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BRIEF COMMUNICATION

Microcomputer Adaptation of the Wheel-Shaped Activity Monitor: Effects of Lindane

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LLORENS, J., C. SUÑOL AND J. M. TUSELL. *Microcomputer adaptation of the wheel-shaped activity monitor: Effects of lindane.* PHARMACOL BIOCHEM BEHAV **35**(4) 1003–1006, 1990.—The development of microcomputers with increased power and memory capacity may allow for the spreading of the techniques of microanalysis of animal behavior in neurotoxicology. The present work describes the adaptation of the wheel-shaped activity monitor to a personal computer system (IBM-PC/XT/AT-compatible). The system has been used to study the effects elicited by a single 30 mg/kg dose of the organochlorine insecticide γ-hexachlorocyclohexane (lindane) on the spontaneous behavior of rats. Lindane induced complex changes in behavior, the most prominent being a disruption of the temporal pattern of activity and changes in the local activity/locomotor activity ratios and in place preferences in the monitor. Effects on body weight and number of fecal boluses were also observed.

Spontaneous behavior Microcomputer Activity monitor (wheel-shaped) Rat Microanalysis of behavior
Lindane Organochlorine insecticides γ-Hexachlorocyclohexane

THE study of the spontaneous activity of laboratory animals has been acknowledged to be a great source of information in experimental neurotoxicology. Since the work of Stata Norton and associates (8), complex maze-like devices where the activity of the animals is monitored by optical gates have been used to assess the behavioral effects of many chemicals (10).

In 1979, Elsner and co-workers (4) described a wheel-shaped activity monitor (first called residential maze) in which infrared optical gates, located in each alley segment of the monitor, were connected to a digital computer. This allowed for a continuous recording of the activity of the rat, and a large amount of varied behavioral information was obtained from each animal. The usefulness of the wheel-shaped activity monitor has been demonstrated by the detection and description of alterations in the patterns of behavior of rats following exposure to several toxicants, drugs and teratogens [e.g., (3, 5, 7)]. Similar computer-based approaches have also been used in other kinds of behavioral studies, such as schedule-controlled behavior (3,11). These kinds of systems lead to the so-called microanalysis of behavior [e.g., (11)], in which the detailed temporal and spatial resolution obtained and the ability to manipulate large arrays of data may reveal patterns of behavior susceptible to neurotoxic alteration.

The spreading of the use of such techniques is limited by the requirement of specific hardware and software. The development of inexpensive microcomputers with increased power and memory capacity makes it possible to adapt these methods to this kind of equipment. The present paper describes an adaptation of Elsner's residential maze using an inexpensive computer, an IBM-PC/XT/AT-compatible with MicroSoft-Disk Operating System (MS-DOS) version 3.1.

This system has been used to study the effects of a low single dose of the organochlorine insecticide γ-hexachlorocyclohexane (lindane). Lindane acts on the mammalian central nervous system as a potent convulsant (12). Increasing evidence links this convulsant action to a blockade of the GABA-gated chloride channel by binding to the picrotoxinin/TBPS site (1, 2, 9). In previous work (6), we studied the effects of several subconvulsant doses of the chemical on the spontaneous activity of rats in 4-hour sessions using the wheel-shaped activity monitor and electronic counters. In the present work, we have studied the effects of lindane in more detail through the use of the computer-based system, and by the extension of the sessions up to 23 hr.

