SC (1 mg/kg) after 90 min and recorded for another 60 min. The animals were carefully removed from the test box, injected with saline, and again placed in the test box in the midpart of one of the four arms. They were allowed a 30 sec calming down period before the start of the automatic recordings. There was no significant effect of the injection procedure as compared to the group which was not removed from the test box whether measured 0–5 min after the injection or 0–60 min after the injection.

Reliability of the Automatic Recording of Gnawing

The reliability of the automatic system for recording gnawing was tested in two ways:

(A) Animals that showed compulsive gnawing were carefully observed and a micro-switch was pressed by the observer every time the animal showed a gnaw. The accumulated manually counted gnaws and the automatic recorded gnawing during 5 min periods were then correlated. Three different pharmacological treatments were given to induce compulsive gnawing. Apomorphine 5 mg/kg SC; reserpine 10 mg/kg 24 hr + apomorphine 0.1–0.4 mg/kg SC; Ro 4-4602 50 mg/kg 30 min + L-DOPA 200 mg/kg. The gnawing was recorded and counted directly after the injection and periodically during the whole duration of the behaviour. The counts were evenly distributed with a range of 11–546 counts/5 min (>2 counts/min). The observed and counted gnawing was highly positively correlated, $r_s>0.98$ for all three treatments (Fig. 4).

(B) Animals that did not show compulsive gnawing were observed and all counts that were wrongly recorded on the gnawing channel were noted. It was found that some other behaviours may cause similar sound/vibration as gnawing. The most common cause was either sudden jumps or that the animal while walking around fell down with a leg into a hole. The intensity of this behavioural noise in the system was estimated by studying the maximal number of counts elicited from these sources. One hundred animals were studied during the initial 15 min after being placed in the test box. Ninety-five percent of these animals caused less than 1 gnawing count/min and none of the animals caused more than 2 counts/min. This analysis was extended to 54 animals from various other pharmacological experiments where animals showed stereotypies without gnawing but there was still not more than 2 counts/min registered on the gnawing channel.

Descriptive Ability of the Automatic Recording System

The accuracy of the recording system for describing changes in behavioural patterns was tested by giving the animal some well known drug treatments. The change in behaviour, as compared to control animals (see Part 1), was judged by observations and compared to the automatic recordings. The animal to be tested was weighed, put alone into a clean cage and moved to the weakly illuminated experimental room 4 hr (Experiment A) or 1 hr (Experiment B) before the start of the experiment. It was pretreated with the drug, carefully transferred to the test box and allowed a 15 sec calming down period before the start of the registration.

(A) The behaviour was recorded during the initial 10 min in the test box. After d-amphetamine 2 mg/kg (50 min) the animals walked in a more automatic and non-varied way than control animals and showed some repetitive head and forelimb movements. They showed in-

