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A remote control training system for rat navigation in complicated environment*

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Abstract: A remote control system has been developed to deliver stimuli into the rat brain through a wireless micro-stimulator for animal behavior training. The system consists of the following main components: an integrated PC control program, a transmitter and a receiver based on Bluetooth (BT) modules, a stimulator controlled by C8051 microprocessor, as well as an operant chamber and an eight-arm radial maze. The micro-stimulator is featured with its changeable amplitude of pulse output for both constant-voltage and constant-current mode, which provides an easy way to set the proper suitable stimulation intensity for different training. The system has been used in behavior experiments for monitoring and recording bar-pressing in the operant chamber, controlling rat roaming in the eight-arm maze, as well as navigating rats through a 3D obstacle route. The results indicated that the system worked stably and that the stimulation was effective for different types of rat behavior controls. In addition, the results showed that stimulation in the whisker barrel region of rat primary somatosensory cortex (SI) acted like a cue. The animals can be trained to take different desired turns upon the association between the SI cue stimulation and the reward stimulation in the medial forebrain bundle (MFB).

Key words: Remote control, Brain, Navigation, Stimulator, Reward stimulation, Whisker
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INTRODUCTION

Electrical stimulation in the animal brain is an indispensable means to investigate brain mechanisms in many experimental setups. However, the cable connections between the stimulation instruments and the animal brain always limit the animal's freedom of movement and thereby cause entwisting and breaking of the cables, especially in freely moving animal experiments. In order to solve this problem, telemetry systems have been designed to deliver the stimulation signals as substitute for cables (Xu *et al*., 2004; Wang *et al*., 2006; Song *et al*., 2006). By using the state-of-the-art technology of microelectronics, the remote controlled micro-stimulator can be made small and light enough to fit small animals such as a rat. In addition to facilitating experiments on freely moving animals in labs, this development of the wireless stimulator has also brought a bright future for animal robots (Talwar *et al*., 2002; Wang *et al*., 2006). By delivering stimulations into specific brain regions as steering cues (Romo *et al*., 2000) and as rewards (Reynolds *et al*., 2001; Hermer-Vazquez *et al*., 2005), animals are expected to be able to explore complex environments to fulfill some types of work that are

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difficult for human beings or machine robots.

Stimulations in the medial forebrain bundle (MFB) of brain have been used as a type of reward to animals in behavior trainings (Hermer-Vazquez *et al.*, 2005). In addition to the MFB stimulation acting as a movement motivation, stimulations in the somatosensory cortices of the left and right half brains have been used as steering cues for controlling rat navigation (Talwar *et al.*, 2002; Wang *et al.*, 2006). In order to investigate the process of animal training, as well as the effect of wireless stimulations in the MFB and in the somatosensory cortices of rat brain with different stimulation intensities, the present study developed an integrated system for the training of remote controlled rats. The system provides changeable amplitude of pulse output with various stimulation modes and has worked stably and effectively in rat navigation training.

METHODS

Surgery and electrode implantation

All procedures used in this study were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (China Ministry of Health). Adult Sprague Dawley rats (230~340 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.) (Lou *et al.*, 2004) and placed in a stereotaxic apparatus (Stoelting Co.). The scalp was incised at the midline. One mm diameter holes were drilled in the skull for inserting three stimulating electrodes into the brain and for anchoring stainless steel screws. Stimulating electrodes were made from pairs of insulated nichrome wires (80 μm in diameter) with a 0.5~1 mm vertical tip separation. One of the bipolar stimulating electrodes was placed in the MFB (AP –3.8, ML +1.6, DV +8.2) (Hermer-Vazquez *et al.*, 2005). The other two stimulating electrodes were implanted symmetrically in the whisker barrel fields of left and right somatosensory cortices (SI) (AP –1.8, ML ±5.0, DV +2.8) (Paxinos and Watson, 1997). Dental acrylic was used to fix the electrodes and screws to the skull. At least 7 d were allowed for post-operative recovery.

