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A computerized system for the simultaneous monitoring of place conditioning and locomotor activity in rats

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Abstract

Place conditioning is one of the most popular behavioral methods for assessing the rewarding properties of various substances. Many substances that are rewarding also influence motor activity. This report describes a computerized system designed to simultaneously monitor both place conditioning and locomotor activity. The system consists of 4 independent conditioning boxes, each equipped with 6 pairs of photosensors connected to an Experiment Controller, an electronic board containing a microprocessor, a programable timer, and 16 K of RAM used to store both instructions and data. The effects of the stimulant (+) – amphetamine were assessed using this system and found to produce a place preference comparable to that obtained from a previously utilized mechanical timer system. The computerized system also demonstrated that amphetamine increased unconditioned activity. There are a number of advantages and broader applications of the new methodology.

Keywords: Place conditioning; Conditioned place preference; Locomotor activity; Experiment controller; (+) – Amphetamine

1. Introduction

The place conditioning paradigm has been used widely to demonstrate the rewarding properties of a variety of substances including: food, psychomotor stimulants such as (+) – amphetamine and cocaine, and opiates such as morphine and heroin (refer to Carr et al., 1989; Hoffman, 1989). Recently, caffeine and intra-accumbens neuropeptide Y have been added to this list (Brockwell et al., 1991; Josselyn and Beninger, 1993). The rationale of place conditioning is simple: following several pairings of a drug injection with a distinctive environment, animals in a drug-free state display an increase in the amount of time spent in that environment, compared to an equally distinctive alternate environment. This shift in preference is generally regarded as evidence for the rewarding properties of the drug (Carr et al., 1989). One of the advantages of place conditioning is that subjects are tested in a drug-free state; thus possible unconditioned motor effects of the drug do not affect the dependent measure.

Unfortunately, although many place conditioning studies have provided excellent evidence of reward, none have examined locomotor activity during either the conditioning or test phase. It is extensively documented that drugs such as amphetamine, cocaine, and caffeine produce enhanced locomotor activity (e.g., Costall and Naylor, 1979; Snyder et al., 1981). Some of these compounds have also been found to act as effective unconditioned stimuli in studies employing classical conditioning paradigms to show that stimuli associated with psychomotor stimulants acquire the ability to elicit enhanced activity (e.g., Beninger and Hahn, 1983). A record of activity levels during the conditioning and test sessions of conditioned place preference experiments would, therefore, be valuable in examining the relationship between reward and both unconditioned and conditioned activity.

The purpose of this paper is to describe a computerized monitoring system, designed and built in our laboratory, which has the capability of simultaneously measuring motor activity and place conditioning in rats. This will be followed by a comparison of the experimental results obtained using this system to those previously obtained using a mechanical timer system. The advantages and broader applications of the new methodology will also be outlined.

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2. The system

Place conditioning and activity are monitored in four similar rectangular boxes (84 × 27 × 36 cm high) constructed of wooden sides and removable Plexiglas covers. Each box consists of two chambers joined by a small tunnel (8 × 8 × 6 cm high) which can be blocked by the insertion of two Plexiglas guillotine doors. The chambers differ in wall pattern and floor design. In two of the conditioning boxes, one chamber has brown walls and a wire mesh floor (1 × 1 cm), while the other chamber has black and white vertically striped walls (stripes are 1 cm wide) and a floor consisting of wire rods spaced 1 cm apart. In the other two chambers the floor and wall pairings are reversed. Each box is housed in an outer plywood shell which is insulated with sound attenuating Styrofoam, illuminated by a 7.5 W light, and ventilated with a small fan.

