An automatic recording system of the positional behavior of mice for experimental analysis

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A device for automatic recording of a mouse positional behavior within a circular corridor-like cage is described. The animal's position is successfully monitored by capacitor detection and its time spent at each position measured; the results are punched on the paper tape as binary coded numbers, and the data are processed off-line by a general-purpose computer.

The positional behavior of a small animal, such as a mouse, confined in a cage with numbered unit spaces, can be represented by a sequence of addresses of unit spaces. Three measures were considered. (1) The length of a passage measured by the number of unit spaces traversed by the animal in a bout (see below). This measure was independent of cage size. (2) The frequency of passage in a given time, which depended, in part, upon the circadian rhythm of activity level. (3) And the multinomial distribution of the time spent in each unit space. This could be evaluated and expressed as "entropy," showing the nonrandomness of the animal's preference for the spaces in the cage.

Hirao and Utena (1962) described an apparatus (the "cloister") by which the position of a mouse could be monitored by capacitor detection and recorded automatically. The device consisted of a circular corridor divided into unit spaces; each space was a little larger than the animal and was coded by number (Figure 1). Hirao, Shinozaki, Furuya, and Ishikawa (1973) improved the system so that the data were processed and analyzed with a general-purpose computer. Lewin (1938) described a similar analytical method. The Lewin method was designed to study the "direction of psychological forces" by tracking the subject's movements projected onto a "hodological space" divided into unit spaces. The method, however, was not tested experimentally.

The purpose of this paper is to describe a useful method that has undergone a series of tests to eliminate technical difficulties originally associated with the system.

The Apparatus and Circuitry

The apparatus was designed for a mouse. The mouse was placed in a circular corridor, where it

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Figure 1. Cloister from the top.

could run freely or stay in place. Its presence in any segment of the corridor was monitored by a capacitor-detection technique. The roof and wall of the corridor were made of transparent Plexiglas. The vertical section was rectangular (height, 7.5 cm, and width, 10 cm), and the corridor-like cage was divided into numerical unit spaces determining the size of the cage. Metal meshes of $14 \times 2 \text{ cm}^2$ were placed on the outer surface of the roof 15 cm apart; these were used as reference electrodes. The floor was of rough metal mesh through which waste material could fall. The floor served as the ground for the electrical circuitry. Thus, each space between the ceiling electrode and the floor was the unit space with a monitor system (Figure 1). The volume of unit space in which a mouse could be measured was $7.5 \times 10 \times 15 \text{ cm}^3$.

Mice were housed in this cage for several months without external disturbance except for feeding and watering.

A block diagram of the monitor system is illustrated in Figure 2. A capacitor between the reference electrode on the ceiling and the floor forms an LC resonator with the input coil; it is tuned at 1.8 MHz. When a mouse enters a unit space from a neighboring

Figure 2. Block diagram of the detect system. Details in the text.

space, the resonator is detuned and the signal voltage decreases. When the mouse leaves this space, the signal voltage returns to the original high level. The signal wave is demodulated, amplified, and converted to a standard 1-kHz wave by the converter-amplifier to increase the S/N ratio. These signals, coming from the inputs of all the unit spaces, are led to a logic circuit where only an on-state signal of the mouse's presence in a unit space is allowed to occur. The off-state signal is made when the on-state signal of the neighboring spaces occurs. Such on and off signals, together with the address code of the unit space, are encoded in the data select circuit. Whenever, the mouse changes position, the information is punched

onto 8-bit paper tape as a binary coded number. All punched data are processed by a general-purpose computer OKITAC-5090.

Capacitor-Detector Circuit

As shown in Figure 3, the 1.8-MHz signal wave, amplitude-modulated at 1 kHz, is supplied from an external oscillator to the input coil, T, of each LC resonator. The resonance is adjusted with a variable capacitor, C_1 , and the resonance voltage is demodulated by a diode, D_1 , amplified by an operational amplifier, A_1 , rectified by a diode, D_2 , then changed to direct current (dc) voltage through an LC filter. This system provides high stability and sufficient sensitivity to detect the position of the mouse and adjustments are unnecessary. Of course, the higher the frequency and amplitude of the carrier wave, the more remarkable is the mutual interference, because of the paralleled input LC resonators nearby. Trial and error indicates that the carrier wave should be fixed at a frequency of 1.8 MHz and at a voltage of 180 mV. The amplitude modulation (by 1-kHz sine wave) is convenient to obtain the high gain and stable amplifying.

The dc voltage signal after an LC filter is injected to the noninverting input of the operational amplifier, A_2 , and the 1-kHz reference sine wave (V_2) is fed to the inverting input of A_2 through an FET follower, Q_3 . The V_2 amplitude is adjusted by a variable

