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Automating the Measurement of Locomotor Activity[1]

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BENINGER, R. J., T. A. COOPER AND E. J. MAZURSKI. *Automating the measurement of locomotor activity.* NEUROBEHAV TOXICOL TERATOL 7(1) 79–85, 1985.—General locomotor activity is assessed in many ways ranging from observer ratings to sophisticated computer-assisted electronic monitoring with the aim of determining the unconditioned and conditioned effects of various pharmacological compounds. A system was constructed utilizing 6 independent test chambers each equipped with 14 infrared emitters and detectors arranged in two tiers and controlled by a microcomputer. The effects of the stimulant, *d*-amphetamine and its antagonism by the tranquilizer, pimozide, on unconditioned and conditioned activity as assessed using this system were found to be almost identical to results obtained using observer rating techniques. Other data showed that activity assessed by the system was subject to habituation and that this learning interacted with the effects of some drugs. Activity as measured by the system showed dose and time effects of various stimulants assessed over a 6 hr test period. Finally, the breakdown of activity into counts on the upper and lower tier of beams resulted in the finding that these two components of behavior were differentially affected by stimulants. These results demonstrate the validity and reliability of automated systems for assessing locomotor activity and show their promise for gaining further insight into the elements of behavior that are affected by various drugs.

Locomotor activity Automated measurement systems *d*-Amphetamine Pimozide LY 141865 SKF 38393

UNCONDITIONED locomotor activity is frequently measured in assessing the behavioral effects of various pharmacological agents or physiological manipulations [15]. Although appearing to be a straightforward and relatively simple behavioral dependent measure, locomotion, like other unconditioned and conditioned responses is influenced by many variables. These include features of the apparatus such as size, temperature and level of illumination and temporal variables including time of day and duration of testing [5]. There are obvious species differences. The measurement of locomotor activity may be affected by age differences [13]. Sex differences may reflect the influence of hormones; another motivational factor that may affect activity is level of food or water deprivation [10]. Conditioning also has an effect. Activity may be influenced by the handling history of the animal, its familiarity with the testing environment [1] and its prior experiences there; for example, stimuli in environments associated with feeding, drinking, sexual activity, rewarding brain stimulation or various psychoactive drug states may become conditioned incentive stimuli that have an altered ability to influence response systems [2, 3, 7, 16].

There have been many different approaches to the measurement of activity. Observing animals and rating them on ordinal scales requires no sophisticated test apparatus and is perhaps the simplest [2, 4, 6]; however, precautions must be taken as this approach is particularly susceptible to experimenter bias. One example is a nine-point scale for rating rat behavior ranging from a score of "1," defined as "asleep" (lying down, eyes closed), through "4," "normal, alert, active" (moving about cage, sniffing, rearing), to "7," "fast patterned activity" (repetitive exploration of the cage with hyperactivity), with "9" being defined as "dyskinetic reactive" (backing up, jumping, seizures, abnormally maintained postures, dyskinetic movements) [4]. This scale includes the range of activities normally seen in undrugged rats as well as the hyperactivity, stereotypies and dyskinesias seen with increasing doses of psychomotor stimulants.

Another simple approach that requires no automation but is labor intensive involves counting line-crossings in an activity monitor with a grid painted on the floor. This type of apparatus can be easily automated by placing light emitters and detectors at an appropriate height at the ends of the lines and using electronic counters to detect crossings. Others have constructed "jiggle cages" by, for example, placing a cage on a centrally located fulcrum with microswitches attached to electronic counters located at each end; as the animal moves from end to end in the cage, switch closures are counted. The running wheel has also been used extensively and is easily automated by placing studs on the outside edge that trigger a microswitch or break a light beam that is attached to a counter. A more recent technique involves the detection of capacitance changes produced by an animal's movement; these are digitized and counts are accumulated. A somewhat similar approach is to transmit an ultrasonic signal throughout the test environment; changes in the signal at a receiver can be digitized and counted. These latter two approaches have the advantage of being sensitive to fine

