INTRODUCTION OF NEW INDICES INTO CONTINUOUS AVOIDANCE TEST —A TRIAL TO DETECT THE EFFECTS OF SODIUM CHLORIDE AND MANNITOL ON AVOIDANCE BEHAVIOR—.

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Abstract.....A personal computer was introduced in conditioned lever-press avoidance experiment in Sidman situation. Experimental data were analyzed with both classic indices such as response rate and shock rate and new indices introduced in this experiment. New indices include sum of squares of shock-shock intervals, mean of shock-response time and number of shock-response time less than 5 sec. Results obtained in diazepam-treated mice were consistent with those reported previously by the classic method. In mannitol-treated mice, significant changes of the avoidance behavior could be detected by both the classic index and our new indices. By the new indices other than classic indices, the effect of administration with sodium chloride on the avoidance behavior could be detected, whereas the classic indices could not detect the effect. Though the meanings of these indices in physiological and toxicological aspects should be appreciated in future study, the experimental system reported in this paper might be applicable in the toxicological research to detect the effect of administration with various substances on the avoidance behavior.

Key words : Continuous avoidance, new indices, personal computer.

INTRODUCTION

Conditioned lever-press avoidance behavior of rat has been widely utilized to study the psychotropic effect of drugs (Seiden and Dykstra, 1977). Antipsychotic

drugs specifically suppress the avoidance responses of rat, thus the avoidance-suppressing activities in rats correlate positively with the clinical potencies and negatively with the daily doses of drugs in psychotic patients (Cook and Catanina, 1964 ; Kuribara and Tadokoro, 1981).

On the other hand, mice are demonstrated to acquire well the conditioned lever-press avoidance responses in both Sidman situation (Sidman, 1953) and discriminated avoidance situation. The avoidance response once established in mice is shown to be stable for a long time (Bovet et al., 1969 ; Renzi and Sansone, 1971 ; Sansone et al., 1972 ; Davidson, 1982 ; Kuribara and Tadokoro, 1983 ; Kuribara and Tadokoro, 1984). Kuribara and Tadokoro (1983) showed that lever-press avoidance behavior in mice was applicable for the study of antianxiety drugs as well as of antipsychotic drugs.

However, these methods have several disadvantages including necessity to use expensive equipment, limited informations from the avoidance test when these methods were applied to toxicological research.

In the present paper, the authors introduced a personal computer into the conditioned lever-press avoidance situation to overcome these disadvantages. Experimental data were analyzed with both the classic indices such as response rate and shock rate and our new indices introduced in this study. We have tried to find out new indices which are able to distinguish the effects of a substance from the others by using diazepam, sodium chloride and mannitol. Our data suggest that the effects of a substance on the avoidance behavior may be distinguished from those of others by using our new indices.

MATERIALS AND METHODS

Animals The animals were 22 male mice of dd strain weighing 22 ± 2 g. The mice were housed in groups of 3 or 4 in plastic (Polycarbonate) cages, 21.0 (W) $\times$ 32.0 (D) $\times$ 13.5 (H) cm, with a wooden-flake mat. Solid diet (MF ; Oriental Yeast Co., Tokyo) and tap water were freely available except during the avoidance test. The room temperature was artificially regulated to 22 ± 3 °C. When the mice were at the age of 5 weeks, training of the lever-press avoidance in Sidman Situation was started.

Apparatus A shock generator (10 channels) and eight equivalent sets of operant chambers for mice 18 (W) $\times$ 9 (D) $\times$ 10 (H) cm, were purchased from O'hara Co., Ltd., Tokyo. A computer (Apple II plus, equipped with a 16 K RAM CARD) with a software which was consisted of machine language and BASIC was used for the behavior controlling and data recording and processing. The original Sidman situation received a modification on the recognition time of a lever-pressing. In the original method, a lever-pressing was recognized when lever was depressed down (lever switch : "ON"). Our behavior controlling program recognized a lever-pressing when the switch was "OFF".