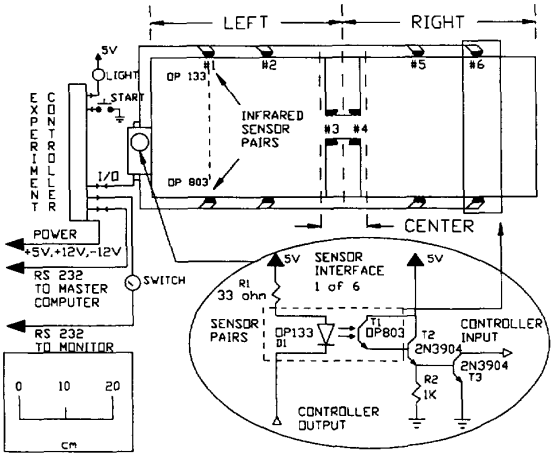

Fig. 1. A schematic diagram depicting the organization of the system hardware. Six infrared photosensors each consisting of 1 infrared diode (D1) and 1 photo-transistor (T1) are mounted in holes drilled through the sides of the the conditioning box. The infrared diodes are biased with a single resistor (R1). Each photo-transistor is connected to 2 other transistors, T2 and T3. T1 and T2 act as a Darlington pair that amplifies the current available at the base of T3. When the infrared light is blocked, a current transition at T3 is registered by the EC Board which records that a sensor interruption has occurred. The start button is used to initiate experimental sessions, and a light, mounted next to the start button, remains illuminated while the EC Board is actively collecting data. System hardware is powered by a three output power supply. The EC Board and photosensors utilize a 5 V supply, while the circuitry used to communicate with the Macintosh Plus uses + 12 and 12 V supplies. Each EC Board is connected via a serial RS232 connection to the Macintosh's serial port. The second serial port on each EC Board is used to send real-time data to a standard ASCII monitor. All 4 EC Boards are connected through a manual select switch to a single monitor. The hardware setup is modular in nature and all major components are interconnected with quick release connectors. The interface housing is simply an alluminum box with DB25 connectors. All electronic components are readlily available from any electronics supplier. The supplier used by this laboratory was Electrosonic (Toronto, Ontario, Canada) and part numbers for components are included in the diagram. The EC Boards were built in our labortory based on information provided in Walter and Palya (1984); however, a new generation of more powerful EC Boards is now commercially available from W.L. Palya and D.E. Walter, Department of Psychology, Jacksonville, AL 36265, USA.

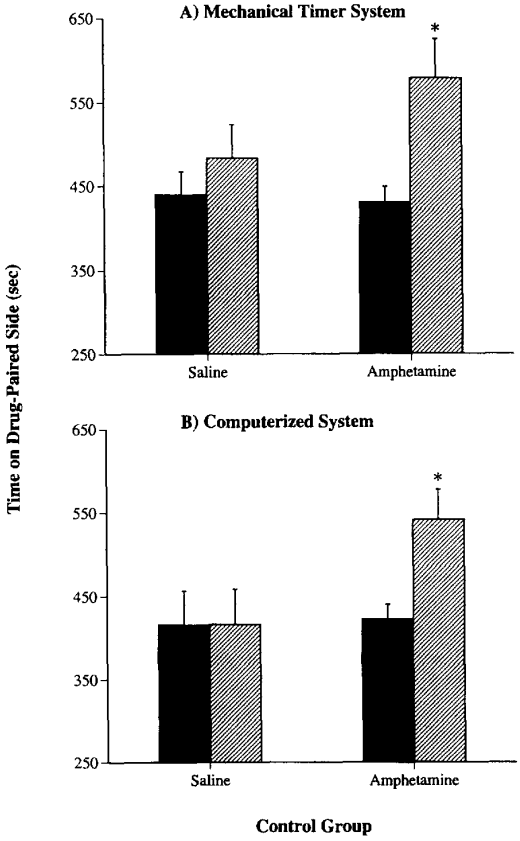

Fig. 2. Comparison of place conditioning using the mechanical timer (A) and computerized (B) systems. Average (± SEM) amount of time spent on the drugpaired side of the conditioning box during the preconditioning (black bar) and test (striped bar) phases is shown. The data have been averaged across the 3 preconditioning sessions. * $P < 0.05$, differs significantly from the preconditioning phase.