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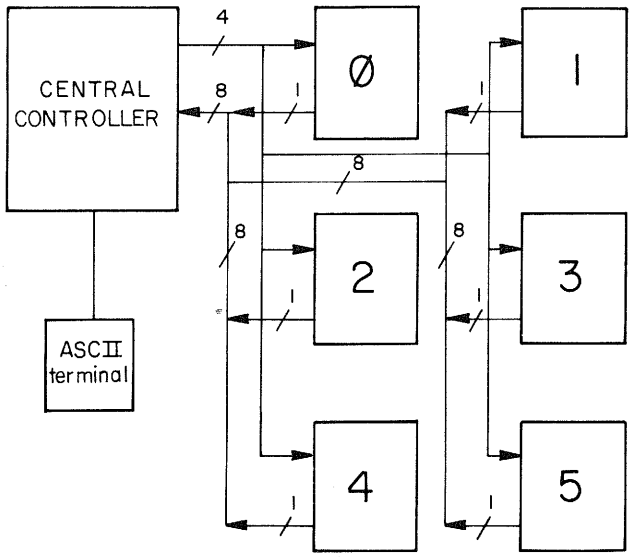

FIG. 1. The configuration of the automated activity monitoring system. Boxes numbered 0 through 5 indicate individual test chambers. Each receives the same 4 input lines from the central controller and each has a single output line that forms part of an 8-line cable (only 6 are used) connecting the chambers to the central controller. The operator interface is a standard ASCII terminal.

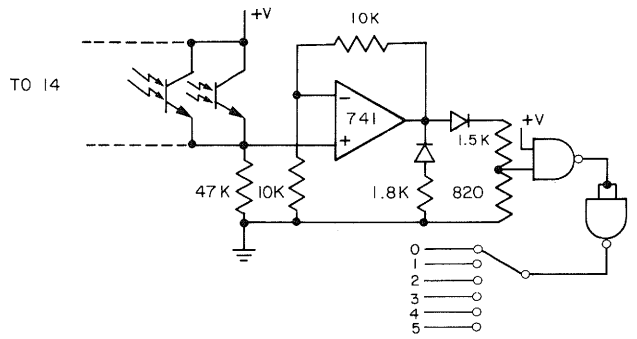

FIG. 2. Circuit to detect the infrared beams; there is one in each chamber. Each of the 14 detectors (only two are shown) is connected in parallel to a single amplifier (741) that provides the one output for each chamber. Therefore, there must be no more than one beam per chamber active at any time (see text).

movements but cannot efficiently discriminate horizontal from vertical movements.

Of these automated approaches to monitoring locomotor activity, photocell cages are among the most widely used in studies of rats. They offer not only an objective quantification of activity but also the opportunity to discriminate horizontal movements from rearing and jumping. Furthermore, with the use of computers, software can be created to allow a virtually continuous record of the animal's location and activities in the experimental space. The purpose of the present paper is to describe one such system that was designed and built in our laboratory. The description of the system will be followed by a comparison of experimental results obtained using a rating scale with those obtained using the automated activity monitors. The effects of familiarity with the test environment will be discussed briefly as will the results of continuous monitoring of activity over a six hr period. Finally, the changing pattern of results seen when total counts are broken down into horizontal counts and rearing counts will be reviewed.

THE SYSTEM

The system (Fig. 1) consists of a central controller, an ASCII terminal and six Plexiglas chambers (41×50×37 cm high) each housed in a styrofoam-insulated wooden sound-attenuating box illuminated by an overhead bulb (2.5 W) and ventilated by a small fan that also provides constant masking noise. Each chamber is outfitted with 14 infrared (IR) emitters (LED's) and detectors, 8 being spaced at 10 cm intervals along the length of the chamber and 6 along the width at a height of 5 and 15 cm above the grid floor. A light beam is formed by placing an IR LED on one side of the chamber and an IR detector on the other. To prevent possible spillage from one LED illuminating two adjacent detectors, the beams alternate direction along the length or width of the chamber.

To detect the IR beams all of the detectors for one chamber are connected in parallel to one amplifier (Fig. 2) eliminating the need for 14 amplifiers. Therefore, there must not be more than one beam active at any time; one beam is activated, it's state checked and it is turned off, then the next beam is activated, etc. The circuit to do this (Fig. 3) uses a 4 to 16 line decoder to convert a 4 bit binary code from the central controller to indicate which one of the 14 beams is to be turned on.