Informations of the avoidance test among the computer, the shock generator and eight of operant chambers were reciprocally transmitted through Shock Generator Controller which was made by the authors.

Avoidance schedules The temporal parameters of Sidman situation were as follows : response-shock interval of 30 sec ; and shock-shock interval of 5 sec. The shock was an electric current 50 V, 0.5 mA, 50 Hz AC and was given to the mouse through the stainless steel floor grid of the operant chamber for 0.3 sec.

Experimental data records of the avoidance test Experimental data of a session consisted of avoidance informations concerning eight mice in each operant chamber at intervals of a second. The informations included the time of lever-pressing and of delivered shock as well as the number of lever-pressings per second. Parts of experimental data which were obtained by the avoidance test were printed out and were shown in Table 1. By the presentation in Table 1, experimental data of a session consisted of nine columns and of 3,600 lines. Columns were arranged from the left in order of memory address of operant chamber No. 1, operant chamber No. 1 to No. 8. Lines were in order of time from the start of a session at intervals of a second.

Informations of the avoidance behavior were presented by digital signals. A

Table 1 Experimental data records of the avoidance test. Part of experimental data of the avoidance test during 11 sec were printed out from saved floppy disk. Experimental data of a session consisted of 9 columns and 3600 lines. Columns were arranged from the left in order of memory address of operant chamber, operant chamber No. 1 to No. 8. Lines were in order of time from the start of a session at intervals of a second. Lever-pressing chains (operant chamber No. 4) and delivered shock (operant chamber No. 3) were observed during 11 sec (see text).

Memory Address	Operant Chamber number							
	1	2	3	4	5	6	7	8
3788-	00	00	00	00	00	01	00	00
3790-	00	00	00	01	00	00	00	01
3798-	00	00	00	01	00	00	00	00
37A0-	00	00	00	01	02	00	00	01
37A8-	00	00	00	02	00	00	00	02
37B0-	00	00	00	00	00	00	00	00
37B8-	00	00	80	00	00	00	00	00
37C0-	00	00	00	00	00	00	00	01
37C8-	01	01	00	01	00	00	00	00
37D0-	02	02	00	02	00	00	00	00
37D8-	00	00	01	01	00	00	00	00

digital signal behavior consisted of two figures of hexadecimals in which upper figure dealt with delivered shock (8 : shock delivered, 0 : no shock), whereas lower figure presented number of lever-pressings. Experimental data of a session were saved in a floppy disk.

Treatment of mice Substances tested were diazepam (Cercin Inj. : Takeda Pharm. Co., Osaka), sodium chloride and mannitol (Wako Pure Chemical Industries, LTD., Osaka). Sodium chloride and mannitol which are widely used in experiments and clinical treatment were selected to find out new indices, because these chemicals are well known as to be not so toxic and also because psychopharmacological and behaviological effects of these substances have not been reported.

Commercial preparation of diazepam was diluted with a physiological saline vehicle and was injected intraperitoneally to mice (0.1 mg/kg). Control mice were injected with the saline vehicle. Injection volume was fixed at 1 ml per 100 g body weight. Administration of diazepam was carried out 30 min before the start of avoidance session, and avoidance behavior was observed for 1 h thereafter.

Sodium chloride (0.7 %) and 5.0 % mannitol solutions were prepared by dissolving in distilled water. Mice were housed in groups of 3 or 4. They were given ad libitum either solution as a drinking water during the treating period of two weeks. Control mice were given distilled water.

Intervals among the treatments of mice were held at least for two weeks.

Metabolic experiment During the treatment of sodium chloride and of mannitol, metabolic experiment was simultaneously carried out to estimate the doses of them. Six mice which were not selected for the avoidance test, were housed in each metabolic cage and were divided into three groups. Mice of each group were given ad libitum distilled water, 0.7 % sodium chloride or 5.0 % mannitol, respectively, as a drinking water.

Sodium chloride in the solid diet was determined by the standard addition-flame emission method with atomic absorption/flame emission spectrophotometer (Shimadzu AA-640-12), wave length at 5890 Å as reported previously (Fukushima et al., 1957).

