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Enhancement of Avoidance-Suppressing Effect After Repeated Administration of Haloperidol and Serum Haloperidol in Rats

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HAYASHI, T., S. TADOKORO, H. HASHIMOTO AND M. NAKASHIMA. *Enhancement of avoidance-suppressing effect after repeated administration of haloperidol and serum haloperidol in rats.* PHARMAC. BIOCHEM. BEHAV. **17**(1) 131–136, 1982.—Adult male rats of the Wistar strain, which were trained under a discriminated lever-press avoidance schedule (intertrial interval; 25 sec, presentation of conditioned stimuli; 5 sec), were given SC 0.025–0.05 mg/kg of haloperidol at fixed intervals of 1, 3–4 and 7 days. The avoidance-suppressing effect of haloperidol was enhanced in parallel to the number of drug administrations until it attained a maximum level. The intensity of the maximum effect tended to be stronger, and the number of administrations necessary to attain it was smaller, when a higher dose was given. When the administration interval exceeded one day, the enhanced effect remained irreversible one month after withdrawal of drug administration. The enhancement of the effect was produced after repeated administrations in an experimental chamber, but not in a home cage. Temporal changes in serum haloperidol concentration were determined 30–90 min after 0.035 mg/kg given SC to the haloperidol-pretreated and saline-pretreated groups. No significant difference in the pharmacokinetic change was detected between the two groups. These results suggest that learning during the drug effect under repeated exposure to a fixed experimental situation influences the enhancing effect.

Haloperidol Repeated administration Avoidance response
Enhancement of avoidance-supressing effect Serum haloperidol Rats

EXPERIMENTAL and clinical effects of psychotropic drugs are often modified by repeated administration. Studies have hitherto been made mainly as regards tolerance, while in recent years the focus has shifted to the enhancement of drug effects. Increased CNS sensitivity to stimulation by cocaine following chronic administration was first demonstrated in dogs by Tatum and Seevers [30]; this phenomenon is called reverse tolerance [24]. Similar phenomena with other CNS stimulants have been reported [19, 22, 25, 26, 32]. The enhancing effect of CNS-depressants such as antipsychotic drugs, has been also noted by us [7,14] and other investigators [18,20] after repeated administration.

We also observed that the repeated administration of d-amphetamine [6, 8, 17, 29], methamphetamine [1, 8, 9] or morphine [6,29] at fixed intervals of 1–7 days produced marked enhancement of their acute effects on locomotor acceleration in mice. In addition, we reported that the enhancing effect was detected only when the animals were placed in the activity cages where they were able to move freely during the stage of the acute effects [1,9]. These results suggest that phenomenon of the enhancing effect is influenced by environmental situations in which an animal is exposued while under the drug effect. A similar phenomenon was observed in the effect of diazepam on punished responding in rats [12]. In the present work, the effect of repeated administration of haloperidol on a conditioned avoidance response (CAR), especially with regard to an involvement of environmental factors, was investigated in rats.

METHOD

Experimental Animals

Male rats of the Wistar strain were provided by the breeding colony of Gunma University Medical School. They were given a solid diet MF (Oriental Yeast Co., Tokyo) and tap water ad lib. The training of rats was begun at 70 days of age when the rats weighed an average of 270 g. Rats which showed a stable behavioral baseline in both avoidance (90% and over) and response rates for at least 5 consecutive sessions were used.

Conditioned Avoidance Schedule

Rats were trained to press a lever under a discriminated avoidance schedule in an experimental chamber 27(D)×20(W)×18(H) cm [5]. The schedule consisted of a 25 sec intertrial interval and a 5 sec conditioned stimulus (CS) presentation (small lamp and buzzer) without escape contingency. A shock of 110 V, 0.5 mA AC for 0.5 sec was delivered through the floor grid. Each session consisted of 1 hr of

TABLE 1
EXPERIMENTAL SCHEDULES FOR REPEATED ADMINISTRATION OF HPD

Groups	Doses of HPD (mg/kg)	Administration intervals (days)	Number of animals
I	0.025	7	6
II		1	6
III	0.035	3–4	6
IV		7	6
V	0.05	7	6
VI	1st=0.2 2nd~10th=0.035	3–4	8
VII	0.035	3–4*	5
VIII	Saline 1 ml/kg	3–4	8

*The 1st and 5th–8th administrations were performed in the experimental chamber and the 2nd–4th in the home cage.

training per day, and the training was held every other day until establishment of the behavioral baseline. All the experiments were performed from 10:00 to 20:00. The indices of CAR were the response rate (lever presses/min) and the avoidance rate (avoidance responses/numbers of CS presentation). Behavior controlling and recording apparatuses (GT 7705 and GT 7715, O'hara Co., Ltd., Tokyo) were placed in a separate room. Gross behavior was observed by a TV monitor.