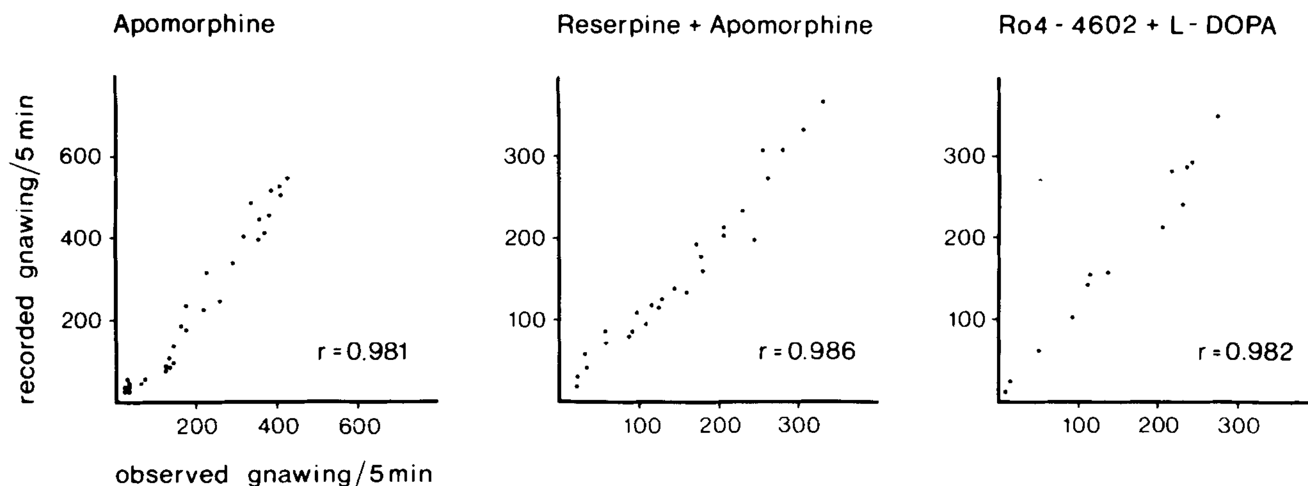

FIG. 4. Apomorphine 5 mg/kg SC; reserpine 10 mg/kg IP (24 hr) + apomorphine 0.1–0.4 mg/kg SC and Ro 4-4602 50 mg/kg IP (30 min) + L-DOPA 200 mg/kg IP were used to induce compulsive gnawing. The compulsive gnawing was counted, both manually and with the automatic system to record gnawing, during periods of 5 min. The high reliability of the automatic system to record gnawing is shown by the high positive correlation for all three treatments between the manually counted gnawing and the automatically recorded gnawing.

creased sniffing on the floor and the walls and made head dips into the holes. The animals moved over the entire test box and were also active in the vicinity of the cube in the middle i.e. not only in the periphery as the controls. There was a non-significant increase in the total locomotion as compared with the controls. The increase in repetitive movements and sniffing was reflected in the increase in activity per total locomotion count and the more automatic pattern of locomotion was reflected by more forward locomotion counts per total locomotion counts than in control animals. The changed position of the d-amphetamine animals in the test box was seen as a decrease in corner count and corner time. The head dips into the holes caused an increase in both hole count and hole time (Fig. 5).

Apomorphine 1 mg/kg IP (5 min) induced a behavior characterized by slow automatic locomotion on somewhat straight legs and with a slightly hunched back which was reflected as a decrease in the recorded locomotion with a relatively greater decrease in the total locomotion. The animals showed continuous sniffing on the floor between the holes and sometimes also at the edges of the holes. However, they made few head dips into the holes. This was reflected as a decrease in both hole count and hole time. The animals also showed some repetitive movements of the head and forelegs but did not show licking, biting or compulsive gnawing (no recorded gnawing and only a non-significant decrease in activity). The stereotyped animals were mostly active away from the corners but made some short entries into the corners (decrease in both corner count and corner time, Fig. 5).

The pretreatment with reserpine 10 mg/kg (24 hr) + apomorphine 1 mg/kg IP (10 min) changed the sniffing and low locomotion after apomorphine 1 mg/kg to a stereotyped behaviour characterized by compulsive gnawing performed on the edges of the holes with only little locomotion and sniffing. This change in behavioural pattern was clearly reflected in the automatic recordings (Fig. 5) as a further decrease in locomotion (both total and forward) and as a strong increase in gnawing. There was still only a non-significant decrease in recorded activity as compared to the controls. The animals were mainly active away from the corners and showed few head dips into the holes which was also reflected in the automatic recording. Even if the animals were gnawing intensively at the edges of the holes they would only occasionally gnaw deep enough to interrupt a hole photobeam causing false hole count.

(B) After the animal was transferred to the test box it was habituated for 90 min before the injection of the drug. The data were accumulated during 15 min periods during the whole drug duration.

Apomorphine 5 mg/kg SC (injection ampoule) induced a strong activation of the habituated animals. At short time intervals after the injection (0–30 min) it consisted of intense, highly automatic, locomotion which was reflected in the automatic recordings as a strong increase in locomotion with a relatively small difference between forward and total locomotion (compare the varied locomotion seen in exploring animals 0–15 min after being placed in the test box, Fig. 6). The locomotion was accompanied by sniffing, repetitive head and forelimb movements and fast biting movements. However, only few periods of intense compulsive gnawing was observed (Fig. 6). After about 30–45 min this pattern of behaviour changed and the animals showed less locomotion, less repetitive head and forelimb movements but instead an increase of strong compulsive gnawing which was nearly always performed on the edges of the holes (see gnawing peak in Fig. 6). The apomorphine injection did not cause any head dipping into the holes. The few hole count recorded were recording errors due to interruption of the photobeams when falling down with the leg in a hole or when gnawing very deep at the edge of a hole (no increase in recorded hole time). The animals were mainly active toward the centre of the open field area but made many short entries into the corners which is reflected as a strong decrease in corner time and as an increase in

