



A Methodological Improvement and System Validation to Obtain Precise Behavioral Parameters for Schedule-Induced Polydipsia

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Abstract

A Schedule-Induced Polydipsia (SIP) animal behaviors monitor system was devised. The system included a software package to acquire data and to format the data storage of the animal behavior in the experiment was designed. Data analysis software will also extract the necessary information from recorded data. The SIP experimental apparatus consists of a number of subsystems. They are operant chambers, event counters, signal acquisition and storage system. The operant chamber is equipped with hopper, pellet and lick sensor and locomotion detector. The data acquisition and storage subsystem are custom designed that run on IBM-PC. The discrete time markers for pellet drop, bar pressing and licking water were recorded at 200 Hz. The image of rat locomotion was recorded at 10Hz. A test of four different stages of animal to verify the accuracy of the system was reported in this article. The food-deprived rats that were exposed to the intermittent food schedule (1 per minute) exhibit an excess drinking behavior. The animal behaviors that were monitored during the scheduled events are adjunctive, facultative and terminal behavior. The volume of water intake was also recorded for reference. The real time data is stored chronologically into two types of data file. Therefore, a special data analysis procedure is designed to extract the results for off-line statistic analysis. The extracted parameters for animal behaviors analyzing including drinking efficiency, inter-lick intervals, number of drinking bursts, size of burst, temporal distribution of licking, temporal distribution of bar pressing and locomotion. The system can be employed in many different SIP studies such as investigating the effect of acute and chronic influence of amphetamine.

Key Words: schedule-induced polydipsia, locomotion, frequency modulation, voltage control oscillation, image processing, parallel interface

Introduction

Schedule-induced polydipsia (SIP) is a well-developed paradigm in studying animal behavior (1-12). A system that will acquire and analyze animal behavior using SIP paradigm is needed (10, 13, 14, 16). The phenomena of schedule-induced animal behavior can be separated into two aspects, the emotional factor and motor factor. The observation of schedule-induced polydipsia shows that it is not

caused by homeostatic imbalance (15). The emotional aspect is related to the anticipatory anxiety and motivated state eager for food. The motor aspect is the body movement that uses to cope with the intermittent delivery of food. The animal behaviors that are monitored during the scheduled events are adjunctive, facultative and terminal behavior. In rats, animal behavior is corresponding to drinking, locomotion and bar pressing. The adjunctive behavior is the drinking or licking after eating food. The

terminal behavior is the bar pressing that anticipates the food delivery. The facultative behavior is the animal behavior between licking and bar pressing. The behavior being monitored is locomotion. The SIP paradigm is specially focusing on evaluating the change of animal behavior. For example, it is important to monitor the change of animal behavior in the effect of pharmacology intervention. It may also be used to monitor animal behavior and compare result.

The paradigm of SIP has generated considerable interest in theoretical and applied study. The investigation that using SIP paradigm must accommodate the actual setting that running the experiment as well as the collecting and analyzing the data. For example, the SIP was used to characterized behavior for monitoring the heightened arousal in a morphine dependence and withdrawal reaction (6). The data to be examined is the pattern of competition in the exhibited animal behavior of the scheduled events. For example, the dramatically increase of the adjunctive behavior will proscribe some of facultative behavior or terminal behavior. The increase of facultative behavior will relegate the importance of adjunctive and terminal behavior. This means that the increase of locomotion will suppress the activity of licking and bar pressing. Hence, the importance of experimental outcome may be contributed from the analyzed result of locomotion. For the purpose of monitoring the animal behavior and examining the outcome of treatment, three types of animal behavior are to be analyzed. The primary stage of data analysis is to tally the licking, bar pressing with the synchronization of pallet drop. For the adjunctive animal behavior, first, the number of licking within a bin, i.e., one second, five seconds or any duration, is needed to monitor the effect of the treatment. One can analyze the efficiency of drink, the pattern of drinking and frequency of licking per second. These data can be used to interpret the effects of treatment. For the observation of terminal behavior, one can record and analyze the data from bar pressing. This data reveals the anxiety and anticipation of animal in the scheduled events. For example, the data can be used to illustrate consistence of the animal. For the observance of locomotion, the data can show and reveal the states of treatment (17-20).