Drug Used and Administration Schedules

Haloperidol (HPD) (Serenase Inj., Dainihon Pharmaceutical Co.) was used. Doses were made up to the volume of 1 ml/kg of body weight with physiological saline solution (saline) and were given SC. CAR was observed for 90 min immediately after HPD and for 60 min after saline in Groups I–VI and VIII only when the acute effects were tested.

All treatments studied are listed in Table 1. Groups I, IV and V were given 0.025, 0.035 and 0.05 mg/kg of HPD, respectively, at a fixed interval of 7 days. In Groups II and III, a fixed dose of 0.035 mg/kg was given at different intervals of 1 and 3–4 days, respectively. In Group VI, 0.2 mg/kg of HPD was given only in the first administration and thereafter 0.035 mg/kg was given at a fixed interval of 3–4 days. Group VIII was given 1 ml/kg of saline at a fixed interval of 3–4 days. In order to elucidate the relationship between the modification of the effect produced by repeated administration of HPD and the environmental factors to which the animals were exposed, the following experiment was performed: In Group VII, 0.035 mg/kg of HPD was given in the first administration and the acute effect on CAR was observed for 90 min immediately after administration in the experimental chamber. The animals were run in the avoidance procedure for 60 min at a fixed interval of 3–4 days in sessions 2nd–4th, and were given the same dose (0.035 mg/kg) of the drug each time immediately after they were returned to the home cage. In the 5th–8th administration, CAR was observed again in the experimental chamber after each administration.

Determination of Serum HPD Concentrations

For determination of serum HPD, 14 rats in Groups III and VI, which showed a stable enhancement of the effect, were used 3–4 days after the 11th administration of HPD. Eight rats of Group VIII, which were given 1 ml/kg of saline for 10 times at a 3–4 days interval and showed a stable CAR after each administration, were used also for this experiment. All rats were restrained on their backs by CFK-1S type fixation apparatus (CFK Co., Tokyo) without anesthesia, and blood samples of 0.5 ml each were obtained by a puncture from the jugular vein 30, 60 and 90 min after 0.035 mg/kg of HPD. This method was specifically established, and it was not difficult to obtain a blood sample within a short time. The serum was separated by centrifugation and kept frozen (−40°C) until assayed for HPD. Serum HPD concentrations were determined by radioimmunoassay according to Suzuki *et al.* [28]. The procedure was briefly as follows: The assay was carried out in plastic tubes. One tenth milliliter of sample or control serum and 0.2 ml of phosphate buffer (0.075 M, pH=7.4) or a standard solution were added to 0.1 ml of ^{3}H-haloperidol solution (1.86×10^4 dpm, 300 pg). Then, 0.3 ml of the diluted antiserum was added to all the assay tubes except for blank tubes to which normal rabbit serum (×100) was added. After adjusting the volume of each tube to 1.1 ml with the phosphate buffer, the contents were mixed and allowed to stand at 4°C for 4 hr. To separate the antibody-bound and free drug, 0.2 ml of dextran-coated charcoal suspension was added to each tube, and the tubes stood at 4°C for 10 min. After centrifugation, 1 ml of the supernatant was pipetted into a counting vial containing 10 ml of scintillation cocktail (Riaflour, New England Nuclear Corp., Boston, MA), and the radioactivity was counted in a scintillation spectrometer (Aloka LSC-671). HPD concentration was calculated using the standard curve obtained.

Statistical Analysis

Results obtained were analysed by use of Student's t-test. They were considered significant when p was equal to or less than 0.05.