Since there is only one beam active at any time there is only one output per chamber (Fig. 1). This is easily accommodated on an input port of the computer using a selector to place the output from a chamber on one of 8 lines. These lines (only 6 are used) are contained in a multi conductor cable connecting the chambers to the central controller. Four lines of this cable are used for the select code and this single cable connects all the chambers to the controller. Some of the advantages of this design are the requirement of only one simple cable, lower power consumption and simpler circuitry. With this design one scan determines the status of all chambers simultaneously. The only major disadvantage is that the detector circuit is very sensitive to ambient light; to reduce this problem the insides of the chambers are painted flat black.

The central controller is a microcomputer (Cromemco) with a control program for this specific task. The design of the hardware means that the software has some extra work to do. The software selects a beam then reads the results from all the chambers as a 6 bit binary code. By comparing this code to the previous one the system can determine if there are any changes. A beam break is the only change of concern; the software can decode the 6 bit number to find the chamber(s) in which a change occurred and record the change(s) as a count. A complete scan takes 85 msec. To count a beam break the software requires that the beam be broken in 2 consecutive scans. This double scan eliminates tail flicks or quick nose pokes thus requiring the animal to move into the beam to be counted.

Several simple commands are provided which allow the experimenter to control the system. Any session can be started, stopped or the results printed independently for each chamber. There are several parameters which can be set for

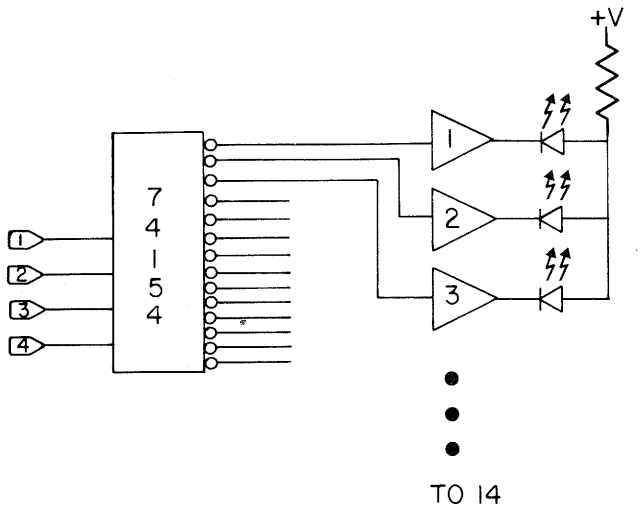

FIG. 3. Circuit to activate the infrared emitters. The central controller selects one of the 14 emitters (only 3 are shown) using a 4 bit binary number that is sent to a 4 to 16 line decoder (74154). The output of the decoder simultaneously activates emitters in all 6 chambers.

each chamber. These are such things as session duration and duration of each data accumulation period from one sec to 18 hr. Headers and comments may also be entered to identify the animal or state any relevant event that occurred during the session; these are printed as part of the report. There are also commands which test the system or help in finding any problems that may occur. There is a complete system test which is run each time the system is powered up so that sessions will not be begun on faulty equipment.

All of this interaction with the system is carried out from a standard ASCII terminal. A printing terminal is useful for keeping hardcopies of the results.

COMPARISON OF RATING SCALE AND BEAM BREAKS

Prior to constructing the automated activity monitors, drug effects were evaluated with the use of the 9-point rating scale already described briefly [4]. To avoid possible experimenter bias, ratings always were made by two independent observers, one of whom was unaware of the treatment conditions; inter-rater correlations ranged from 0.7 to 0.9 and always were highly significant. Test boxes were constructed with wooden floor and sides and a Plexiglas front and were of the same dimensions as the chambers in the automated system.