A number of automatic measuring and recording systems have been reported (17-20). There was a system that specifically targeted for recording event for a long period (24 hrs) (18). This system used PC to acquired licking and locomotion data at 100 Hz. The locomotion was registered using photocell technology. However, this device only recorded location of rat while licking. The path of rat movement was not recorded. The collected data was transferred

to VAX via 1200-baud serial line for data processing, analysis and storage.

To record full course of locomotion is valuable in studying animal behavior. Rat spontaneous spatial behavior is temporary. However, the spatial movement of SIP trained rat is highly predictable. Therefore, the recording of locomotion is important in studying facultative behavior. For this purpose, video-recording device is frequently used (17). The video information can only be qualitative analyzed by the researcher. To automatic tracking rat movement quantitatively using video information requires intensive computation. It is not time efficiency. A lesser data redundant recording system that will meet the constrain of accuracy and efficiency of computation is needed. There was a system that used touch panel technology as spatial grid to register the location of rat (20). However, using touch panel technology can only record a point at a time. The spatial orientation of the rat can not be registered. This article reports a special devised system that records the completed course of SIP animal behaviors during experimental runs in digital format. The data recorded are time markers of licking, pallet drop, bar pressing and images of locomotion. The system will also help researcher to extract the necessary information from recorded data.

The paradigm of SIP has generated considerable interest in theoretical and applied study. The investigation that using SIP paradigm must accommodate the actual setting as well as the collection and analysis of the data. A test of four different stages of animal to verify the accuracy of the system was reported. The food-deprived rats that were exposed to the intermittent food schedule (one per minute) exhibit an excess drinking behavior. The animal behaviors that were monitored during the scheduled events are adjunctive, facultative and terminal behavior. The volume of water intake was also recorded for reference. The real time data is stored chronologically into two types of data files. Therefore, a special data analysis procedure is designed to extract the results for the off-line statistic analysis. The extracted parameters for analyzing animal behaviors are drinking efficiency, inter-lick intervals, number of drinking bursts, size of burst, temporal distribution of licking, temporal distribution of bar pressing and locomotion. The system can be employed in many SIP studies such as investigating the effect of acute and chronic influence of amphetamine.

Materials and Methods

The experimental apparatus was integrated from costume-designed hardware and software. The

developed software includes data acquisition, data analysis and data presentation. The software was written in C++ using Borland C Builder software package. It operates under Windows 95 environment. The hardware was custom designed and integrated with existing installation. The software and hardware circuit diagram can be made available upon requested.

The experimental apparatus consists of a number of subsystems. There are four operants' chambers in this design. Every experimental chamber consists sensors of hopper, pellet drop and licking. The sensors are connected to a set of 12 event counters. One set of custom-made LED motion detector device is facilitated to the operant. A dedicated data acquisition procedure and data storage system is programmed to run the system.

The size of operant chamber by Coulbourn Instruments is 25 cm by 28 cm by 30 cm. The sides are made of 0.2 cm thick of clear Plexiglas. The grid floor is constructed with 0.6 cm diameter stainless-steel rods spaced 1.5 cm apart. A 1 cm by 3 cm food hopper locates above the grid floor and at the center of the front wall. A food magazine locates at the center of the front wall that is connected to a pellet dispenser that will deliver 45 mg of standard formula Noyes precision food pellets. Water is freely available from the drinking tube that is connected to a bottle of water. Infrared light detectors are used to sense the event of hopper being pressed and drinking tube being licked.

The infrared LED transmissions devices for registering the locomotion are placed at the ceiling. The sensors for the locomotion are placed beneath and under grid floor. The data acquisition and data storage are controlled by a PC microcomputer that

uses custom design software. The configuration of instrumental design and signal flow are described below and shown in Figure 1.

The sensor systems consist of locomotion, licking, bar pressing and pallet drop as shown in a box enclosing by dash line. The infrared receiving devices are composed of an array of infrared photocells that sense the frequency-modulated infrared. If the infrared is blocked or the detected frequency from the photo cell is not match to the specified frequency, the signal from the decoder unit will be set to "OFF". Otherwise, the signal from the decoder is normally "ON". The array of 12 by 15 sensed infrared signal is ready to feed into computer. The data acquisition system consists of a parallel interface, multiplex/control unit and a computer.