Organization of the system is depicted in Fig. 1. Each box is equipped with 6 pairs of infrared photosensors: 2 located 5 cm above the floor of each chamber and 2 located 3 cm above the floor of the tunnel, and a signal amplifier circuit. Each set of photosensors is connected to an Experiment Controller (refer to Walter and Palya, 1984) also called an EC Board, that samples the photosensors repeatedly to monitor for sensor interruptions. The EC Board consists of a 6809 microprocessor, 16 K of RAM, 2 serial ports, a programable timer, 40 open collector outputs and 8 digital input lines.

The 4 Experiment Controllers are connected as a network to a Macintosh Plus computer which serves as the master. Communication setup and method is based on Weisman and Palya (1988) and uses Red Ryder 9.3 (Freesoft Co.), a powerful, user-friendly communication pro-

gram that is shareware available on many electronic bulletin boards. Red Ryder was chosen because it has the ability to implement a variety of procedures with a simple macro command, which can be initiated by simply clicking a mouse on graphically displayed 'virtual buttons' on a macro status bar at the top of the computer screen. Although it would be possible to run a fairly small experiment directly from the Macintosh, the use of EC Boards allows many experiments to be run simultaneously. In addition, the use of the EC Boards frees the Macintosh for a variety of other tasks during much of the day including program development, data analysis, and word processing.

All software was written on a Macintosh Plus in ECBASIC utilizing Notepad + (Borland International), a simple fullscreen text editor. ECBASIC (Experiment Controller Basic) is a modified version of standard BASIC and can be easily understood by anyone with programming experience. The present program is designed to allow the user to specify the number of experimental sessions and their duration. Additional information such as the box number and animal identification number are also incorporated into the program. A flowchart outlining the software development is displayed in Fig. 5.

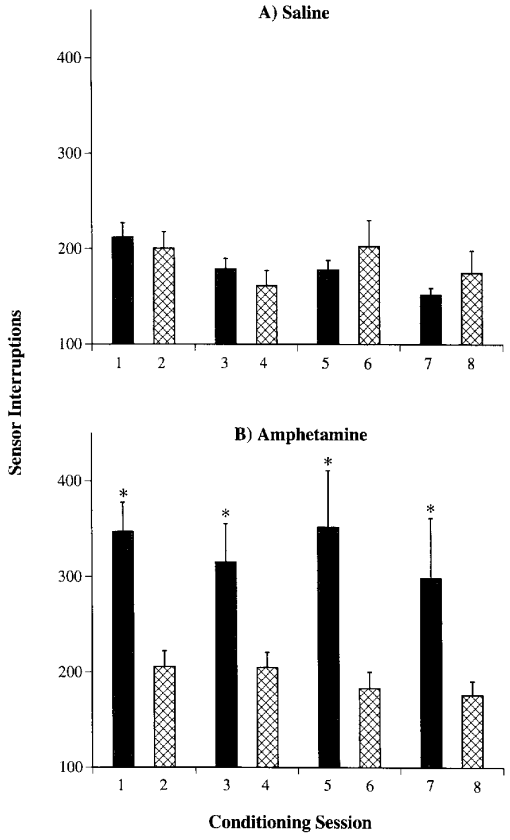

Fig. 3. Average (±SEM) number of sensor interruptions during conditioning sessions for saline and amphetamine groups. Gray bars indicate sessions where either saline (A) or 2.0 mg/kg i.p. (+)−amphetamine (B) was injected and the rats were confined to one side of the conditioning box. Crosshatched bars indicate sessions where both groups received saline and placement in the alternate side. * $P < 0.05$, mean of drug treatment differs significantly from the mean of saline treatment.