METHOD

Hardware

The design of the wheel-shaped activity monitor is that described by Elsner *et al.* (4). Briefly, six concentric alleys emerge from a central area. They are connected near the peripheral ends by a hexagonal alley. This defines three radial alley com-

partments: blind alleys, circular alley segments and inner spoke alleys. Three of the six blind alleys contain the entry door, a water spout and food access. An infrared optical gate is located in each alley segment (18 in total). Photocells are controlled by a special electronic device (Panlab S.L., Barcelona, Spain). The electronic device provides an on/off output signal for each photocell. This output is presented at specific memory addresses in the computer, through an adapter unit (Pb 40035L, Panlab S.L.) and I/O board (Pb 40036, Panlab S.L.). Photocell status can be read in the I/O port, one bit per optical gate (1: break, 0: free), beginning at address 768. The computer used (Falcon, Barcelona, Spain) has a 8088/2 processor running at 8 MHz, with a 20 Mbyte fixed disk drive, and 640 Kbytes of motherboard random access memory (RAM). An independent clock system switches the lights of the activity monitor on and off to provide the appropriate daily light/dark cycle.

Software

Beside other minor programs (allowing for the setting of defaults, checking of the monitors, and data verification), three main programs are used.

The acquisition program (ADQ) was written using Borland's Turbo Pascal (release 4.0). The program supports up to 4 activity monitors. The acquisition loop continuously checks the I/O buffer for differences with previous values. The sampling rate is about 100 cycles per second. If a change in photocell status is detected, the system time is read and stored together with the photocell information. To increase speed, the data are stored in RAM. Each behavioral event (optical gate break or release) is codified as a 9 byte record containing the change found in the I/O port array (2 bytes), the time of the event (3 bytes) and a pointer to the next record (4 bytes). At the end of the session, a subroutine translates the information from input values to break/release information for a specific optical gate in a specific maze and verifies its logic sequence. It then stores the global data (5 bytes per event) in a disc file and the specific information from each single wheel-shaped activity monitor (4 bytes per behavioral event) in separate files. As other devices may be sharing the same interface system, the global data file can be read by other routines to collect information from these devices. The data files from the activity monitors contain the behavioral events (gate number and break/release information), and the time of occurrence, in a format equivalent to the system timer of the computer.

The two other main programs, MZC and MPF, are adaptations of the original Fortran sources written by J. Elsner and R. Looser for a PDP-11/34 computer. At the end of the experiment, these programs read the data streams stored in the disk files and generate ASCII text files containing the behavioral parameters required. These files are then accessible for commercially available program packages for statistical analysis and graphics. For each rat, and as a function of a preselected period of time (usually 1 hr) and monitor location, the main data matrix contains: local activity (defined as the number of successive breaks and releases of the same gate), locomotor activity (number of gate crossings excluding repetitions of the same gate), time spent in the individual maze locations and transition frequencies between different gates (4). A separate file stores the path iteration frequencies for the whole session as a measure of stereotypy (3). A path is defined as a sequence of successive gate-crossings, after eliminating back and forward movements between two gates. Path iteration frequencies are defined as the number of replications of identical paths divided by the number of match opportunities, and are computed as a function of path length. Another file stores one-minute local and locomotor activity data in the whole monitor for the quantitative analysis of their temporal patterns (6).

Animals and Experimental Procedure

Male Wistar rats (160–200 g) were purchased from Iffa-Credo-Panlab S.L (Barcelona, Spain). Rats were housed in standard conditions for at least 7 days before experimentation. On the day of the experiment, one rat was weighed and administered with lindane (30 mg/kg, Merck) or control vehicle (olive oil) by gavage (PO), 1 ml/kg, 30 min before the test began. The rat was introduced at 10 a.m. into the illuminated maze and left undisturbed for 23 hr. Lights were off from 7 p.m. to 7 a.m. At the end of the session, the rat was removed from the monitor and weighed. The number of fecal boluses was recorded and the plastic sheet covering the floor of the monitor was replaced.

At the end of the experiment, MZC and MPF programs were run to obtain the basic behavioral parameters. Two commercially available program packages, SPSS/PC + and HG, were then used to compute secondary variables (cumulative values, local/locomotor ratios, etc.) and to perform statistical analyses and graphics. Data were analyzed, where appropriate, using Student's t-test or two-way ANOVA (2×23) including repeated measures, with treatment as the grouping factor and time as the within-subjects factor.