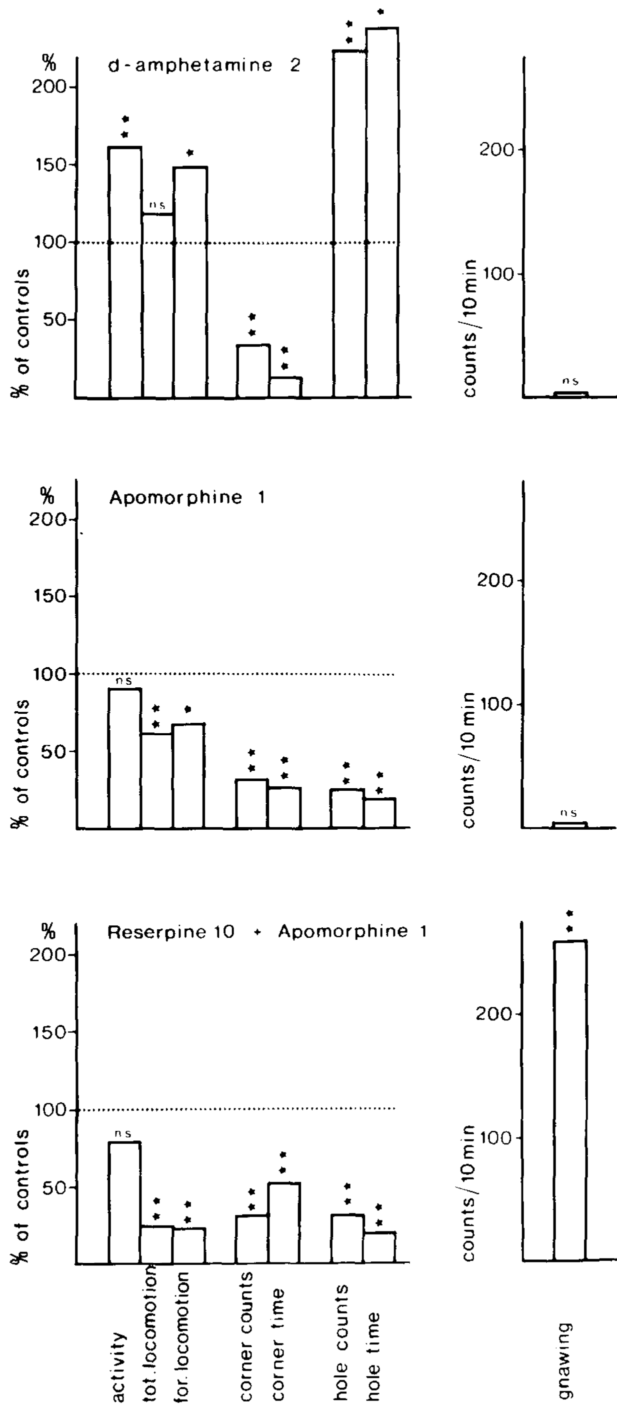

FIG. 5. The behavioural pattern for three pharmacological treatments as detected by the automatic recordings are shown: D-amphetamine 2 mg/kg IP (50 min), apomorphine 1 mg/kg IP (5 min) and reserpine 10 mg/kg IP (24 hr) + apomorphine 1 mg/kg IP (10 min). The recorded gnawing is presented in absolute counts/10 min while the other channels are presented as per cent of saline injected control animals (* = $p<0.05$ and ** = $p<0.01$).

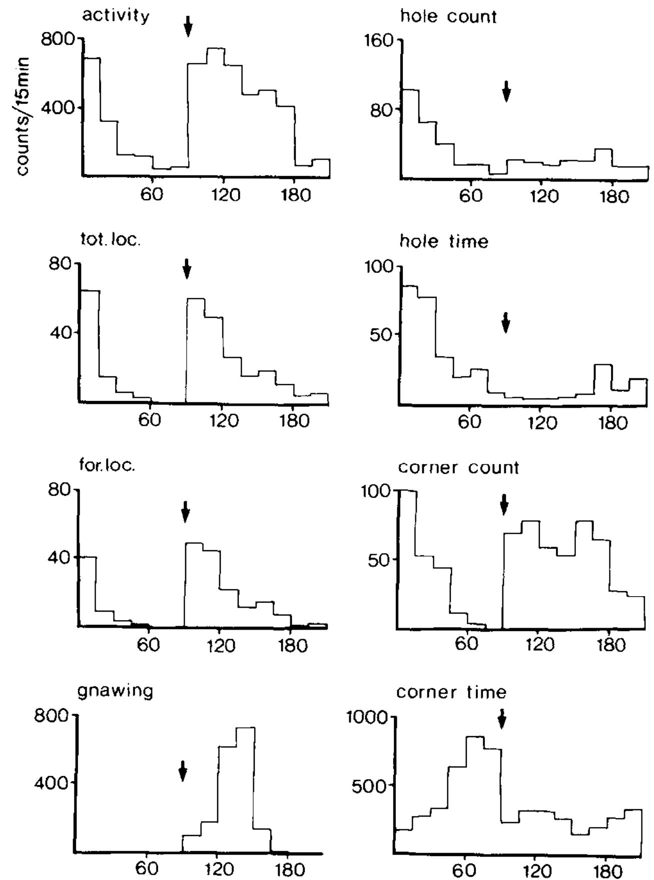

FIG. 6. The animals were hatibuated for 90 min in the test box before being injected with apomorphine 5 mg/kg SC. The data are presented as median counts/15 min (n = 12).

corner count. After 90–105 min the drug induced activation wore off and the animals again laid inactive in the corners.