The PC equips with a 24-bits parallel interface adapter and three programmable timers. A custom designed data acquisition software controls the devised multiplex to read the 12 bits of data at 200 Hz. For each box, the data are bar pressing, licking of water tube and the pallet drop. The program scans the locomotion device for each operant at 10 Hz. This means that the locomotion of each operant is recorded 10 image frames per-second. The incidences of events that are first picked up by the detector will, then, be read into PC via parallel interface adapter and recorded into two types of file chronologically. One is to record the SIP parameters and the other is to record the locomotion (one file per operant).

The data acquisition (sampling) routine is diagrammed in the Figure 2. For each data sampling, the PC tests the programmable timer to determine whether the timer marker of one millisecond has occurred. The SIP parameters are sampled every 5 counts. The locomotion at each row of operant is

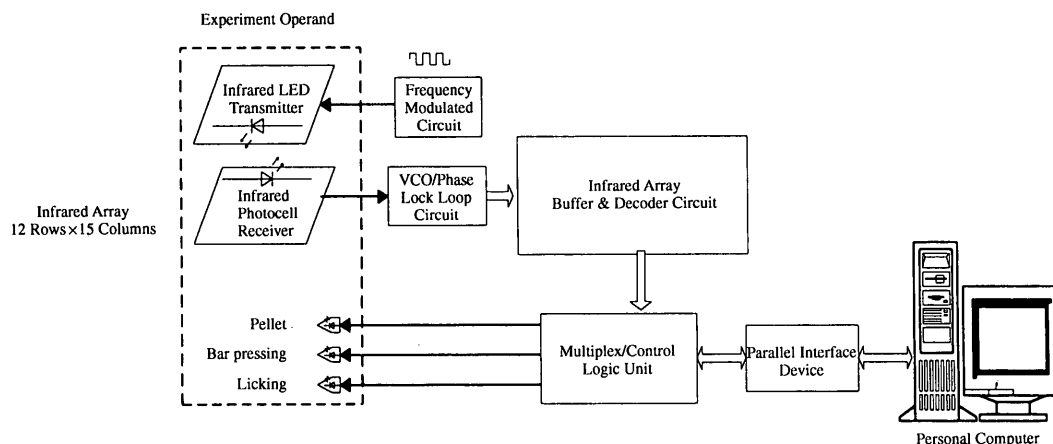


Fig. 1. System Block Diagram

In this figure, one operant chamber of data acquisition system is illustrated. The experimental apparatus consists of four operant chambers. Each operant chamber has sensor that will register bar pressing, pellet drop and licking. One set of custom-made infrared LED motion detector devices is equipped for each operant chamber. A dedicated data acquisition procedure is programmed to record and store the data.

The Result Report..

File name : 5-21.sip
 Date : 5/18/1994
 Time : 12:42:22
 Sampling Time (hr:min) : 0:15
 Total Pellet Number : 14
 Total Bar Press Number : 674
 Total Lick Number : 2232
 INTER_PELLET INTERVAL (sec) : 60
 Observation from PRE_PELLET (sec): 0
 to POST_PELLET (sec) : 60
 Duration (sec): 5

The '*' marks the time for pellet

Box No.1

<<<Pressing Hopper>>>

Time\Bin	1	2	3	4	5	6	7	8	9	10	11	12
Food Dispense	*											
P 1 12H42M48S	2	4	0	1	0	2	0	0	0	1	1	6
P 2 12H43M49S	1	1	1	5	12	8	8	11	12	7	13	9
P 3 12H45M51S	2	0	0	0	0	0	0	2	1	10	13	12
P 4 12H46M52S	4	0	0	0	0	1	0	0	6	12	15	16
P 5 12H47M53S	2	0	0	0	2	0	1	0	5	13	13	13
P 6 12H48M54S	3	0	0	0	0	0	0	1	0	4	11	13
P 7 12H49M55S	3	0	0	0	0	2	3	0	6	11	14	15
P 8 12H50M56S	1	0	0	0	0	0	0	0	0	0	0	3
P 9 12H51M57S	3	0	0	0	0	3	4	10	4	12	13	13
P10 12H52M58S	1	0	0	0	3	1	2	13	10	13	16	13
P11 12H53M59S	1	0	0	0	2	0	0	0	2	8	10	11
P12 12H54M59S	2	0	0	0	2	2	1	6	13	14	15	12
P13 12H56M 0S	3	0	0	0	1	0	0	0	2	11	13	12
Mean	2.2	0.4	0.1	0.5	1.7	1.5	1.5	3.3	4.7	8.9	11.3	11.4
Stdev	1	1.1	0.3	1.4	3.3	2.2	2.4	4.9	4.6	4.6	5.1	3.5
Rate	0.5	0.1	0	0.1	0.4	0.3	0.3	0.7	1	1.9	2.5	2.5