RESULTS

One 23-hr session in the wheel-shaped activity monitor generates about 10,000 records per animal, which is equivalent to 90 Kbytes in RAM and 40 Kbytes in the final disk file. The text data files generated by MZC and MPF programs occupy about 400 Kbytes of disk memory in the present experiment, which involved 18 animals.

Figure 1A shows total activity (local + locomotor) of control and treated rats in the whole maze. Control rats display a definite pattern of activity in the monitor. After an hour of high "exploratory" activity, the number of gate breaks decreases and stays low until the light is turned off at 7 p.m. A high level of nocturnal activity is maintained until the lights are turned on again at 7 a.m. This circadian pattern is disrupted in the animals treated with lindane, for which a flatter plot is obtained. Statistical analysis revealed a significant effect of the treatment per time interaction, $F(22,352) = 3.72$, $p<0.001$. Lindane strongly decreased locomotor activity in all areas of the monitor during the nocturnal period, but increased it during the diurnal period. These changes were similar to those illustrated in Fig. 1A for the total activity, and even more pronounced. Statistic results showed significance for the treatment-per-time interaction in blind alleys, $F(22,352) = 4.19$, $p<0.001$, circular alleys, $F(22,352) = 4.90$, $p<0.001$, and spoke alley segments, $F(22,352) = 3.66$, $p<0.001$. Global treatment effects on the locomotor activity were robust in the circular alleys, $F(1,16) = 5.21$, $p<0.05$, nearly significant in the spoke alleys, $F(1,16) = 3.92$, $p = 0.065$, but not seen in the blind alleys, $F(1,16) = 2.80$. In contrast, a global treatment effect on the local activity was only observed in the blind alleys, $F(1,16) = 4.63$, $p<0.05$, for which the treatment-per-time interaction was also significant, $F(22,352) = 2.73$, $p<0.001$. Data plots of local activity in the circular and spoke alleys (not shown) revealed a slight increase during the postexploratory period, and treatment per time interactions reached statistical significance: $F(22,352) = 1.70$, $p<0.05$, for the circular alleys, and $F(22,352) = 1.59$, $p<0.05$, for the spoke alley segments. The strong interaction between the compartment of the monitor and the effects of the lindane treatment on local or locomotor activities were clearly demonstrated by the local/locomotor ratios. Lindane strongly increased