In one study [2] the ability of the psychomotor stimulant, *d*-amphetamine to produce environment-specific conditioned activity was evaluated. Two groups (each n=12) were tested for 30 min each day, ratings being made at 5, 10, 20 and 30 min. One group was injected with *d*-amphetamine (2.5 mg/kg, IP) and the other, saline. The results on the fifth conditioning day for each group are shown in Fig. 4A. Group medians differed significantly ($p<0.01$) at each time. When both groups were injected with saline and tested on the sixth day (Fig. 4B), environment-specific conditioned activity was observed, the group previously receiving amphetamine there having significantly higher ratings at 5, 10 and 30 min ($p<0.05$). The ability of pretreatment (4 hr before) with the

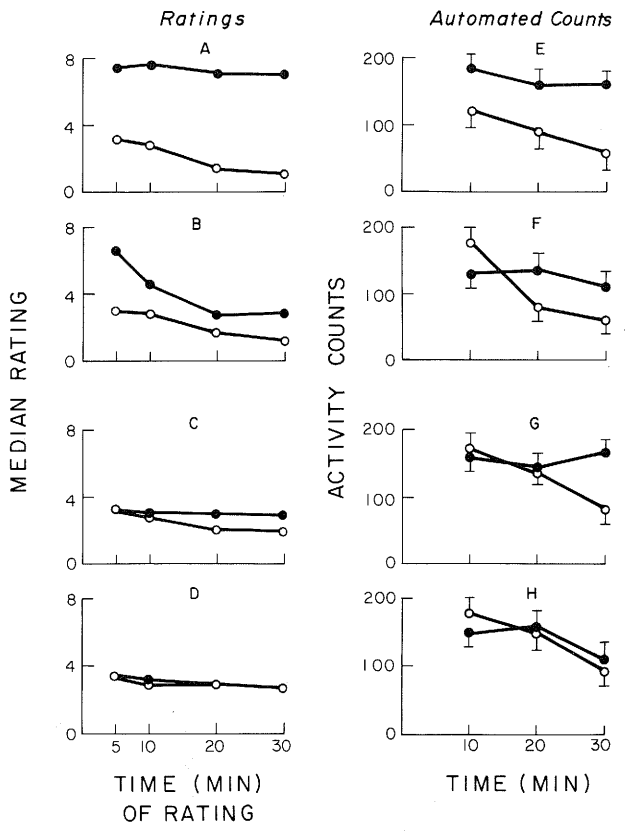

FIG. 4. Comparison of rating scale and beam breaks. The left column shows the median ratings on a 9 point scale on the fifth day (A) for a group (n=12) given 2.5 mg/kg amphetamine (solid symbols) and a saline control group (n=12; open symbols). B shows the same groups on day 6 following saline. C shows two different groups (each n=12) on day 10 following 0.4 mg/kg pimozide plus amphetamine (solid symbols) or pimozide alone (open symbols). D shows the same groups following saline on day 11. The right column shows mean (±SEM) activity counts (counts/10 min) on both tiers of beams combined for two groups (each n=12) on day 4 (E) following 2.0 mg/kg amphetamine (solid symbols) or saline (open symbols). F shows the same groups after saline on day 5. G and H show data for 2 groups (both n=12) that correspond to C and D but represent days 8 and 9, respectively.

dopamine receptor blocker, pimozide (0.4 mg/kg) to antagonize the stimulant effects of amphetamine in another group of rats is illustrated in Fig. 4C. Shown is the tenth session with pimozide; amphetamine produced only a marginal stimulant effect that was significant at the 20 and 30 min rating ($p<0.05$). Figure 4D shows that no environment-specific conditioned activity was observed on a saline test day following 10 conditioning days with amphetamine plus pimozide. For further details see [2].

To evaluate the comparability of these rating data to data generated using the automated activity monitors, the experiment was repeated using two new groups of rats (each n=12). Figure 4E shows the mean (±SEM) total counts for each group for each 10 min of the fourth conditioning session. Animals receiving amphetamine (2.0 mg/kg) were significantly more active than controls ($p<0.001$). When both groups were tested with saline on day 5 (Fig. 4F), environment-specific conditioned activity was observed;

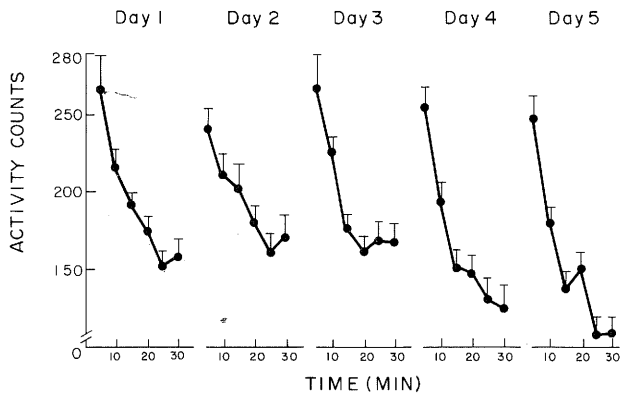

FIG. 5. Mean (±SEM) activity counts (counts/5 min) on the lower tier of beams for a group (n=24) of rats during each of 5 30-min sessions. Decreases both within and across days were significant.