Hardware and software

A PC was used as the main controller with an integrated program. Bluetooth (BT) modules were used for the transmitter and the wireless receiver. The Mixed-Signal ISP FLASH MCU (C8051F020) was used as the processor of the remote controlled micro-stimulator.

An operant chamber with a bar-press was designed to test the reward effects of wireless MFB stimulations (Bear *et al.*, 2001; Reynolds *et al.*, 2001). The operant chamber was connected with the computer through a COM port. Each bar-pressing action produced a negative pulse that was delivered to the COM port by an interface circuit (MAX232). When the computer detected the pulse signal, it turned on a cue light located on the wall of the operant chamber and sent a command string to trigger the remote control stimulator. At the same time, the computer also counted the number of the bar-pressing.

An eight-arm radial maze was made for steering training of animals' locomotion.

RESULTS

System design

The training system includes the following parts (Fig.1): a PC, the wireless modules (including a transmitter connected to the PC through a serial port and a receiver connected to the stimulator on the rat backpack), a remote controlled micro-stimulator which outputs to the implanted electrodes in the rat brain, as well as an operant chamber and an eight-arm maze for trainings. The PC program starts by initiating the hardware and opening a protocol file associated with stimulating parameters as well as a log for a specific rat. Then the program waits for user's instructions and executes by sending commands to the transmitter with given parameters. The transmitter then transfers the commands and parameters to the remote controlled receiver and the stimulator. Finally, the stimulator produces trains of pulses which are delivered into the implanted electrodes in the specific brain sites (Fig.1).

1. Hardware

BT modules are used for the transmitter and the wireless receiver. The BT modules can work within 100 m distance with its max Baud rate of 1.384 Mbps.

The Mixed-Signal ISP FLASH MCU (C8051F020) is the main processor of the micro-stimulator in the rat backpack. The processor has the features of high speed, small size and low power consumption important for a small animal backpack

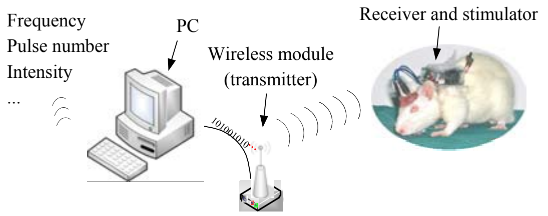


Fig.1 System structure including a PC, a transmitter module connected to the PC, a wireless receiver module and a stimulator on the rat backpack

system. In addition, it has two 12-bit digital to analog converters (DACs), which output simultaneously to timers for jitter-free waveform generations. In order to minimize the weight and size of the micro-stimulator, most of its components are made of surface mounted devices (SMD 0402 package). The whole backpack weights only 20 g, with a size of 36 mm×22 mm×15 mm.

The electrical stimulation pulses are originated from the DACs of the C8051 MCU. The outputs of the two DACs control the constant voltage drive circuit and the constant current drive circuit, respectively, to produce mono-polar pulses (Fig.2a). Before the pulses of constant voltage/current are fed into the stimulating electrodes, they go through three analog switches. The first switch is used for mode selecting between the constant voltage and the constant current modes. The second switch is used to produce bipolar pulses from the mono-polar pulses by reversing every half of the positive pulse into a negative phase. The third switch is used to choose the output channel among the four connected to the electrodes (Fig.2a). Therefore, with the use of accessorial analog switch circuits, the stimulator works as a voltage pulse generator or as a current pulse generator with either mono-polar or bipolar output pulses. The amplitude of the pulses is changeable. By setting a digital data form for the 12-bit DACs, the stimulator can produce an output signal with varied waveforms to meet the different needs of experiments.