RESULTS

Enhancement of the CAR-Suppressing Effect Following Repeated Administration of HPD

Relation with doses given. The correlation between changes in mean response and avoidance rates for 90 min and the number of the drug administrations is represented by the histograms in Fig. 1. The mean response and avoidance rates after saline are also shown in the same figure. Small changes in the CAR-suppressing effect of HPD were observed in the first administration and thereafter progressive enhancement of the effect was produced independent of the doses given. The doses used in this experiment did not induce any excessive physical symptom such as marked sedation or catalepsy even after 0.05 mg/kg in a home cage. The response rates after the 9th administration of 0.025 mg/kg, the 7th of 0.035 mg/kg and the 4th of 0.05 mg/kg were significantly lower when compared with those in the first administration of each corresponding dose. Significant differences in the avoidance rate were observed after 8th administration of 0.035 mg/kg, and the 4th of 0.05 mg/kg, when compared with those in the first administration of each corresponding dose, but no significant difference was seen after administration of 0.025 mg/kg. The CAR-suppressing effects were enhanced by repeated administration regardless of dosage, while the

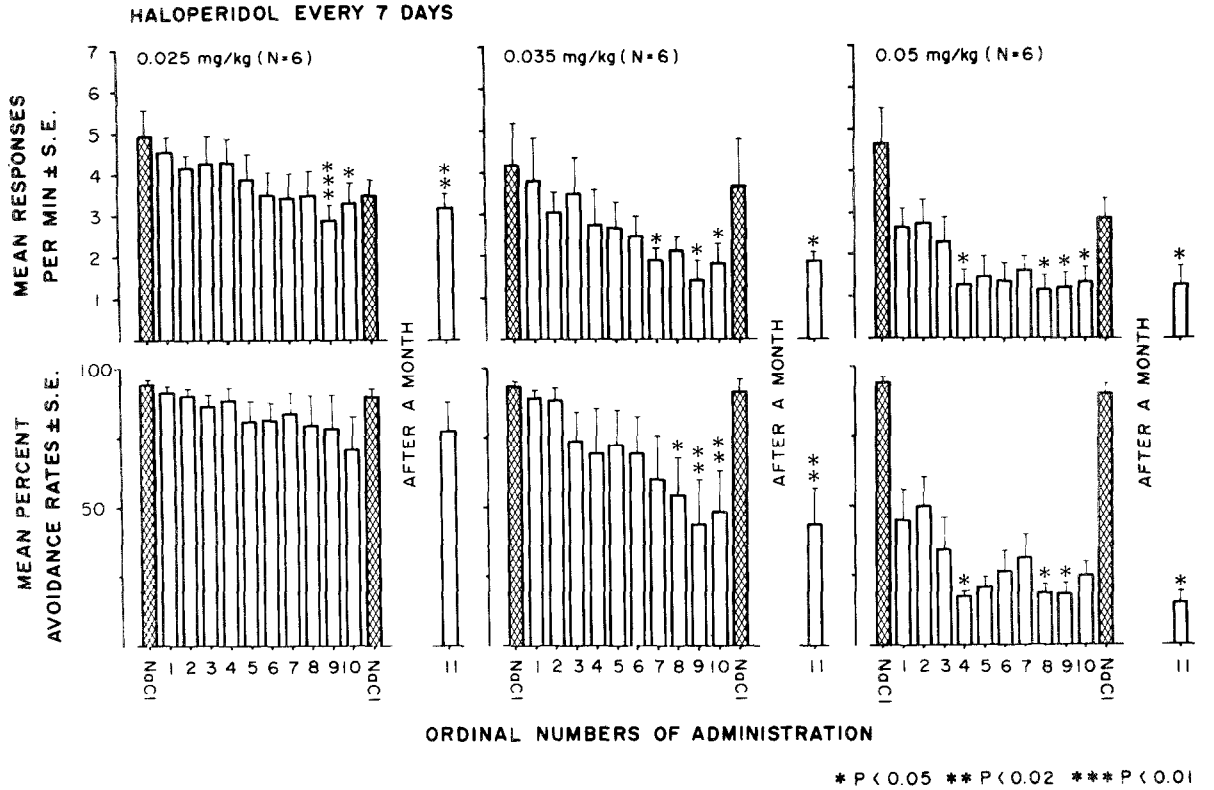

FIG. 1. Changes in CAR-suppressing effect of HPD given at a fixed interval of 7 days in Groups I, IV and V, respectively. The asterisk denotes significant difference from the value obtained in the first administration of each corresponding dose.