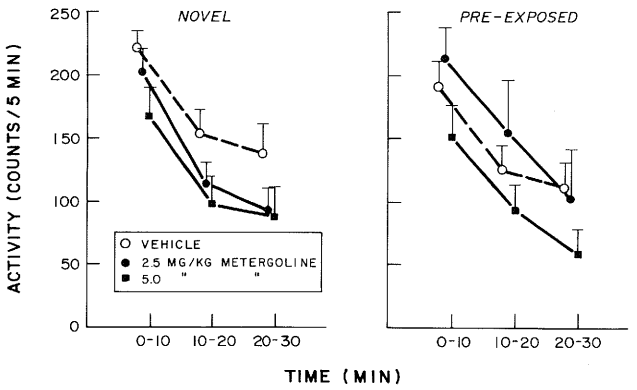

FIG. 6. Mean (±SEM) lower tier activity counts (counts/5 min) averaged over 10 min segments of 3 test sessions for groups (all n=6) receiving metergoline and tested in novel (left) or familiar (right) chambers. Both conditions showed significant effects of time but the drug dose was significant only for the novel condition.

analysis of variance revealed a significant group by time interaction ($p<0.03$) and post hoc tests showed that the group previously receiving amphetamine in the test box was marginally more active for the last 20 min ($p<0.09$). In another experiment with two groups (both n=12) the ability of pimozide (0.4 mg/kg, IP) to antagonize the stimulant effects of amphetamine was tested. Analysis of variance of the eighth conditioning day (Fig. 4G) revealed a significant group by time interaction ($p<0.05$) and post hoc tests showed that the amphetamine plus pimozide group differed from the controls only in the final 10 min ($p<0.01$). When these two groups were tested with saline on day 9 (Fig. 4H), no environment-specific conditioned activity was observed, the groups not differing significantly ($p>0.10$) and the time by group interaction being insignificant ($p>0.10$).

These findings suggest an excellent concordance between results obtained with ratings and the automated activity monitoring system. As shown in Fig. 4, both methods revealed a significant stimulant effect of amphetamine over 30 min (Panels A and E) and a significant environment-specific conditioning effect following saline (Panels B and F). Both methods showed that pimozide reduced the group differences produced by amphetamine (Panels C and G) and that following conditioning sessions with pimozide plus amphetamine, no significant environment-specific conditioned activity was seen (Panels D and H).

EFFECTS OF FAMILIARITY

In many instances, the repeated presentation of a particular stimulus to, for example, a rat results in the orderly decline of the response initially elicited by that stimulus; this learning phenomenon is termed habituation [9]. When animals are tested repeatedly in activity monitors, habituation effects may occur and, if they do, precautions must be taken in designing experiments to control for these effects. As many animals tend to explore novel stimuli, it might be hypothesized that activity counts would be high during the initial period of exposure to novel activity monitors and would be seen to decline over the course of exposure and on subsequent exposures.

To test this hypothesis, 24 experimentally naive male rats weighing 200–225 g each received one daily 30-min session of exposure to the activity monitors over 5 consecutive days, the sessions commencing at approximately the same time each day for each animal. Results (Fig. 5) revealed both within and across session declines in activity counts accumulated on the lower tier of beams; analysis of variance yielded significant effects of time and day ($p<0.01$). These results show that repeated tests of activity in the same monitoring apparatus can lead to significant habituation effects.