The biphasic charge-balanced current signals can minimize the polarization of the electrode as well

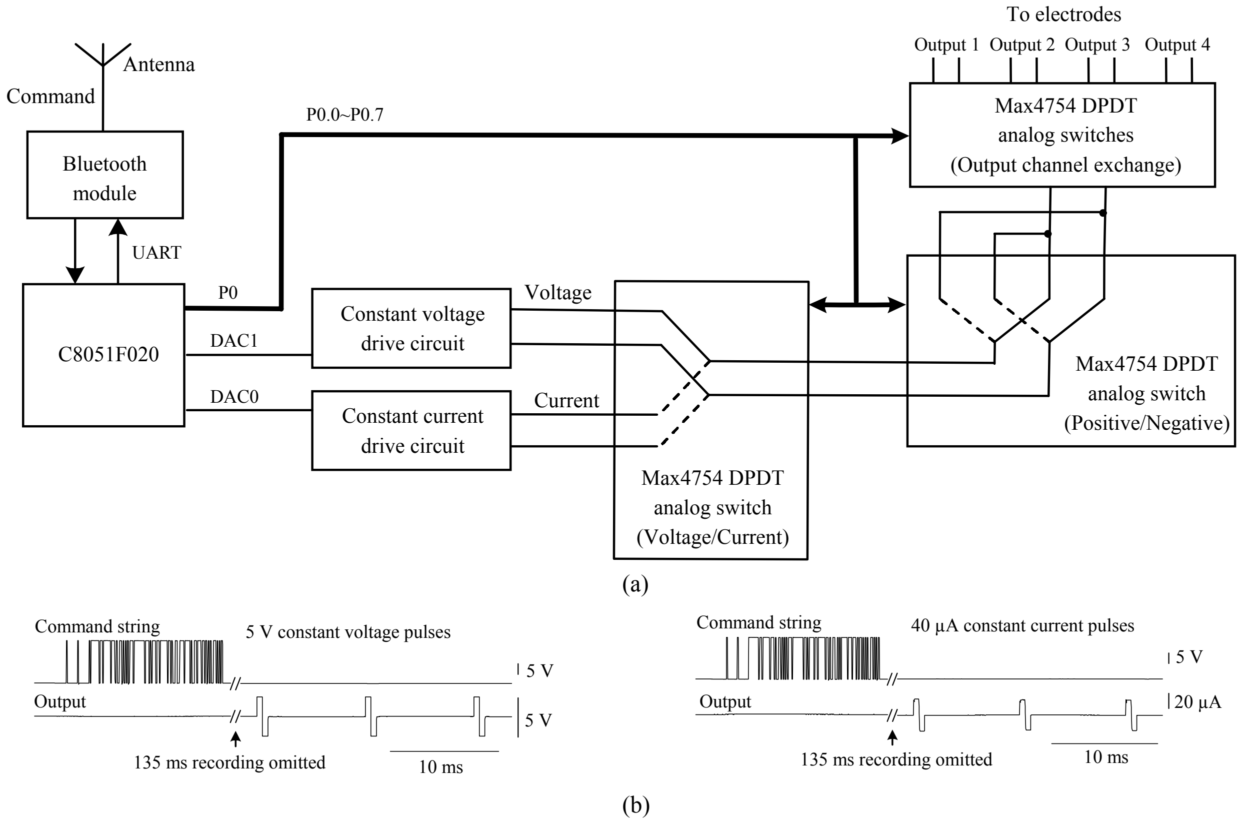


Fig.2 Circuit schematic diagram of the micro-stimulator and the input and output signal waveforms. (a) The micro-stimulator includes a Bluetooth module as the wireless receiver, a C8051F020 MCU as the main processor, constant voltage/current drive circuits, as well as analog switch circuits to produce bipolar or mono-polar stimulation pulses; (b) The waveforms of input command strings and output constant voltage and constant current pulses

as the permanent lesion to the brain tissue, which is caused by the charge accumulation (Tehovnik, 1996). Therefore, bipolar pulses were used in the present study. The duration and amplitude of the positive phase and negative phase in the bipolar pulses are equal. Examples of the waveforms of input command strings and the output constant voltage and constant current pulses are shown in Fig.2b. The errors of pulse duration and inter-pulse interval are less than 0.01 ms. The errors of pulse amplitude for the constant voltage and the constant current modes in the given ranges (Table 1) are less than 1.3% and 12.0%, respectively. The coefficients of variance of the constant voltage and constant current pulses at a given amplitude value are less than 0.2% and 0.4%, respectively. The control program is written in C51 language and is saved in the internal memory of C8051 MCU.