Analytical program of the avoidance behavior Three analytical programs of the avoidance behavior were written. All programs were designed to take out informations from the experimental data in floppy disks.

Definitions of the used terms were as follows : Experimental time : the time which was duration of one sec, was counted in order, from the start of a session. Experimental time numbers were from zero to 3599.

Lever-pressing chain : one of the avoidance behaviors of mice, in which one or more lever-pressings were observed during three or more successive experimental times.

S-R time : an interval of the experimental times between zero and the time when the behavior response started or between the time when the latest shock had delivered and the time when the behavior or response was recognized.

Three analytical programs of the avoidance behavior were as follows :

1. Classic indices (response rate and shock rate) : Total number of lever-pressings and of delivered shocks were counted from the experimental data of session. Response rate (No. of lever-pressings/min) and shock rate (No. of delivered shocks/min) were calculated with a computer.
2. Sum of squares of shock-shock intervals : On the assumption that shocks were delivered at zero and at 3599 experimental time, shock-shock intervals were squared and sum of squares was calculated.
3. Lever-pressing chain : On the assumption that a shock had delivered at zero experimental time, lever-pressing chain was picked out together with the experimental times when the chain started and the times when the latest shock had been delivered before the chain. Means of S-R time and number of S-R time less than 5 sec were calculated.

Appreciation of an effect on the avoidance behavior Experimental data which belonged to the last three sessions before a treatment, were regarded as control and shown as "before" in Table 2 and 3. Means and standard deviations (SD) of control data on each index were computed.

If the data of an index after the treatment were considered to be larger/smaller than $\text{mean} + \text{SD}/\text{mean} - \text{SD}$ of control (before treatment), the index of the mouse was regarded as "increased/decreased". And if three or more indices in a group of mice were increased/decreased, then the index of the group was regarded as "increased/decreased" ($p < 0.05$). In addition, probability of an event was calculated by the binomial distribution.

RESULTS

Doses of sodium chloride and mannitol were calculated from the results which were obtained by the metabolic experiment. Mice in control group ingested 0.50 ± 0.03 g/kg/day sodium chloride from the solid diet during treating period. Estimated dose of sodium chloride was 1.60 ± 0.23 g/kg/day in mice which were given 0.7 % sodium chloride for two weeks. Mannitol-treated mice ingested 4.2 ± 1.1 g mannitol/kg/day from the drinking water by the calculation from the ingested volume.

Effects of administration with diazepam on the avoidance behavior in mice were shown in Table 2. The avoidance behavior was analyzed with several indices including classic indices. Experimental data were compared with the data after the treatment. Increases in the shock rate and decreases in the sum of squares of shock-shock intervals were observed among diazepam-treated mice.

Effects of sodium chloride or mannitol administration on the avoidance behavior in mice were shown in Table 3. Sodium chloride (0.7 %) or 5.0 % mannitol were given ad libitum as a drinking water for one or two weeks. Indices of a mouse after the administrations were compared with indices of controls.

Table 2. Effects of diazepam administration on the avoidance behavior in mice.
 The avoidance behavior was analyzed with several indices including classic indices.
 Diazepam (0.1 mg/kg) was administered intraperitoneally. Control mice were injected with saline.

Treatments	Mouse No.	Indices									
		Response rate		Shock rate		Sum of squares of shock-intervals		Mean of S-R time		No. of S-R time less than 5 sec	
		Before	Treated	Before	Treated	Before	Treated	Before	Treated	Before	Treated
		lever-pressings per min		delivered shocks per min		(x10 ⁶ sec ²)		(sec)			
Control Group	2	10.3±0.6	9.6	0.19±0.06	0.37	1.65±0.38	1.02	264±198	135	2.67±1.53	5
	3	10.4±1.3	11.2	0.24±0.08	0.25	1.50±0.46	1.66	151±87	282	4.33±2.31	4
	4	5.4±1.1	5.9	1.62±0.03	1.68	0.16±0.01	0.19	6.4±6.2	7	9.67±5.51	13
Diazepam Group	5	13.9±2.9	12.4	0.23±0.13	0.57*	1.74±0.44	1.20*	261±142	206	6.00±1.00	14
	6	11.3±0.3	11.2	0.15±0.00	0.22*	2.52±0.32	1.89*	332±65	293	0.67±0.58	2
	7	12.5±2.8	11.2	0.12±0.08	0.23*	2.97±1.31	1.56*	413±306	209	2.67±3.06	5

*p<0.05, as compared with the results before treatment.