<<<Licking>>>

Time\Bin	1	2	3	4	5	6	7	8	9	10	11	12
Food Dispense	*											
P 1 12H42M48S	0	0	0	0	0	0	0	0	0	0	0	0
P 2 12H43M49S	0	0	0	0	0	0	0	0	0	0	0	0
P 3 12H45M51S	9	51	55	54	51	62	29	0	0	0	0	0
P 4 12H46M52S	0	51	49	64	0	0	0	0	0	0	0	0
P 5 12H47M53S	10	52	50	43	0	0	0	0	0	0	0	0
P 6 12H48M54S	0	41	40	30	0	0	0	0	0	0	0	0
P 7 12H49M55S	1	28	45	39	27	0	0	0	0	0	0	0
P 8 12H50M56S	0	0	61	49	42	43	41	15	63	28	20	34
P 9 12H51M57S	0	14	54	37	29	4	0	0	0	0	0	0
P10 12H52M58S	14	51	33	9	0	0	0	0	0	0	0	0
P11 12H53M59S	0	36	40	37	7	0	0	0	0	0	0	0
P12 12H54M59S	2	42	50	32	0	0	0	0	0	0	0	0
P13 12H56M 0S	4	45	43	31	9	0	0	0	0	0	0	0
Mean	3.1	31.6	40	32.7	12.7	8.4	5.4	1.2	4.8	2.2	1.5	2.6
Stdev	4.8	20.9	19.2	19.6	18.2	20	13.4	4.2	17.5	7.8	5.5	9.4
Rate	0.7	6.8	8.7	7.1	2.8	1.8	1.2	0.2	1.1	0.5	0.3	0.6

Fig. 2. The display of text format of extracted SIP data. The result of data extraction is in three parts. The first part is the statistics of event and the specified information. The second part is the tally of counts for pressing the hopper within the duration (5 sec). The third part is the tally of count for licking the water tube within the duration (5 sec).

sampled every 2 counts. The parameters and locomotion of SIP events are recorded throughout the test session synchronously and continuously. A comprehensive off-line data analysis procedure has been programmed to extract the data for statistic analysis. The data extraction process includes some image processing routine for imaging the infrared locomotion detection. The routines are functions of dilation, erosion, opening, closing and morphological smoothing. The dilation and erosion are to filter out and combine the shape. The opening and closing are to clear up the shape of rat. And, the morphological smoothing is to take out the rough edges.

To verify the system, four stages of animal condition are tested. Male Sprague-Dawley rats weighted 300(50g for either at the condition of untrained, training, trained SIP rat or chronic amphetamine (1mg/kg) administrated to the SIP trained rat. These tests are to demonstrate the accuracy and sensitive of the system.

Male Sprague-Dawley rats are first fed in boxes on 12 hours' day and night cycle for one week before starting SIP training. The weight, food consumption and water intake were recorded. In training SIP rat, rats are limited to food consumption for one week until its weight reaches 85% of initial weight. For the rats met the criteria will be put in Skinner operant box to learn one pallet per minute scheduled event. The water is freely intake. The time span for the training courses is approximately 9 to 14 days. The well-trained SIP rat will exhibit licking water tube more than 1000 times in 30 minutes and the water intake is more than 10 ml.

The system is planned to employ in many pharmacological intervention studies such as investigating the acute and chronic effect of amphetamine. In this article, a test to verify the affectedness of SIP monitor system will be reported. A rat with chronic effect of amphetamine was prepared for the test. That is to select a SIP trained rat and prepare it for amphetamine studies. The rat is exposed to amphetamine for 5 days with (1mg/kg) administrated. Then, the amphetamine is stopped for three days. Before putting rats into Skinner operant boxes for SIP monitor, one shot of amphetamine, 1mg/kg, was administrated to the rat. The result of the validation is follows.