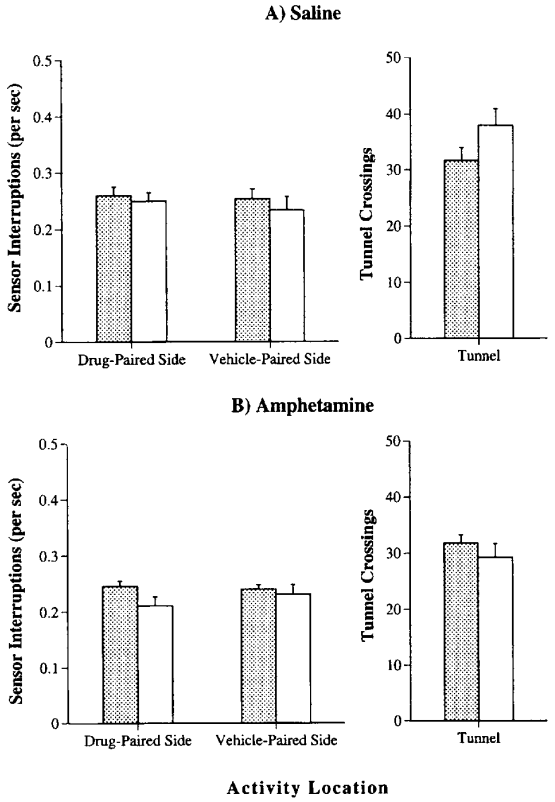

Fig. 4. Average (±SEM) number of sensor interruptions (per second) and tunnel crossings during the preconditioning (stippled bar) and test (white bar) phases for saline (A) and amphetamine (B) groups. All animals were in a drug-free state and had access to all areas of the conditioning box during these phases of the experiment.

After the program has been downloaded to the EC Boards, they can run independently of the master. A single microswitch, connected to the input of the EC Board, can be used to start experimental sessions. Alternatively, sessions can be controlled from the master computer. Data are collected and stored on each EC Board until the master computer requests a data upload. Data files are then stored in the master computer as a text file but may be converted easily to hardcopy or transferred to a spreadsheet, statistical, or graphics program. Each EC Board possesses a second serial port that is used to monitor the progress of experimental sessions. A manual switch is used to select any of the EC Boards which in turn send realtime data to a standard ASCII terminal. The terminal displays the ongoing number of sensor interruptions and cummulative time in each area of the conditioning boxes.

For each conditioning box the software monitors the position of a rat in the following manner. Photosensors 1, 2 and 3 signify the left chamber while photosensors 4, 5, and 6 signify the right chamber. Simultaneous interruption of photosensors 3 and 4 signifies the tunnel. While monitoring is in progress all of the photosensors are always enabled, and the software continually scans for sensor interruptions. A full scan of all photosensors takes approximately 100 ms. The system is analogous to three stopwatches. Interruption of photosensor 1, 2 or 3 activates the left chamber timer and simultaneously stops all other timers. Simultaneous blockade of photosensors 3 and 4 activates the tunnel timer and stops all other timers, while interruption of photosensor 4, 5 or 6 serves to activate the right chamber timer and stops all other timers. Thus, each EC Board maintains a continual cumulative record of the time (s) spent within each area of the conditioning box.

Activity levels in each chamber are measured using the same set of photosensors. Thus, the cumulative number of interruptions of sensors 1 and 2 would indicate the level of activity in the left chamber, whereas cumulative interruptions of sensors 5 and 6 would indicate the level of activity in the right chamber. In addition, the number of tunnel crossings, indicated by the cumulative number of simultaneous interruptions of sensors 3 and 4, is recorded as a separate entry in the data record. A sample data printout is presented in Fig. 6.

3. Place conditioning: comparison of mechanical timer and computerized systems

The previous method for evaluating place conditioning in this laboratory used identical conditioning boxes but utilized a mechanical device for monitoring the time spent in each chamber. The floor of each box was positioned on a fulcrum such that the weight of a rat in either chamber would close a microswitch and activate a timer in an adjoining room. This system was used in a number of published reports from this laboratory (Hoffman et al., 1988; Hoffman and Beninger, 1988, 1989; Brockwell et al., 1991).