Table 1 Protocol of wireless module communication

Byte index	Data
0,1	0xFF, 0xFF
2	Stimulation mode (Voltage/Current)
3	Pulse polar (Mono-/Bipolar)
4,5	Inter-pulse interval 0~40 ms
6,7	Pulse number 1~100
8,9	Pulse duration 0~10 ms
10,11	Pulse amplitude 1~10 V/20~120 μA
12,13	0xAA, 0xAA

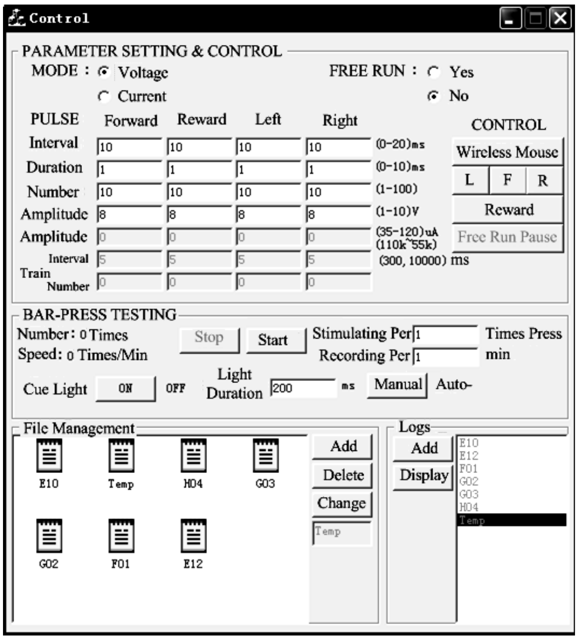
The power for the stimulator is provided by two lithium cells (3.7 V, 120 mA·h), which can continuously run approximately 8 h. For saving power, the stimulator processor is set idle automatically as soon as it is neither producing stimuli nor communicating with the transmitter.

2. Software

The PC program is written in Visual C++ 6.0. Its main functions are shown in the graphical user interface (Fig.3), which includes parameter setting for the wireless micro-stimulator, operation choices, control and automatic data statistics for the bar-press testing of the operate chamber, file management for system configurations and for experimental data and logs. The protocol of wireless module communication is shown in Table 1.

The wireless micro-stimulator delivers stimulation pulses at a constant voltage mode or a constant current mode. Also, it can work on "free run" mode that delivers a series of pulse trains with given frequency and given train numbers. The parameters of stimulation pulses that are delivered to the left and right SI and to the MFB location can be set independently, which include inter-pulse interval, pulse duration, pulse number, pulse intensity (peak-to-peak voltage or current amplitude of the pulse) (Fig.3 and Table 1). In addition, two different sets of stimulation parameters can be delivered to the same MFB stimulating electrode during the same test run for two purposes, named "forward" and "reward". This design resulted from the fact that the rewards for the rats to make correct turns on their navigation routes usually needed stronger MFB stimulation than the MFB rewards just for the rats to walk forward. Therefore, different stimulation trains delivered to the same MFB site under different situations can train the animal more efficiently.

The options in CONTROL panel allow the user to guide animals through a wireless mouse without sitting in front of a computer.