Results

The validation for the system starts at the rat training in Skinner operant boxes. The events of bar press, pallet drop, licking and locomotion are continuously recorded. A special data analysis procedure is designed to extract the results for the

off-line statistic analysis. The procedure will extract the result by examining the complete data files and by the requested operant chamber. Therefore, the user must supply the information of operant chamber, screening interval and duration. The time marker of the pallet drop was first used to align the scheduled event. The period of the screening interval is the sum of pre and post-pellet interval. The duration is the period that counts will be summed. The procedure will use the supplied information to mark the pellet drop and will tally the count of hopper pressed and the lick of water tube for a screening inter-pellet interval.

The report is stored in an ASCII data file. As shown in Figure 2, the results can be organized in three sections. First, the header section, the description contains the list of the specified information and the statistic of the examining chamber. The second and third sections are the tally of events during one seconds' bin. The data are histograms of the bar pressed and the licking occurrences. The first row of the table represents the tally interval (time/bin). The first column represents the scheduled events. The numbers in the column are the recorded occurrences. The mean in the last third row is the average of occurred event for 13 scheduled tests. The data shows (licking data, bottom panel) that, after food dispensed (bin 1), the SIP trained rats exhibit a high occurrence adjunctive behavior of licking (bin 2 to bin 5). For the terminal behavior toward the end of schedule, the rats exhibit a high occurrence of bar pressing (bin 10 to bin 12).

The progress of data analysis and the coherence graphical representation of one minute of events can be observed from the monitor as shown in Figure 3. The coherence graphical display includes the history recording of SIP behavior, the temporal position of

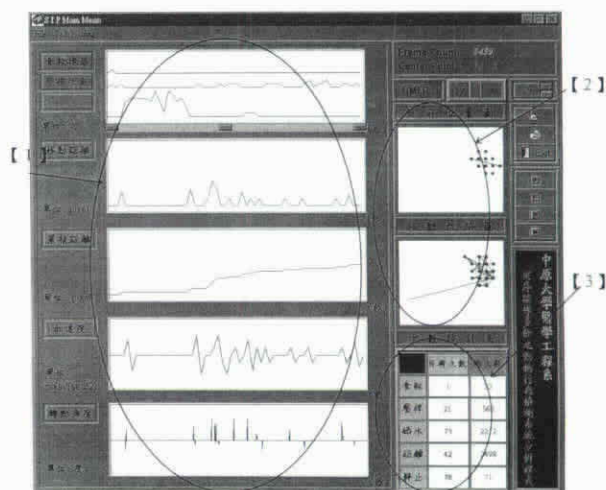


Fig. 3. The screen display of SIP analysis and monitor.

rat, and the text representation of tallied SIP events. In the history recording of SIP behavior, there are five panels (Figure 3-1). The first panel is the graphical display of SIP events. In this panel, there are three lines representing the pallet drop marker, the bar pressing, and the number of licking. Panel two to five is the time recording of four different measurements of locomotion. There are the coherence moving distance per frame (velocity), the accumulated moving distant per scheduled events, the acceleration per frame, and the turning angle. The second part of the display is the temporal position of rat at that scheduled instant which includes two panels (Figure 3-2). The top panel shows the position of the rat. On the other hand, the lower panel is the accumulated moving path. Parameters such as numbers of pallet drop, bas press, and licking, moving distance and the number of frame count of no movement are displayed in the text representation of tallied SIP events. As shown in the figure, the current scheduled event has 78% of frame time and 71% of the previously accumulated scheduled events frame time that the rat is not moving. In the current system, one frame time is 96 milliseconds. The history of scheduled incidence within the recording period can be replayed and reconstructed with the real time clock. From the results of these post analyses, the reported SIP behavior monitor is capable of extracting the SIP events. As shown in Figure 3, the SIP trained rat exhibited the adjunction licking event, the locomotion and terminal event of bar pressing.