The study of Brockwell et al. (1991) included data showing the ability of (+) − amphetamine to produce place conditioning. During three 15-min preconditioning sessions, rats were given access to the entire box. During the 8 session conditioning phase, the experimental group was injected i.p. with 2.0 mg/kg (+) − amphetamine ($n = 9$) and the control group with 0.9% saline ($n = 10$). Immediately following injections, rats were confined to

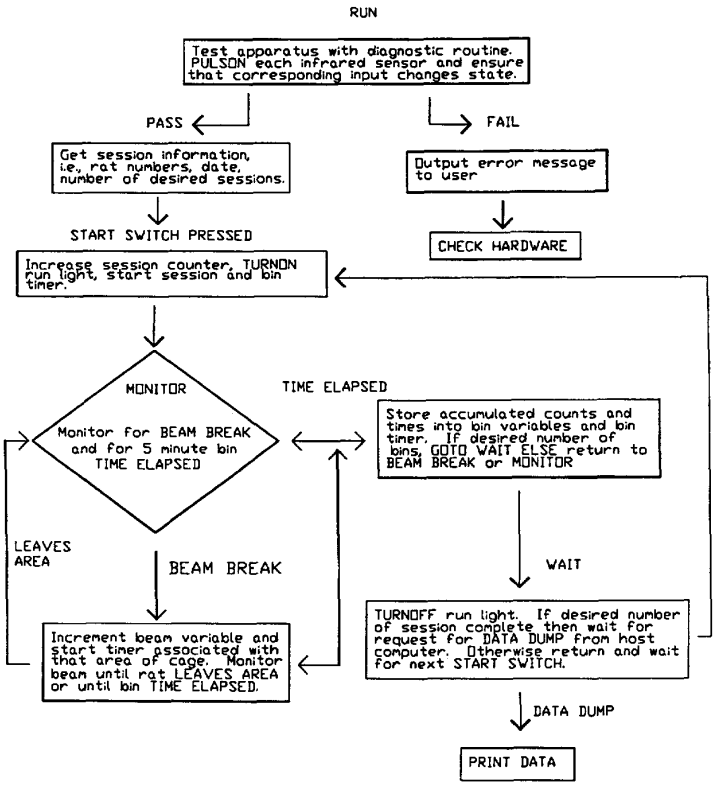

Fig. 5. A flowchart outlining the process used to develop the system software. Examples of ECBASIC code can be found in Walter and Palya (1984) and Weisman and Palya (1988).

one of the chambers for 30 min. On alternate sessions, both groups were administered saline and confined to the opposite chamber. During the single 15-min test session, animals in a drug-free state were again allowed access to the entire conditioning box.

Fig. 2A presents the average (±SEM) amount of time spent on the drug-paired side of the conditioning box during the preconditioning and test phases for both groups. One-way analyses of variance (ANOVAs), with phase (preconditioning vs. test) as a repeated measure, revealed that amphetamine produced a significant place preference ($F_{1,8} = 10.64$, $P < 0.05$) whereas saline administered exclusively failed to induce conditioning.

To evaluate the comparability of the two systems, the experiment was repeated with 2 new groups ($n = 8$) of rats using an identical experimental procedure but this time using the computerized system. Fig. 2B presents the average (±SEM) amount of time spent on the drug-paired side of the conditioning box during the preconditioning and test phases. ANOVAs again indicated that rats given amphetamine on one side showed a significant preference for that side ($F_{1,7} = 5.60$, $P < 0.05$), whereas saline failed to induce conditioning. Statistical analyses were then conducted to compare the place conditioning produced in the two systems. For the amphetamine groups, a 2-way ANOVA, with system (mechanical vs. computerized) as a between factor and phase as a repeated measure, revealed a main effect of phase ($F_{1,15} = 15.62$, $P < 0.01$), but failed to reveal either a main effect of system or system by phase interaction. Thus, the place preference produced by amphetamine in the mechanical timer system was replicated by the group that received amphetamine in the computerized system. A 2-way ANOVA, conducted for the 2 saline groups, failed to reveal any significant effects indicating that the results produced by the 2 methods were not significantly different.