PARAMETER SETTING & CONTROL

MODE： ● Voltage ○ Current

FREE RUN： ○ Yes ● No

PULSE	Forward	Reward	Left	Right	
Interval	10	10	10	10	(0-20)ms
Duration	1	1	1	1	(0-10)ms
Number	10	10	10	10	(1-100)
Amplitude	8	8	8	8	(1-10)V
Amplitude	0	0	0	0	(35-120)uA (110k~55k)
Train Interval	5	5	5	5	(300, 10000) ms
Train Number	0	0	0	0	

BAR-PRESS TESTING

Number: 0 Times

Speed: 0 Times/Min

Stimulating Per 1 Times Press

Recording Per 1 min

Cue Light ON OFF Light Duration 200 ms Manual Auto-

File Management

E10 Temp H04 G03 G02 F01 E12

Temp

Logs

E10
E12
F01
G02
G03
H04
Temp

Fig.3 The graphical user interface of the control program coded in Visual C++ 6.0. The PARAMETER SETTING & CONTROL panel sets the parameters for stimulation modes, stimulating pulses and a wireless mouse used for the computer. The BAR-PRESS TESTING panel is used for controlling the operate chamber and data statistics for bar-press tests. The FILE MANAGEMENT panel is used for animal logs

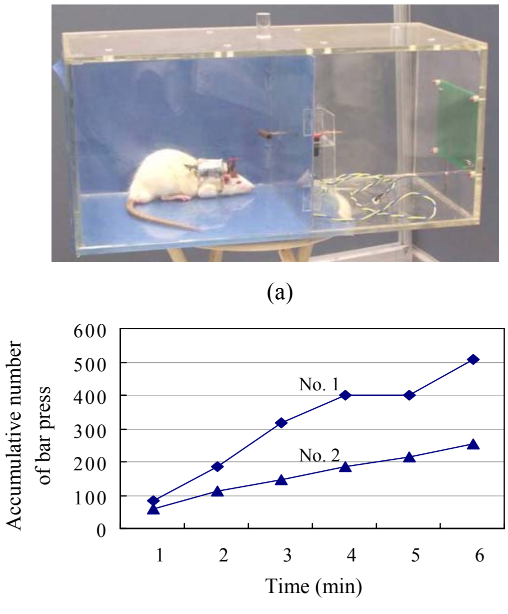
The file management function of the PC program facilitates the running of the system. The system files automatically save the experimental protocol, parameter settings and test data for every experiment of each individual animal. The files are saved in text format that can be opened and read by any commonly used editor software. Each experimental protocol can be easily retrieved for reuse or edit, which simplifies the manipulation of experiments. All of the files are available for off-line data analysis.

MFB reward effect testing

MFB stimulation has long been known as a way to reward animals in behavior trainings (Hermer-Vazquez *et al*., 2005). We tested the reward effects of wireless MFB stimulations by two behavior experiments: the self-stimulation in an operant chamber with a bar-pressing and the freely roaming in an eight-arm radial maze.

When the rats first started training to press the bar in an operant chamber, they would behave by exploring, rearing, grooming or sitting. Occasionally the rats touched the bar mounted on one side of the wall. MFB stimulation reward was immediately given through the remote control system for the bar press (Fig.4a). After several training sessions, most rats could press the bar continuously to obtain the MFB stimulation reward shortly after they were placed in the operant chamber. Fig.4b shows the accumulative number of bar press of two rats (No. 1 and No. 2) during a 6 min test run. The total numbers of bar press of the two rats were 510 and 253, which correspond to the pressing rates of 84 min^{-1} and 42 min^{-1}. Each stimulation train was set as ten biphasic pulses at 100 Hz with pulse duration 1 ms and peak-to-peak pulse amplitude 8 V in the test runs.