The details of time course of any experiment can be examined using this reported system. For example, the event time relationship among pallet drop, licking event, bar pressing and locomotion can be examined and validated. The typical results were shown in Figure 4. In this figure, accumulated 35 minutes SIP events are displayed. The event of pallet drop is shown in panel (4a). The bar-pressing event is shown in panel (4b). The licking event is shown in panel (4c) and, the moving distance is shown in panel (4d). In this figure, the rat exhibited the high occurrence of bar pressing toward the end of scheduled event, the terminal behavior. However, the rat also exhibited the high occurrence of bar pressing right after the pallet drop (Figure 4b). Significant results shown that the rat exhibited the adjunctive licking behavior after the food was consumed (Figure 4c). Figure 4d illustrates the accumulated moving distance that documents the activity of rat in the scheduled events. It shows that there was intense locomotion between adjunctive behavior and terminal behavior period. From these data, four different analyses can be made. They are 1) the off-line quantitative analysis of different stages of training and the SIP tests; 2) the analysis of locomotion; 3) the analysis of

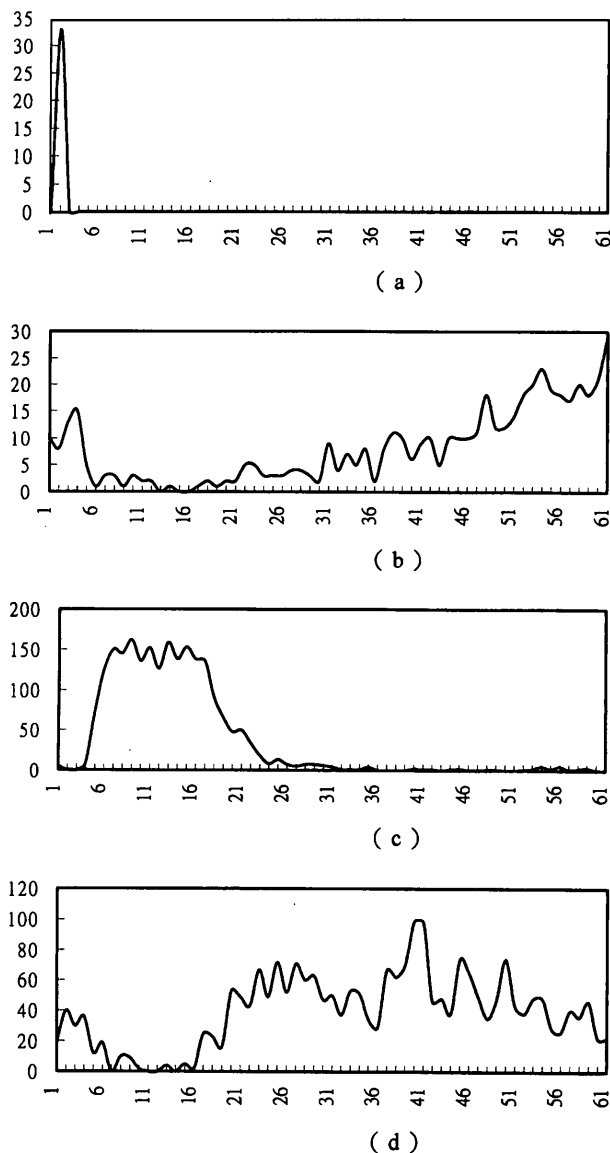


Fig. 4. The accumulated SIP events. The accumulated 35 minutes SIP events are graphed in four different panels. The documented pallet drop is shown in panel (a); the bar-pressing event is shown in panel (b); the licking event is shown in panel (c); and, the moving distance is shown in panel (d).

licking pattern; and 4) the synergetic interpretation of data.

For off-line quantitative analysis of different training stages and the SIP tests, it is possible for the researcher to tally and tabulate the progress of the experimental animal. Performance of rats at three different training stages, untrained, training, and SIP trained, as well as a testing condition, administrated of 1 mg/Kg amphetamine to SIP trained rats, are tabulated in Figure 5. It can be easily observed that, during different training stages, the total count of the bar press (terminal behavior) and the moving distances are held steady. On the other hand, the adjunctive

	Untrained Rat		Training Rat		SIP Trained Rat		1 mg/Kg Administrated of Amphetamine	
	Current	Accumulate d/Average	Current	Accumulate d/Average	Current	Accumulate d/Average	Current	Accumulate d/Average
Pallet	1	33	1	37	1	33	1	11
Bar Pressing	20	674	10	478	21	562	2	367
Licking	0	46	25	953	73	2212	5	367
Moving Distance (cm)	96	3931.5	21	2142	63	3747	261	8025
No Activity	70%	74%	88%	80%	78%	71%	51%	54%

Fig. 5. The tabulated tested SIP events monitored by the reported system.

behavior (licking) increased progressively from 0 to 2212. This remarkably increases of water licking indicate the rat is well trained for SIP experiment. However, after 1 mg/Kg of amphetamine is administrated to a well-trained rat, the adjunctive licking behavior shows different patterns. The rat under test condition shows no motive for food and drink. In addition, the moving distance (locomotion) increased dramatically.