4. Computerized system: measurement of unconditioned and conditioned activity

The computerized system has a distinct advantage in that it simultaneously monitors both the location and activity levels of each subject. For example, activity levels were monitored throughout all phases of the experiment for the 2 groups whose data are presented in Fig. 2B. Fig. 3 presents the average (±SEM) number of sensor interruptions for these groups during each of the 8 conditioning sessions. For the group receiving amphetamine, enhanced locomotor activity was clearly seen on alternate days following drug administration, whereas no such effect was apparent for the group that received saline every day. The results of ANOVAs indicated that amphetamine increased unconditioned activity ($F_{1,7} = 13.57$, $P < 0.01$), whereas saline failed to significantly alter motor activity.

Previously, studies have demonstrated that (+)-amphetamine has the ability to produce conditioned activity. Animals which have repeatedly received amphetamine in a specific environment subsequently display enhanced locomotor activity when placed in that environment in a drug-free state (e.g., Beninger and Hahn, 1983). In the present series of studies, 3 activity measures were available from preconditioning and test sessions; these included the number of sensor interruptions per second in each side of the conditioning box and the number of tunnel crossings. The mean (±SEM) of each of these measures for the 3 preconditioning sessions and the value for the test session are shown in Fig. 4. In the present study no significant conditioned activity was found. This failure to find conditioned activity may be the result of the constraints of the experimental paradigm. The number (4) of conditioning sessions with the drug may not have been sufficient to induce conditioning. Previous evidence suggests that conditioning becomes stronger with repeated pairings (Beninger and Hahn, 1983). In addition, during the test session animals had access to 3 distinct environments, only one of which was associated with the drug.

March 3, 1994 Preconditioning Session 1 Saline group

PLACE PREFERENCE DATA FOR BOX NUMBER 2

SESSION #2
RAT NUMBER 6

SENSOR COUNT	1	2	3	4	5	6	MIDDLE
5 MINUTES	18	25	21	12	35	19	17
10 MINUTES	35	66	32	24	59	38	28
15 MINUTES	56	90	41	33	77	52	38

TIME SPENT IN LEFT, CENTRE AND RIGHT OF BOX

	LEFT	CENTER	RIGHT
5 MINUTES	145	18	136
10 MINUTES	292	37	269
15 MINUTES	431	57	408

Fig. 6. A sample data printout for 1 session in a single conditioning box. Information at the top of the printout describes the experiment in progress. The information below this lists the EC board and rat identification number and indicates that this is the second session of the day. The middle portion of the printout is a record of cummulative activity counts at each of the 6 photosensors. The term MIDDLE refers to simultaneous interruption of photosensors 3 and 4 and is a record of tunnel crossing. The lower portion of the printout is a cummulative record of the amount of time (sec) spent in the left and right chambers of the conditioning box and the tunnel (center). At the conclusion of each 5-min interval the recording of data by the Experiment Controller results in an inactivation of monitoring for ≈ 1 s. However, the loss of 3–4 s over a 15 min session has no significant effect on experimental outcome.

5. Conclusion

The results of the present series of studies suggest that the Experiment Controllers provide a reliable, cost-effec-

tive method for measuring unconditioned locomotor activity and place conditioning in rats. Preliminary studies suggest that with modifications to the experimental design, the system may also be capable of detecting conditioned activity.

The computerized system was effective in demonstrating that a drug which produced enhanced activity during the conditioning phase, subsequently induced place conditioning. It is not surprising that the system demonstrated a relationship between the rewarding and motor activating properties of (+) − amphetamine. However, experiments conducted more recently in this laboratory using the computerized system have demonstrated that it is possible to produce a dissociation of the rewarding and motor activating properties of some substances. We have examined several combinations of adenosinergic agents and have found one combination that produces both a significant decrease in unconditioned activity and a significant place preference (Brockwell and Beninger, 1996). Therefore, this new technology may provide valuable insight into the relationship between the unconditioned motor effects of various pharmacological agents and their ability to produce place conditioning.

Acknowledgements

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