The need to control for apparatus familiarity in evaluating the effects of pharmacological compounds on locomotor activity was shown in a recent study [1]. Three groups of rats (n=6) received 5 30-min exposures to the activity monitors over 5 days and 3 groups (n=6) were simply handled on each day. All groups then received 3 test sessions, one per day on each of the next 3 days; 30 min prior to each, one pre-exposed and one non-pre-exposed group received 2.5 mg/kg IP of the serotonin receptor blocker, metergoline, two groups received 5.0 mg/kg and the remaining two groups received vehicle. Mean (±SEM) activity counts per 5 min, accumulated on the lower tier of beams, for each 10-min block of the 3 test sessions combined, for each group are shown in Fig. 6. Comparison of the two vehicle groups again shows the habituation effect, the pre-exposed rats being less active. Of greater interest is the effect of the drug; noting especially the 2.5 mg/kg groups, it can be seen that this dose resulted in reduced activity in the novel condition whereas activity appeared to increase in the pre-exposed animals. Analysis of variance revealed a significant group (drug dose) effect in the novel condition ($p<0.01$) but not the pre-exposed condition ($p>0.05$). As the data suggest, both conditions also showed highly significant within session time effects ($p<0.001$).

In summary, results obtained with the automated activity monitoring system have been found to be subject to both intra- and inter-session habituation effects. Furthermore, the effects on locomotor activity of centrally acting drugs were found to be influenced by prior exposure to the apparatus; metergoline results are briefly presented here and similar results have been seen with the putative serotonin agonist, quipazine [1]. Future studies of the effects of pharmacological compounds on locomotor activity should employ experimental designs that control for these habituation effects.

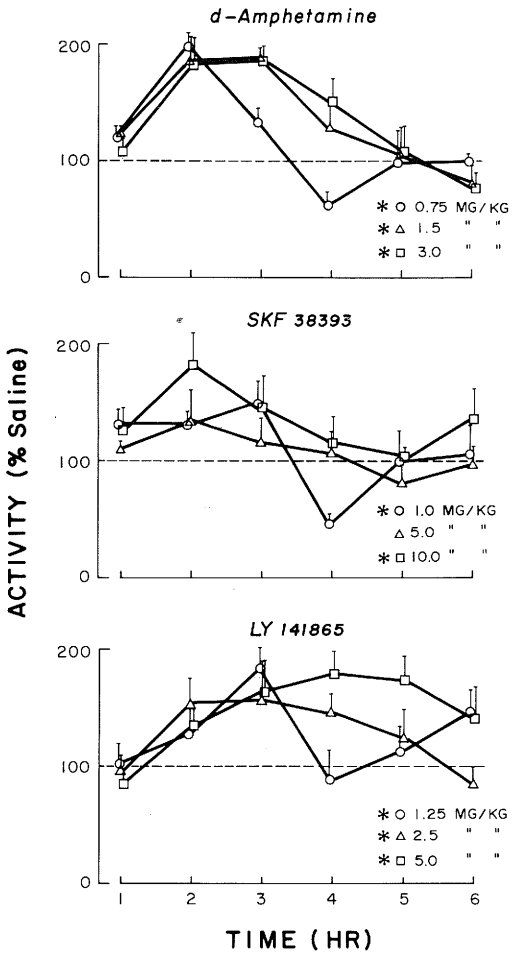

FIG. 7. Mean (±SEM) activity counts per hr over 6 hr, expressed as a percent of saline controls, for groups (all n=6) receiving amphetamine, SKF 38393 or LY 141865 at the doses specified immediately before each session. Asterisks indicate doses that produced significant stimulant effects compared to saline.