The locomotion can be further analyzed using tools that are illustrated in Figure 6. In this figure, the temporal positions and the frequencies of visit at previously described conditions are illustrated in two- and three-dimensional displays, respectively. In the three-dimensional displays, the rows and columns are representing the temporal locations of detectors and the vertical axis is the frequency of visiting. The untrained rat exhibited a temporal pattern of asking food and water (Figure 6a). From the increase of visiting frequency at the bar pressing location, one can observe that the training rat is learning how to asking food (Figure 6b). In Figure 6c, the frequency of visiting water tube location is dramatically increased. This represents that the rat is well trained and exhibits good adjunctive SIP behavior. However, after the administration of 1 mg/Kg of amphetamine, the pattern for locomotion changed. The frequency of visiting water tube and bar pressing location decreased dramatically and the frequency of visiting other sites increased (Figure 6d).

Discussion

The most important aspect of SIP scheduled event is to observe the animal behavior. A single scheduled event (one-minute event) does not show the typical animal behavior. The reported system provides on-line analysis and displays trends of animal behavior by ensemble averaging the accumulated events. That is to build the histogram using the pallet drop to align data.

The reported SIP data acquisition and analysis system are capable of acquiring the SIP behavior at a

rate of 200 samples per second and 10 frames of locomotion per second. Each sample of SIP event consists 3 bits of data and each frame of locomotion requires 180 bits of storage space. In this format, the entire course of experiment was synchronously recorded. At this rate, it is possible to acquire adequate data for analysis of adjunctive behavior pattern. The analysis of licking pattern is shown in Figure 7. In this graph, the horizontal axis is licking frequency (licking count per second). The vertical axis is the number of frequency. Four observations can be made, including: 1. untrained rat has lower frequency of licking; 2. the training rat exhibits the progression of learning the adjunctive behavior; 3. the SIP trained rat shows higher frequency (6 to 7 counts/s) of licking pattern (22); and 4. the rat under the influence of 1 mg/kg of amphetamine shows very high occurrence of low frequency licking. The dramatically effect of pharmacological intervention of learned SIP adjunctive behavior is clearly demonstrated by this graphical illustration.

The synergetic interpretations of above data illustrate the changing pattern of adjunctive behavior, the terminal behavior and the pattern of locomotion. The reported system can provide quantitative data that review the complete view of animal behavior throughout the experiment. For example, the time period and occurrences of adjunctive behavior can be correlated with bar pressing and locomotion data to examine the accuracy of data recording.

The presented data of pharmacological intervention indicates that not only the SIP behavior but also the patterns of locomotion are affected by the administration of amphetamine. The purpose of this article is to report an integrated system for simultaneously acquiring SIP behavior and locomotion. The system provides entire experiment recording with lesser storage requirement. This report has presented data that correlate with previous SIP behavior. This report has also illustrated four stages of rat to test the accuracy of the system. From off-line and post analyzed data, the licking data has shown the