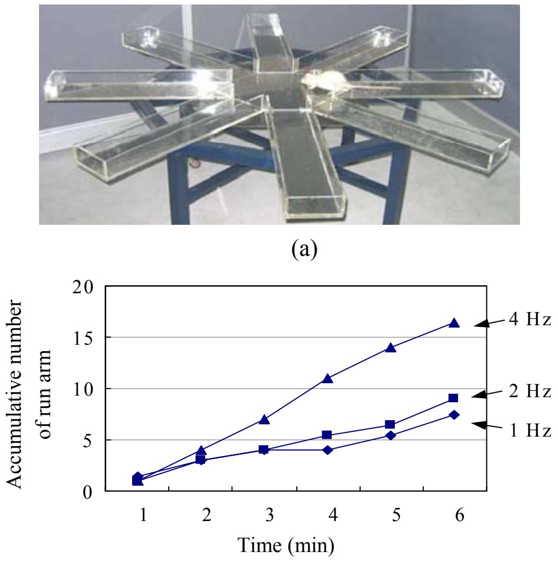
The reward effect of MFB stimulation was also tested in an eight-arm maze (60-cm-long and 12-cm-wide arms with 5-cm-high walls and a 35-cm-wide octagon center) (Fig.5a). Without MFB stimulation, the rat in the maze seldom moved. When MFB stimulation was delivered in a given frequency, the rat underwent reinforced forward movements in the maze arms. In addition, the movement amount increased with the elevation of the MFB stimulation frequency. Fig.5b shows the experimental data of a representative rat. During 6 min test run, the rat walked across 7.5, 9.0 and 16.5 arms with different



(a)

(b)

Fig.4 MFB rewarding test in an operant chamber with a press bar. (a) A photo of the operant chamber; (b) Measurements of bar-pressing number of two rats (No. 1 and No. 2) during a 6 min experiment session. The rats kept pressing the bar in the operant chamber to obtain the MFB rewarding stimulations



(a)

(b)

Fig.5 MFB rewarding test in an eight-arm radial maze. (a) A photo of the eight-arm radial maze; (b) Movement distance measurements of a representative rat during 6 min experiment sessions with different MFB stimulation train frequencies of 1 Hz, 2 Hz and 4 Hz, respectively. The movement amount increased with the elevation of the MFB stimulation frequency

MFB stimulation train frequencies of 1 Hz, 2 Hz and 4 Hz, respectively. Each stimulation train had ten biphasic pulses at 100 Hz with pulse duration 1 ms and peak-to-peak pulse amplitude 10 V. Since one arm was defined as one loop between the maze center, and the end of the arm, the corresponding distances which the rat moved in the experiment (Fig.5b) were approximately 12 m, 14 m and 26 m under the stimulation frequencies of 1 Hz, 2 Hz and 4 Hz.

The results of the two behavior experiments, the bar-pressing in the operant chamber and the roaming in the eight-arm maze, indicated that the MFB train stimulation had significant rewarding effect to reinforce the rat behaviors.

SI stimulation for steering rat roaming

Rats use their whiskers to explore the environment and to discriminate the surfaces of objects. The whisker "barrel cortex" in the left and right somatosensory cortices (SI) receives projections from the contralateral facial vibrissae (Simons, 1978; 1983; Masino, 2003). Therefore, an electrical stimulation delivered to one side of SI would give the animal a sense of object touch in the contralateral side. By using this feeling cue, rats could be trained to turn correctly on their navigation route by associating MFB rewarding stimulation to each correct turn (Talwar *et al*., 2002). After 5~7 d of training, the average correct rate of left and right turns reached as high as (93.6±5.3)% (n=6 rats) in 5 to 7 test runs per rat. In each test run the rats were given ten left and ten right turning instructions during their navigations in the eight-arm maze. The instruction stimulations were 1~2 trains with ten pulses at 100 Hz, pulse duration 1 ms and peak-to-peak pulse amplitude 6~8 V.

At the beginning of each training session, rats were motivated to keep running forwards in an eight-arm maze by receiving MFB rewarding stimulations delivered at a speed around 0.5~1 Hz. Left or right turning instructions were given through SI stimulations when the rats moved into the central area of the maze. The rats would obtain immediately two trains of MFB rewarding stimulations upon each correct turn. An interesting finding in the training procedures is that the SI stimulations can be the cues for different turning directions. When they received SI stimulation trains, some animals can learn to always make ipsilateral turns while the others can learn to always make contralateral turns. The turning direction that the animal established from the training depended on the association of MFB rewarding stimulation to which of the rat turning directions during the training sessions, especially in those initial training sessions. If MFB rewarding stimulations were always given upon ipsilateral turns, the rat could learn to turn ipsilaterally on the SI stimulations, and vice versa.