Table 3. Effects of sodium chloride or mannitol administration on the avoidance behavior in mice.
Sodium chloride (0.7%) or 5.0% mannitol solutions were given ad libitum as a drinking water for one or two weeks.

Treatments	Mouse No.	Indices									
		Response rate		Shock rate		Sum of squares of shock-shock intervals (x10 ³ sec ²)		Mean of S-R time (sec)		No. of S-R time less than 5 sec	
		Before	Treated	Before	Treated	Before	Treated	Before	Treated	Before	Treated
Control Group	17	12.4±1.5	12.2 (16.0) ^{a)}	0.42±0.20	0.35 (0.35)	1.23±0.62	0.65 (1.14)	145±77	123 (172)	9.00±3.46	8 (6)
	18	10.0±0.6	8.1 (10.4)	0.52±0.22	1.18 (0.73)	0.83±0.29	0.50 (0.46)	78±28	56 (69)	5.33±4.16	9 (1)
	21	8.1±2.3	7.4 (11.3)	1.18±1.23	1.05 (0.72)	0.60±0.36	0.82 (0.48)	68±43	40 (76)	7.67±2.31	10 (4)
	22	9.5±1.4	13.8 (9.9)	0.26±0.13	0.25 (0.28)	1.70±0.53	1.37 (1.69)	257±150	279 (191)	2.33±1.53	1 (1)
Sodium chloride Group	6	10.7±1.3	13.1 (10.9)	0.13±0.00	0.12 (0.18)	2.37±0.21	3.89 (1.94)	309±16	325 (285)*	0.33±0.58	3 (2)*
	7	12.1±0.2	9.8 (12.0)	0.40±0.09	0.42 (0.52)	1.09±0.41	0.89 (0.86)	138±69	106 (43)*	6.00±1.00	2 (9)*
	8	14.5±4.7	12.0 (11.2)	0.45±0.04	1.03 (1.65)	0.85±0.05	0.53 (0.29)	112±22	89 (80)*	6.67±1.15	3 (9)*
	9	11.5±1.6	12.3 (11.5)	1.60±0.16	0.98 (1.52)	0.62±0.10	0.96 (0.52)	183±12	179 (85)*	6.33±1.53	9 (9)*
Manni-tol Group	10	13.7±1.2	20.3* (19.7)*	0.37±0.15	0.30 (0.22)	1.33±0.25	1.50 (2.44)*	177±31	223* (299)*	5.33±0.58	4 (4)
	12	4.5±0.5	9.1* (16.1)*	2.51±0.49	0.80 (0.48)	0.21±0.04	0.53 (0.89)*	44±13	63* (135)*	2.67±2.08	3 (5)
	14	9.2±0.7	11.0* (13.9)*	0.41±0.11	0.25 (0.23)	1.03±0.23	1.43 (1.93)*	127±14	259* (215)*	4.67±0.58	0 (4)

a) Parentheses indicate the results in mice treated for 2 weeks. The results without parenthesis indicate those in mice treated for one week.

*p<0.05, as compared with the result before treatment.

Table 4. Summary of the results which are shown in Table 2 and 3.

Treatments ^{a)}	Indices ^{a)}				
	Response rate	Shock rate	Sum of squares of shock-shock intervals	Mean of S-R time	No. of S-R (< 5sec)
Control					
0.1mg / kg Diazepam		* ↑	* ↓		
0.7% NaCl(1W) ^{b)}					
0.7% NaCl(2W) ^{c)}				↓	↑
5% Mannitol(1W)	↑			↑	
5% Mannitol(2W)	↑		↑	↑	

a) Further details of treatments and of indices see Materials and Methods.

b) 1W; a week-treatment.

c) 2W; two weeks-treatment.