Figure 3. Capacitor-detection and amplification. $R_1, R_7, R_{10}, R_{21}, R_{31}, R_{35}$: 10 K Ω ; R_2, R_9 : 100 K Ω ; R_3, R_5 : 2 K Ω ; $R_4, R_{14}, R_{17}, R_{19}, R_{24}, R_{27}$: 1 M Ω ; R_6 : 5 K Ω variorhm; $R_8, R_{15}, R_{33}, R_{36}$: 1 K Ω ; $R_{11}, R_{12}, R_{28}, R_{29}, R_{34}$: 100 Ω ; R_{13} : 500 K Ω variorhm; R_{16}, R_{22} : 5 K Ω ; R_{18}, R_{20} : 20 K Ω ; R_{23} : 220 Ω ; R_{25}, R_{26} : 50 K Ω ; R_{30} : 20 K Ω variorhm; R_{32} : 330 Ω ; C_1 : 15 pF variable capacitor; C_2, C_6 : 0.01 μ F; C_3, C_4 : 2.2 μ F; $C_5, C_{11}, C_{20}, C_{21}$: 0.1 μ F; C_7 : 100 pF; $C_8, C_{13}, C_{14}, C_{16}, C_{17}, C_{19}, C_{22}$: 10 μ F; C_9, C_{10} : 33 μ F; C_{12}, C_{15} : 4.7 μ F; C_{18} : 2.2 μ F; T : Trio T-21; Q_1, Q_3 : MK-10; Q_2, Q_4, Q_5 : 2SC372G-0; D_1, D_2, D_3 : 1S1555; A_1, A_2, A_3 : SN72741; L : ST-30; V_1 : input for the ceiling electrode; V_2 : input for the reference 1-KHz sine wave; V_3 : output to the logic identification circuit; V_{CC} : +15 V; $-V_{EE}$: -15 V.

Data signals are reduced to a set of symbols by applying the appropriate binary-coded address of the data selector inputs (A, B, C, D) taken from the outputs of a binary COUNTER₁. With each CLOCK PULSE whose frequency is 2 kHz, the COUNTER₁ switches to the next state, causing the DATA SELECTOR₁ to select in sequence the information source connected to its inputs, V₇. When data are selected, the output of the DATA SELECTOR₁ changes from a logical "0" to a logical "1," triggering a monostable multivibrator (MM₁) to produce a pulse as a transfer signal, which makes the LATCH₁ function and COUNTER₁ clear and reset through another multivibrator (MM₂). Therefore, the output of the LATCH₁ represents the specific coded address number of the unit space where the mouse is present. The change of mouse position produces a change in the output code of the LATCH₁, inducing a pulse through multivibrators (MM₃) and OR gate (G). This pulse is used as a clutch signal for the tape punch, enabling the LATCH₂ function and resetting the

the increment of humidity, particularly through the reference electrodes on the roof of the cloister.

This apparatus works with find stability at a temperature of 5°C-35°C and a humidity of 20%-90% without adjustment for at least a month.

In order to record economically the data obtained during the experiment (about 2 months), our tape punch was driven by the transfer signal produced by the mice changing position. However, the speed of mouse movement was influenced by the condition of the floor mesh (Hirao & Utena, 1965; Hirao et al., 1973). This was fixed so that the maximum running speed of a mouse was 1 m/sec; that is, a mouse could pass through a unit space in 150 msec. Therefore, any positional change that lasted less than 150 msec or which occurred during the recording period of 240 msec, was not punched on the tape; these were supplemented by the software which measured the duration of a visit to the nearest 100 msec when the data was processed by the computer. Due to the repeated operation of the punch and the rounding errors of timing, there were slight discrepancies in total time elapsed compared with time recorded by the apparatus. However, these can be ignored since they comprise less than 3 min in a day (= 0.2%).

Several calculations can be performed on the raw data. We found four indices to be useful (Hirao & Utena, 1965; Hirao et al., 1973): (1) Length of "passage" (measured by the number of unit spaces traversed by a mouse until it stopped in a unit space for more than 1.5 sec). The length followed the law of geometrical distribution probability, and the mean value was constant for the adult mice of several strains. The choice of criterion time is discussed by Hirao et al. (1973). (2) Frequency of passage in a given time was measured and showed a circadian rhythm with particular characteristics identifiable for each strain. The product of the mean value of (1) and (2) is equal to the locomotory activity level in a given time. (3) The spatial distribution of the animal location is represented by the "entropy" H , as follows:

$$H_{v,n} = - \sum_{i=1}^n p_{v,i} \log_2 p_{v,i} \quad (1)$$

where

$$p_{v,i} = \frac{(\text{total number of visits to the unit space numbered } i)}{(\text{total number of visits to all unit spaces})} \quad (2)$$

and

$$H_{s,n} = - \sum_{i=1}^n p_{s,i} \log_2 p_{s,i} \quad (3)$$

Figure 7. Changes of entropy and daily activity level along the habituation process in the cloister. H_v (open circles) obtained from the visiting frequency, and H_s (closed circles) from the staying time. Daily activity level (xs) is also shown.

where

$$p_{s,i} = \frac{(\text{total time spent in the unit space numbered } i)}{(\text{total time spent in all unit spaces})} \quad (4)$$

The suffix n denotes the total number of unit spaces, i.e., the cage size, and i denotes the particular unit space. Thus, $H_{v,n}$ describes the nonrandomness of the mouse entering a unit space, and $H_{s,n}$ describes the nonrandomness of staying. In order to compare the positional behavior of the animal in various circumstances, and because the entropy depends on the cage-size, (4) relative entropy can be calculated for $H_{v,n}$ and $H_{s,n}$ as a fraction of the maximum value of entropy.

Figure 7 shows an example of the change of the entropy thus calculated during the habituation process of ddN strain in the cloister. In this figure, the abscissa represents the day elapsed since introduction, and the ordinate the entropy H_s and H_v in 24 h. The former entropy decreased gradually for the first 3 days and then attained a stationary level, while the latter entropy kept the maximum level throughout the experiment.

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