DISCUSSION

The different parameters included in the test box recordings have been chosen to describe known components of drug induced behaviour i.e. components of behaviour that from previous observations of animals are known to be related to monoamine neurotransmission. The comparison between the manual observations and the automatic recordings shows that the selected parameters are recorded with high reliability: Activity reflects the total movements of the animal and is non-descriptive unless it is compared with other recorded parameters (see below). Because of the recording principle, activity corresponds to the most commonly used methods of recorded motor activity in commercially available photocell type activity boxes. Locomotion is defined as walking a distance corresponding approximately to the length of the side of the box and reflects how much the animal is walking around in the cage. The ratio forward locomotion/total locomotion reflects how varied or how stereotyped the locomotion is. Normal exploring animals have a ratio of 0.5–0.7 (see Fig. 6, 0–15 min) while animals showing drug induced locomotion often get a ration of

0.8–0.9 (see Fig. 6, 0–30 min after apomorphine injection, see also Fig. 5, d-amphetamine and apomorphine). By forming the ratio activity/locomotion (total or forward) an indirect value of the amount of small repetitive head and limb movements, and sniffing movements can be obtained (see the increase in activity count/locomotion count in d-amphetamine, apomorphine and reserpine + apomorphine in Fig. 5). Corner count and corner time reflects the position of the animal in the test box. Corner count tells how active the animal is in or in the vicinity of the corners and corner time shows the time the animals spends in the corners (as opposed to somewhere else in the test box). This is seen by the changed position in the cage of the animals given the different treatments as illustrated in Fig. 5 as well as the apomorphine 5 mg/kg treated animals (see results in Fig. 6). The holes in the bottom of the test box were included as an environmental stimulus the animal could react to (head dipping into the holes, [1]) and have previously been used as a way of measuring exploration of mice or rats [1, 6, 7]. However, it should be emphasized that in our pharmacological study we hesitate to consider head dipping into holes as a measure of exploration. Instead we regard it solely as a descriptive variable. In our study we found that d-amphetamine increased head dipping (Fig. 5) as also found in previous studies [2,12]. In contrast File [4] found decreased head dipping after d-amphetamine. These discrepancies may be due to differences in the design of the test boxes e.g. number of holes in the box, their size, location and the distance between them. The usefulness of head dipping for discriminating between different drug effects is well illustrated by comparing d-amphetamine and apomorphine which both cause increased locomotion, sniffing and repetitive movements while only amphetamine cause increased head dipping (c.f. Figs. 5 and 6). The holes however also served another important function in that they provided a place where the animal could perform gnawing. If the box where apomorphine treated animals were tested was completely smooth, only some compulsive gnawing was induced. If however, a suitable place was available where the animal could gnaw much more compulsive gnawing was induced [13]. In the present test box almost all compulsive gnawing was performed on the edges of the holes. This gnawing behaviour is recorded with high reliability on the gnawing channel as shown in Fig. 4. With the present technique, drug effects can thus be evaluated quantitatively, i.e. as increases or decreases in one channel but also qualitatively i.e. a drug effect can be illustrated by combining all the recorded parameters making up a descriptive pattern of the effect.

Two principally different ways of designing experiments are evident: (A) Animals may be pretreated with a drug and the effect evaluated by how it changes the normal exploring behaviour when the animal is first introduced into the test box i.e. drug interaction with exploratory behaviour (see Fig. 5). (B) The drug may be given to the habituated, inactive animal i.e. drug induced activity in habituated animals (see Fig. 6).

In conclusion we would like to emphasize that the design of this test box is not an attempt to make a final motor activity recording system. Instead, the intention has been to create a system that measures, as specifically as possible, components of behaviour that we have recognized in the response to drugs altering monoamine neurotransmission. As the needs change the present parameters may be excluded, replaced or supplemented with new ones. Ideally this kind of test box should be an automatic observer. However, due to unavoidable shortcomings in the technique it must always be supplemented with observations to avoid misinterpretation and recording errors.

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