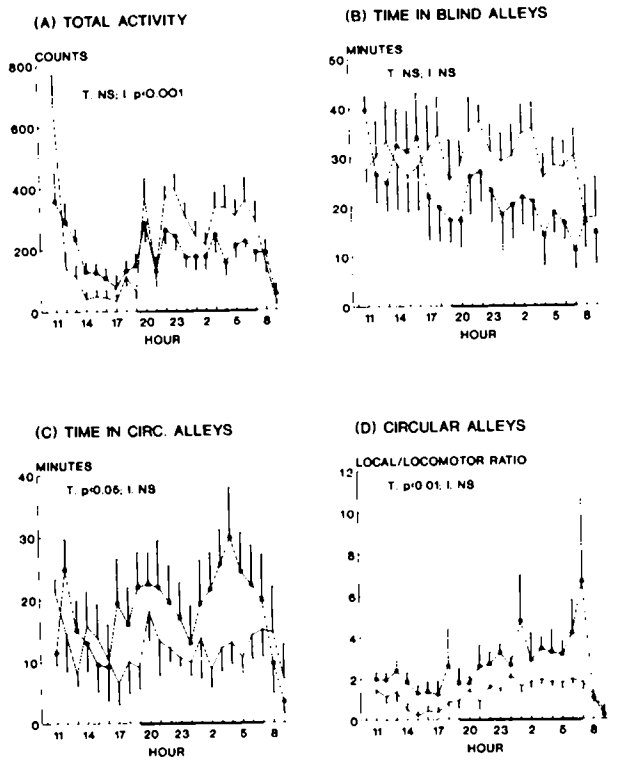

FIG. 1. Effects of 30 mg/kg lindane treatment on some parameters of the spontaneous activity of rats in the wheel-shaped activity monitor. Data are mean ± SEM of 9 animals per group. (■): control rats; (*): lindane-treated. Statistical results are from two-way (2 × 23) ANOVA tests including repeated measures, with time as the within-subjects factor. T: treatment, grouping factor. I: treatment-per-time interaction. Abscissas are hour of the day (from 10–11 a.m. to 8–9 a.m. the day after). (A) Total activity (local + locomotor) in the whole monitor. (B) Mean single values of time spent by the animals in the blind alleys. (C) As B for the circular alley segments. (D) Local/locomotor ratio for the circular alleys.

this ratio in the circular alley segments, $F(1,16) = 10.85$, $p<0.001$, Fig. 1D, and also in the spoke alleys, $F(1,16) = 5.09$, $p<0.05$, but local/locomotor values were not affected in the blind alleys, $F(1,16) = 0.17$.

Another prominent effect following the lindane administration is that of changes in place preference. Lindane treatment increased the time spent by the rats in the circular alley segments, $F(1,16) = 5.15$, $p<0.05$ (Fig. 1C). Although it did not reach statistical significance, $F(1,16) = 2.15$, the decrease of the time spent in the blind alleys (Fig. 1B) was parallel to that effect, whereas time in the spoke alleys was not modified at all, $F(1,16) = 0.04$. Although there seemed to be a slight increase in the path iteration frequencies of path length 3 to 8 as a consequence of lindane treatment, no statistical significance was reached. We also observed a change in the body weight increase [$2.5 \pm 0.7\%$ for control animals against -3.5 ± 1.0 for lindane-treated rats, Student's $t(16) = 4.9$, $p<0.001$] and in the number of fecal boluses [24.1 ± 5.2 and 6.3 ± 2.7 for control and treated animals, respectively, $t(15) = 3.2$, $p<0.01$].

DISCUSSION

The adaptation of the wheel-shaped activity monitor to an IBM-PC-compatible microcomputer allows for the performance of a microanalysis of the spontaneous behavior of rats with a minimum of equipment requirements. Although we have used only one activity monitor, the software developed is ready to record inputs from 4. After loading the ADQ program, the standard 640 Kbytes of RAM can store up to 58,800 9-byte records in the dynamic variable used by the acquisition routine. As control animals generate around 10,000 records in 23-hr sessions, there is enough RAM to store data from 4 activity monitors and can accommodate an increase of almost 50% in animal activity. With the storage of the data in RAM, the acquisition loop written in Turbo Pascal effectively controls byte values in the I/O port at sufficient speed.

The administration of 30 mg/kg of lindane induced several changes in the activity patterns of the rats in the monitor. The activity patterns in the first 4 hours of the experimental session agree with those we reported previously (6), showing that lindane induces an increase in the time spent in activity during the exploratory/postexploratory phases of behavior in the wheel-shaped activity monitor.

The data also showed changes in place preference. One of the main sources for differences between areas in the monitor is that the food access and the water spout are located in two of the blind alleys. This undoubtedly accounts for part of the decrease in the time spent in the blind alleys by the rats treated with lindane. Data showed a 31% and a 34% decrease in the time spent in the alleys containing the food and the water, respectively, and only a 19% reduction in the four remaining blind alleys. Lindane has been reported to reduce food intake (12), and the present results showing a decrease in body weight and number of fecal boluses may be related to this anorexic effect. From the present results we also suggest an effect of lindane on drinking which has not been described previously. Unfortunately, we did not monitor food and water consumption in the monitor. The remaining reduction in the time spent in the blind alleys is likely to be related to the activity/resting structure of the behavior. Rats usually choose the blind alleys for resting and we have previously shown that resting times are shortened during the exploration/postexploration period after lindane treatment (6). The diverse effects of lindane on the local/locomotor ratios as a function of monitor sector may also be linked to the activity/resting patterns. As a whole, the results indicate a specific effect of lindane-reducing locomotor activity (speed) but increasing the time spent in activity.