Six of the rats learned to turn correctly almost every time when they received instruction stimulations, three with ipsilateral turns and the other three with contralateral turns. With the MFB rewarding stimulations and the SI stimulation cues for turn direction, these rats were able to run forward, turn correctly and complete the navigations through a 3D obstacle route (Fig.6).

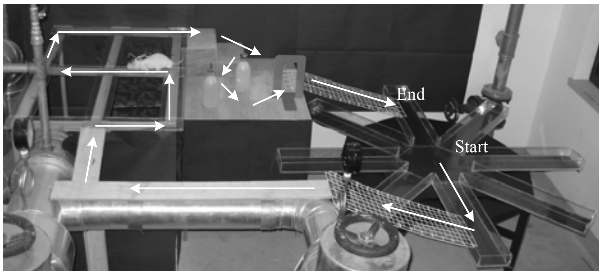


Fig.6 An example of a remote control rat running in a 3D obstacle route. White arrows indicate the movement track. The rat was instructed to start in the center of eight-arm maze, climb a ramp, moved forwards and turned correctly along a complicated course, walk down steps, pass through a hoop, and return to the eight-arm maze

DISCUSSION

A new remote controlled rat behavior training system was developed in the present study. Compared with previous telemetry stimulation systems (Xu *et al*., 2004; Song *et al*., 2006; Wang *et al*., 2006), this system has the following different features.

(1) The remote controlled micro-stimulator provides multiple output modes, such as constant voltage/current pulse, changeable pulse amplitudes and as much as four output channels for bipolar stimulating electrodes or eight output channels for monopolar stimulating electrodes. The unique feature of the changeable amplitude of output pulses provides an

easy way to set the proper stimulation intensity for various training purposes based on the individual animals.

(2) The PC control program is integrated and its friendly graphical user interface is easy to use. It provides functions of parameter setting, transmitter controlling and communicating, operation executing, as well as experimental protocol files and data files managing. In addition, the program controls the operant chamber, by detecting a bar pressing action, to turn on a cue light and trigger the remote control stimulator. The numbers of bar pressing within each given time period are recorded automatically throughout every experiment and are saved into data files for off-line analysis.

(3) The self-made operant chamber, as well as the eight-arm radial maze, makes the present remote control training system not only suitable for animal telemetry navigation training but also useful for other experiments related to investigations about the mechanisms of learning and memory. Animals were first put in the operant chamber to confirm the validity of MFB stimulation by reaching a given value of bar-pressing rate (Lindner *et al*., 1997). Then, they were trained to run forwards and to turn correctly on the associated stimulations of MFB and of SI in the eight-arm radial maze. By using the system, animals were able to learn quickly, to obtain solid memory and to complete complex roams through a 3D obstacle route.

In addition to these special features of the system, the present study shows an interesting phenomenon that has not been reported before. The SI stimulations enable the rats to learn to make both ipsilateral turns and contralateral turns. It has been known that the whisker barrel region in the primary somatosensory cortex (SI) receives projections inputting from the vibrissae on the contralateral face (Simons, 1978). Presumably, stimulation on one side of SI can cause a feeling of touching an obstruction on the contralateral side in the rat brain. The rat is thereby expected to make an ipsilateral turn to avoid the obstruction (Wang *et al*., 2006). However, a different amount of stimulation intensity can evoke the different behavioral responses (Tehovnik, 1996). The results of our study indicated that a moderate stimulation in SI acts like a cue similar to other cues, such as a flash of light or a tone of sound, used in traditional animal training programs. The action type that the rat prefers to take following the cue depends on the associated reward. Therefore, the animals can be trained to take different desired turns on the SI stimulation. However, from our experimental observation, an excessive stimulation could force a rat to turn swiftly or even to whirl. In this situation, the rat behaved like it was avoiding some obstruction urgently.

In summary, the present system is integrated and is useful for the training remote controlled animals and for investigating the learning and memory behaviors of rats.

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