* ↑; increased ($p < 0.05$)

* ↓; decreased ($p < 0.05$)

Decreases of mean of S-R time and increases of No. of S-R time less than 5 sec were observed among mice which were given sodium chloride for two weeks. Increases of response rate and mean of S-R time were observed in mice which were given 5.0 % mannitol for one week. Increase of sum of squares of shock-shock intervals was also observed in addition to the above indices, when mice were given mannitol for two weeks.

On the basis of the above results, a summary of them was shown in Table 4. In the table, an effect of a treatment on index after a treatment was shown by an arrow : ↑ : increase, ↓ : decrease. If an index after a treatment was in the range of controls, it was not shown in the table.

DISCUSSION

In the present experimental system, significant increase of shock rate was observed among diazepam-treated mice on the avoidance behavior, although the effect of diazepam administration on the response rate could not be detected. These evidences were consistent with the results reported previously by Kuribara and Tadokoro (1984). These facts suggested that the experimental system in the present report was comparable with the original method reported by Sidman (1953).

Effects of administration with sodium chloride on the avoidance behavior could be detected by the means of S-R time and No. of S-R time less than 5 sec, although the classic indices could not detect any changes of the avoidance behavior. On the other hand, effects of administration with mannitol on the avoidance behavior could be detected not only by the classic index, the response rate, but also by the new indices such as sum of squares of shock-shock intervals and means of S-R time.

Although the significance of new indices in the behavior toxicology is not clear, it may be possible that new indices detect particular avoidance behaviors which can not be detected by the classic indices. If the significance of new indices was well evaluated, effects of administration with substances on the avoidance behavior might be characterized by combining the classic and new indices. Further studies are needed to appreciate the meanings of these new indices in physiological and toxicological aspects.

REFERENCES

- Bovet, D. Bovet-Nitti, F. and Oliverio, A. (1969) : Genetic aspects of learning and memory in mice. *Science*, **163**, 139-149.
- Cook, L. and Catanina, A. C. (1964) : Effects of drugs on avoidance and escape behavior. *Fed. Proc.*, **23**, 818-835.
- Davidson, A. B. (1982) : Effects of chlorpromazine on continuous avoidance behavior in mice. *Pharmacol. Biochem. Behav.*, **17**, 385-387.
- Fukushima, S., Shigemoto, M., Kato, I. and Otozai, K. (1957) : A relationship between interfering substances in flame spectrophotometry. *Mikrochim. Acta*, **3**, 35-70.
- Kuribara, H. and Tadokoro, S. (1981) : Correlation between antiavoidance activities of antipsychotic drugs in rats and daily clinical doses. *Pharmacol. Biochem. Behav.*, **14**, 181-192.
- Kuribara, H. and Tadokoro, S. (1983) : Comparison of susceptibilities to the effects of antipsychotic drugs on lever-press avoidance responses between mice and rats. *Japan J. Pharmacol.*, **33**, 1127-1133.
- Kuribara, H. and Tadokoro, S. (1984) : Conditioned lever-press avoidance response in mice ; acquisition processes and effects of diazepam. *Psychopharmacology*, **82**, 36-40.
- Renzi, P. and Sansone, M. (1971) : Discriminated lever-press avoidance behavior in mice. *Comm. Behav. Biol.*, **6**, 315-321.
- Sansone, M. Renzi, P. and Amposta, B. (1972) : Effects of chlorpromazine and chlordiazepoxide on discriminated lever-press avoidance behavior and intertrial responding in mice. *Psychopharmacologia*, **27**, 313-318.
- Seiden, L. S. and Dykstra, L. A. (1977) : *Psychopharmacology, a biochemical and behavioral approach*. Van Nostrand Reinhold, New York.
- Sidman, M. (1953) : Avoidance conditioning with brief shock and no exteroceptive warning signal. *Science*, **118**, 157-158.