In conclusion, the system described here is thought to be cost effective, and lends itself to further development. The recording of the activity of the animal in the monitor has been optimized. Data records are kept at minimal length, 4 bytes per behavioral event, and the whole amount of information is not too large for the computer used. Even in laboratories where larger computers are available and data analysis may be faster, microcomputer systems may be used for the acquisition process, releasing the main system from the task of controlling the monitors and data recording. The effects of lindane observed in the present study agree and complement those previously reported. Furthermore, some clues to hitherto unreported putative effects on drinking and place preferences have been obtained.

Readers interested in obtaining more information about the system, and/or in obtaining copies of the programs on floppy diskettes, are welcome to contact the authors.

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REFERENCES

1. Abalis, I. M.; Eldefrawi, M. E.; Eldefrawi, A. T. High affinity stereospecific binding of cyclodiene insecticides and γ-hexachlorocyclohexane to γ-aminobutyric acid receptors of rat brain. Pest. Biochem. Physiol. 24:95–102; 1985.
2. Cole, L. M.; Casida, J. E. Polychlorocycloalkane insecticide-induced convulsions in mice in relation to disruption of the GABA-regulated chloride ionophore. Life Sci. 39:1855–1862; 1986.
3. Elsner, J. Testing strategies in behavioral teratology: III. Microanalysis of behavior. Neurobehav. Toxicol. Teratol. 8:573–584; 1986.
4. Elsner, J.; Looser, R.; Zbinden, G. Quantitative analysis of rat behavior patterns in a residential maze. Neurobehav. Toxicol. 1(Suppl. 1):163–174; 1979.
5. Elsner, J.; Suter, K. E.; Ulbrich, B.; Schreiner, G. Testing strategies in behavioral teratology: IV. Review and general conclusions. Neurobehav. Toxicol. Teratol. 8:585–590; 1986.
6. Llorens, J.; Tusell, J. M.; Suñol, C.; Rodríguez-Farré, E. Effects of lindane on spontaneous behavior of rats analyzed by multivariate statistics. Neurotoxicol. Teratol. 11:145–151; 1989.
7. Looser, R.; Elsner, J.; Zbinden, G. Behavioral effects of cyclazocine on rats assessed in the open field and residential maze. Psychopharmacology (Berlin) 84:323–330; 1984.
8. Norton, S.; Culver, B.; Mullenix, P. Development of nocturnal behavior in albino rats. Behav. Biol. 15:317–331; 1975.
9. Obata, T.; Yamamura, H. I.; Malatynska, E.; Ikeda, M.; Laird, H.; Palmer, C. J.; Casida, J. E. Modulation of γ-aminobutyric acid-stimulated chloride influx by bicycloorthocarboxilates, bicyclophosphorus esters, polychlorocycloalkanes and other cage convulsants. J. Pharmacol. Exp. Ther. 244:802–806; 1988.
10. Reiter, L. W. Chemical exposures and animal activity: utility of the figure-eight maze. In: Hayes, A. W.; Schnell, R. C.; Miya, T. S., eds. Developments in science and practice of toxicology. Amsterdam: Elsevier Sci. Pub. B.V.; 1983:73–84.
11. Weiss, B. Specifying the nonspecific. In: Zbinden, G.; Cuomo, V.; Racagni, G.; Weiss, B., eds. Application of behavioral pharmacology in toxicology. New York: Raven Press; 1983:71–86.
12. Woolley, D.; Zimmer, L.; Dodge, D.; Swanson, K. Effects of lindane-type insecticides in mammals: Unsolved problems. Neurotoxicology 6:165–192; 1985.