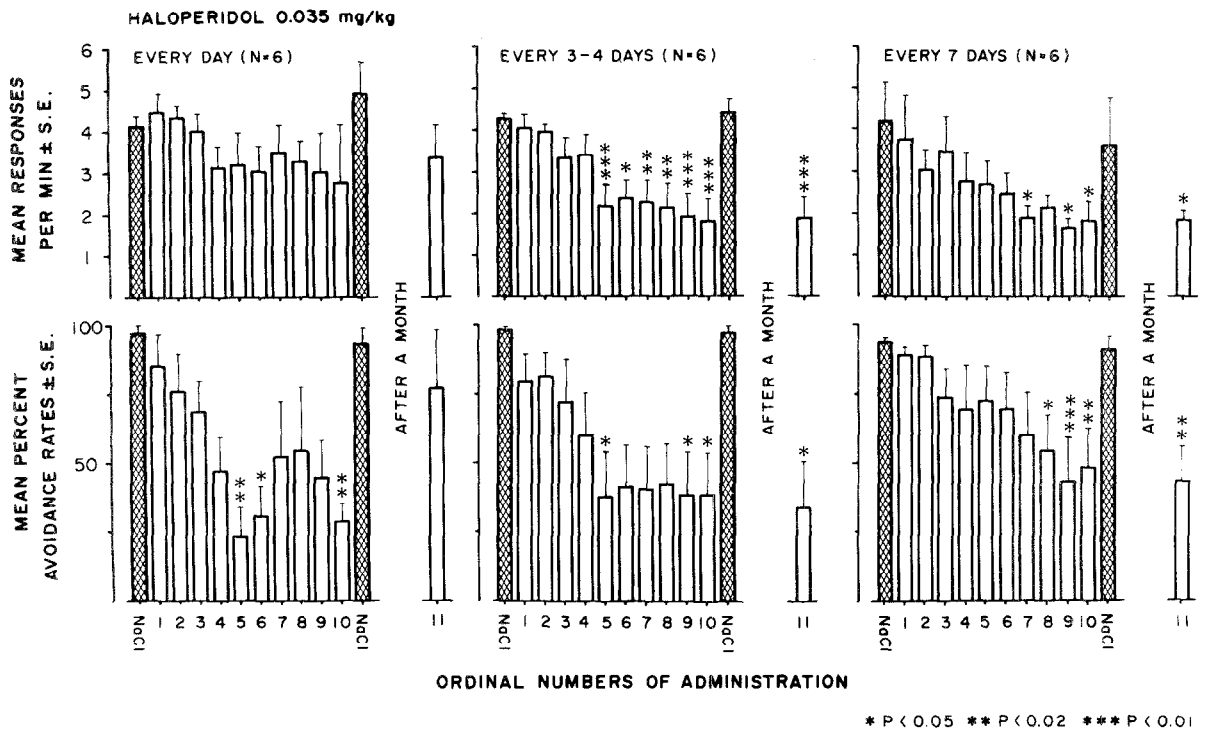

FIG. 2. Changes in CAR-suppressing effect of HPD given at fixed intervals of 1, 3–4 and 7 days in Groups II, III and IV, respectively. The asterisk denotes significant difference from the value obtained in the first administration at each corresponding interval.

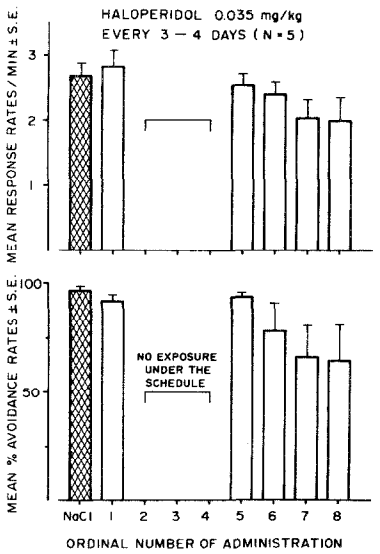

FIG. 3. Effect of environmental factors on the enhancement of CAR-suppressing effect of HPD produced by repeated administration in Group VII. HPD 0.035 mg/kg was given in the first administration and the acute effect on CAR was observed in the experimental chamber. However, the animals were not run in the avoidance procedure after the same dose of HPD at the same interval in the 2nd–4th sessions. In the 5th–8th administrations, the CAR was observed again in the experimental chamber.