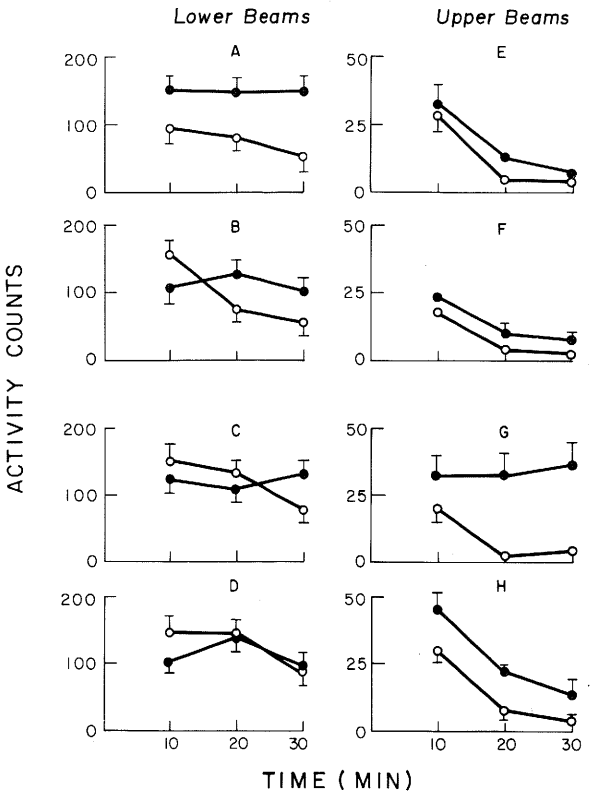

FIG. 8. Mean (±SEM) lower tier (left column) and upper tier (right column) activity counts (counts/10 min) presented separately for the groups depicted in Fig. 4E–H. Note that the scale on the vertical axis differs for the two tiers.

MONITORING LOCOMOTOR ACTIVITY FOR SIX HOURS

Activity is frequently assessed in sessions ranging in length from only a few min to one or two hr although a small number of studies have reported data for longer periods of time, especially those interested in possible diurnal variations [5]. One advantage of automated activity systems is that they allow for continuous, uninterrupted monitoring over long periods of time. The use of this approach in the assessment of drugs may provide new insights concerning their behavioral effects.

In a recent study employing the automated activity monitoring sytem, the effects of 3 compounds known to affect central dopaminergic neurotransmission were assessed over 6 hr test sessions. Brain receptors for dopamine have been shown to be of at least two types: D1 receptors are linked in an excitatory fashion to the enzyme, adenylate cyclase whereas D2 receptors are not [8]. Dopamine is well documented as playing an important role in the control of locomotor activity [15] but the relative contribution of D1 and D2 receptors remains uncertain. Thus, the effects on activity of the indirect-acting dopamine agonist, *d*-amphetamine [11] that would result in stimulation of both D1 and D2 receptors, the D1 agonist, SKF 38393 [12] and the D2 agonist, LY 141865 [14] were assessed.

To establish the reference level of activity in undrugged animals, 6 saline-treated rats received three 6-hr sessions, each from 1000 to 1600 hr on the same day of each of 3 consecutive weeks. Mean total upper and lower counts for the 3 sessions were approximately 600 for the first hr and ranged from approximately 380 to 320 counts for the next five hr thus showing a within-session habituation effect. Each of the 3 dopamine agonists was tested on a different group of 6 rats. Each group received 3 sessions like the saline controls. Immediately prior to each a different IP dose of the appropriate agonist was given; doses were: 0.75, 1.5 and 3.0 mg/kg *d*-amphetamine, 1.0, 5.0 and 10.0 mg/kg SKF 38393 and 1.25, 2.5 and 5.0 mg/kg LY 141865. Of the 6 rats in each group, one received the doses in each of the 6 possible orders, thus controlling for any effects due to repeated testing.

Results for each hr for each rat at each dose were converted to a percent of the corresponding score for the saline group (Fig. 7). For each drug there appeared to be both time and dose-dependent effects. With the exception of 5.0 mg/kg SKF 38393, each dose of each drug produced significantly more activity than saline ($p<0.05$). Analysis of variance comparing the doses of *d*-amphetamine yielded significant effects of time ($p<0.01$), dose ($p<0.04$) and their interaction ($p<0.01$). For SKF 38393 only the time effect was significant ($p<0.01$). For LY 141865 there was a significant effect of time ($p<0.05$) and a time by dose interaction ($p<0.05$).

These results provide a number of interesting findings. Most importantly, they suggest that both D1 and D2 receptors may participate in dopamine's locomotor function. They also show the time course of the stimulant action over 6 hr. Note that the effect in the first hr was small; this might be due in part to novelty effects in the saline group that showed high levels of activity in the first hr (approximately 600 counts) compared to the next 5 (320–380 counts). For each agonist the lowest dose appeared to produce a precipitous decline in activity in the fourth hr; this was not seen with the higher doses. The cause of this low-dose effect remains to be determined. Further studies of the time course of other dopamine agonists, antagonists and their interactive effects may further elucidate the role of this neurotransmitter in the control of activity. Conversely, the profile of effects of, for example, a known dopamine agonist can be used to evaluate the similarity of behavioral action of other drugs thought to have comparable pharmacological effects.