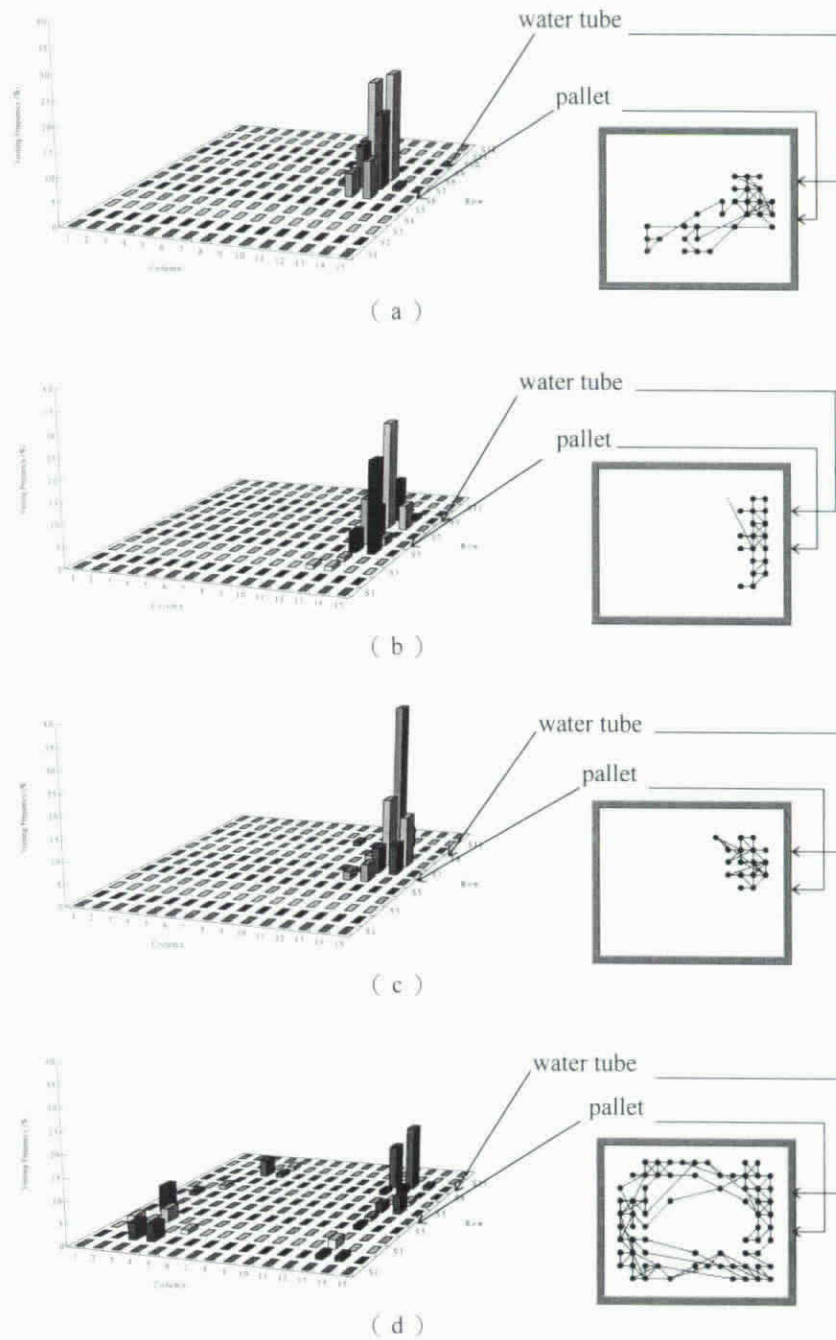


Fig. 6. The comparison of locomotion. The following are the results of four different stages of locomotion (a) untrained rat; (b) training rat; (c) trained SIP rat and (d) 1 mg/Kg administrated of amphetamine testing condition.

accuracy of acquiring the SIP behavior and the feasibility of utilizing data. The data is discussed with SIP behaviors that shows the reliability of the system.

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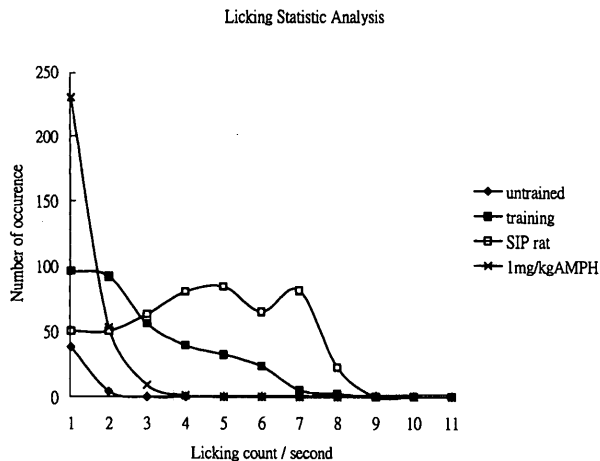


Fig. 7. Analysis of licking pattern. This graph shows that the licking pattern at the different stages of animal has different pattern of distribution.

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