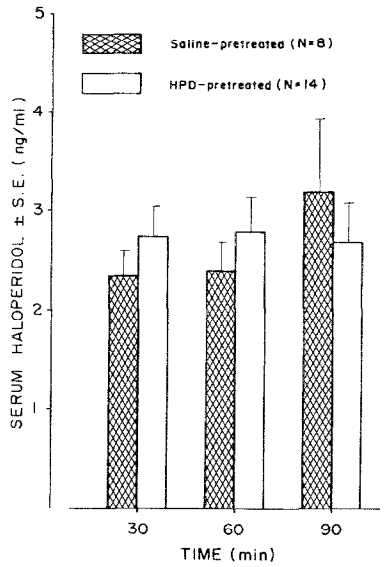

FIG. 4. Temporal changes in serum HPD concentration in HPD-pretreated (Groups III, VI) and saline-pretreated (Group VIII) groups.

maximum effects were marked in a dose-dependent manner. Thus the number of administrations necessary to attain the maximum effect tended to be fewer after higher doses of HPD. As shown in Figs. 1 and 2, the response and avoidance rates after saline following the 10 times of HPD administration did not differ significantly from those obtained after saline preceding the first drug administration. Thus the behavioral baseline did not change following the repeated administration of HPD.

Relationship to administration intervals. Figure 2 represents changes in response and avoidance rates following 10 times of administration of 0.035 mg/kg of HPD at fixed intervals of 1, 3–4 and 7 days. The progressive enhancement of the CAR-suppressing effect was observed in all the groups regardless of the intervals, and attained the maximum decrease in the 5th administration at intervals of 1 and 3–4 days, and in the 9th at an interval of 7 days. No significant differences were observed among the nadir of avoidance in all the groups.

The CAR was markedly suppressed in Group VI when 0.2 mg/kg of HPD was given only in the first administration. However, the suppressing effects obtained from the 2nd to 10th administration of 0.035 mg/kg of the drug at a fixed interval of 3–4 days did not differ significantly from those obtained by repeated administration of the same dose in the initial administration. The maximum effect was obtained in the 6th administration and stabilized thereafter.

CAR one month after withdrawal of HPD. The animals in Groups I–V were withdrawn from HPD after repeated administration and kept in the home cage for a month after the 2nd administration of saline. Then they were given HPD again in the same doses used previously (11th administration, Figs. 1 and 2). The maximum effects were reproduced in all the groups except for one group which was exposed to daily administration. The CAR-suppressing effect once enhanced was well maintained after a long period of withdrawal of the drug.

Environmental Factors and Enhancement of the CAR-Suppressing Effect of HPD

When Group III was repeatedly given HPD at a fixed interval of 3–4 days in the experimental chamber, the CAR-suppressing effect had already attained the maximum level by the 5th administration, as shown in Fig. 2. However, in Group VII, which was not placed in the experimental chamber while under the influence of the drug during the 2nd to the 4th administrations, the enhancing effect after HPD was not produced in the experimental chamber following the 5th administration of the drug (Fig. 3). Enhancement of the effect was observed only when the animals under the drug influence were exposed repeatedly to the avoidance situation in the experimental chamber.

Temporal Changes in Serum HPD Concentration

Maximum serum HPD concentrations were obtained within 30 min after administration of the drug in both HPD-pretreated (Groups III, VI) and saline-pretreated (Group VIII) groups, and the level was maintained for at least 60 min thereafter. No significant pharmacokinetic difference in the concentrations was detected between the groups. The results are represented in Fig. 4.

DISCUSSION

The present experiment demonstrated that the CAR-suppressing effect of HPD was progressively enhanced after various schedules of repeated administrations of the drug, and finally attained the maximum level. Moreover, the number of administrations necessary to attain the maximum suppression was dependent on the dosage and the interval between the repeated administrations. Although the dose was limited to a range in which no excessive physical symp-

toms were elicited, the effect once enhanced was considered to be irreversible.

The present experiment also showed that the enhancing effect was not produced when the animals were given HPD repeatedly in their home cages without exposure to the avoidance situation. Furthermore, significant differences were neither produced in behavioral baselines between experiments in the 1st and the 2nd administration of saline in Groups I–V, nor were significant differences observed in serum HPD concentration between HPD-pretreated and saline-pretreated groups. These results suggest that the enhancing effect may be not associated with either accumulation or change in absorption of HPD. However, the enhancement may be closely related to the environmental situations to which the animals have been exposed while under the drug effect. Schuster [23] emphasized that interaction between the contingencies of reinforcement and the drug effect was an extremely important variable in behavioral changes. In fact, psychotropic drugs sometimes show different behavioral effects in different circumstances, and the effects are also influenced by past or present experiences [21, 27, 31].