SEPARATION OF UPPER AND LOWER COUNTS

The studies discussed in the preceding section and the automated activity study discussed in the comparison with ratings employed total counts on the 14 beams as the dependent measure. However, the current software provides accumulated counts on the upper and lower beams separately. In this section the mean total counts per 10 min sample period for the groups shown in Panels E–H of Fig. 4 are discussed separately for upper and lower counts.

Lower beam counts presumably represent horizontal activity including pivoting, walking and running. As shown in Fig. 8A, amphetamine appeared to stimulate horizontal activity and analysis of variance revealed a significant group effect ($p<0.01$). Horizontal activity (Fig. 8B) showed environment-specific conditioning in the group previously receiving amphetamine in the test box, analysis yielding a significant time by group interaction ($p<0.04$) and post hoc tests revealing marginally significant differences in the last 20 min ($p<0.10$). When pimozide was given prior to conditioning sessions (Fig. 8C) the stimulant effects of amphetamine were almost totally blocked; analysis revealed a marginal time by group interaction ($p<0.09$) and post hoc tests of group differences at each time suggested that the only difference may have been in the last 10 min ($p<0.10$). The test session that followed conditioning sessions with amphetamine plus pimozide (Fig. 8D) revealed no significant main effects or interactions. This overall pattern of significant, marginal and insignificant results is almost identical to that obtained when both levels were combined and analysed as outlined in the preceding section comparing ratings of activity to beam breaks.

Counts on the upper tier of beams would require the animal to jump or rear either in the center of the field or against the wall. When these counts were analysed separately, amphetamine was found to produce a small but insignificant ($p<0.10$) increase (Fig. 8E; note the change in scale on the vertical axis). However, on the saline test day the group previously receiving amphetamine in the test chamber had significantly more counts ($p<0.05$), as shown in Fig. 8F. Animals treated with pimozide prior to each conditioning session with amphetamine (Fig. 8G) showed significantly higher upper tier counts ($p<0.01$) and on the following saline test day (Fig. 8H) showed environment-specific conditioned activity ($p<0.02$).

Comparison of these lower and upper tier data reveals a number of differences. Amphetamine produced a significant stimulant effect on horizontal activity but not on rearing, although the latter dependent measure showed a small insignificant effect (Figs. 8A and E). Both measures showed environment-specific conditioned activity on the saline test day (Figs. 8B and F). Pimozide almost totally blocked the stimulant effect of amphetamine on horizontal activity but not rearing (Figs. 8C and G) and conditioning was not observed on the former measure but was seen on the latter (Figs. 8D and H). The pimozide results might suggest that horizontal activity and rearing constitute aspects of general locomotor activity that are differentially influenced by dopaminergic neurotransmission.

CONCLUSIONS

Animals' general level of activity provides a reliable measure of the effects of various drugs whether assessed by subjective ratings or automated photocell apparatus. The latter approach has the advantage of being relatively free of experimenter bias, providing almost continuous monitoring of activity and allowing the simultaneous testing of many animals. One disadvantage is that subtle behavioral changes such as stereotyped head movements may not be detected by automated systems. Activity as a dependent measure is influenced by many variables in addition to various drugs. Some of these include apparatus familiarity and duration of testing as reviewed above.

Computer software can be developed to independently assess components of overall activity. The possiblity that this may provide further insight into drug effects on behavior was suggested by the analysis of upper and lower tier counts in the amphetamine conditioning experiment. Thus, although pimozide blocked the effects of amphetamine on horizontal activity and no conditioning was subsequently observed, pimozide failed to block the unconditioned effects of amphetamine on rearing and the establishment of conditioning occurred. With additional software it will be possible to determine, for example, horizontal movements or rears along the walls of the chamber versus those away from the walls, speed and direction of movements, total duration of immobility, etc. Possibly, these measures will lead to a further refinement of activity data and a better understanding of the components of behavior that are influenced by drugs.

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