We have observed progressive enhancement of motor-accelerating effects of d-amphetamine, methamphetamine or morphine in mice, when appropriate doses of the drugs were given at intervals of 1–7 days [1, 6, 8, 9, 17, 29]. However, the enhancing effect was not detected when the animals were placed individually in narrow jars where their movement was strongly impaired during the stage of acute effects of the drugs [1,9]. Our results suggest that the enhancing effects are detectable for many psychotropic drugs only when appropriate experimental determinants are provided. Thus, the drug experiences associated with these conditions, i.e., learning associated with the drug influence, is important for the enhancing effect.

Kuribara and Tadokoro [13] indicated a clear correlation between the critical doses for CAR-suppressing effect of various antipsychotic drugs in rats and their daily clinical doses. The present results are also important for the preclinical evaluation of antipsychotic drugs, since the effect is sometimes weak and unstable when the drugs are given acutely to drug-naive rats. Data are more reliable from animals exposed to sufficient drug experiences to show a stable susceptibility to the drug.

The neurochemical mechanism of the enhancing effect observed in the present experiment has not yet been elucidated. Increased dopamine turnover or receptor sensitivity in dopamine neurons in the brain is considered to be a possible mechanism for behavioral changes produced by chronic administration of antipsychotic drugs [2–4, 10, 11, 15, 16]. Among these works, there are several investigations in which CAR was used as a indicator [3, 11, 16], but in contrast with the present results, only tolerance to the CAR-suppressing effects was reported. In these works, however, relatively high doses and prolonged pretreatment were employed. Moreover, the animals were not exposed to a conditioned avoidance situation while under the drug effects. Correlation between the enhancement phenomenon of the behavioral effects of HPD and the neurochemical changes in the brain needs to be elucidated.

REFERENCES

1. Alam, M. R. Enhancement of motor-accelerating effect induced by repeated administration of methamphetamine in mice: Involvement of environmental factors. *Jap. J. Pharmac.* **31:** 897–904, 1981.
2. Christensen, A. V., B. Fjalland and I. M. Nielsen. On the supersensitivity of dopamine receptors induced by neuroleptics. *Psychopharmacology* **48:** 1–6, 1976.
3. Danneskiold-Samsoe, P. and V. Pedersen. Inhibition of conditional avoidance response by neuroleptics upon repeated administration. *Psychopharmacology* **51:** 9–14, 1976.
4. Fjalland, B. and I. M. Nielsen. Enhancement of methylphenidate-induced stereotypies by repeated administration of neuroleptics. *Psychopharmacologia* **34:** 105–109, 1974.
5. Hayashi, T., H. Kuribara and S. Tadokoro. How to observe acquisition using operant conditionings in rats. *Biol. Sci.* **30:** 127–134, 1978. (In Japanese)
6. Hayashi, T., K. Ohashi and S. Tadokoro. Conditioned drug effects to d-amphetamine- and morphine-induced motor acceleration in mice: Experimental approach for placebo effect. *Jap. J. Pharmac.* **30:** 93–100, 1980.
7. Hayashi, T. and S. Tadokoro. Environmental factors and effect change in psychotropic drugs when given repeatedly. *Biol. Sci.* **23:** 75–82, 1980. (In Japanese)
8. Hirabayashi, M., F. Iwai, M. Iizuka, T. Mesaki, M. R. Alam and S. Tadokoro. Individual differences in the accelerating effect of methamphetamine, d-amphetamine and morphine on ambulatory activity in mice. *Folia pharmac. jap.* **75:** 683–693, 1979. (In Japanese)
9. Hirabayashi, M. and M. R. Alam. Enhancing effect of methamphetamine on ambulatory activity produced by repeated administration in mice. *Pharmac. Biochem. Behav.* **15:** 925–932, 1981.
10. Hyttel, J. Changes in dopamine synthesis rate in the supersensitivity phase after treatment with a single dose of neuroleptics. *Psychopharmacology* **51:** 205–207, 1977.
11. Irwin, S. Differential tolerance development to the avoidance and locomotor suppressant actions of perphenazine and chlorpromazine. *Pharmacologist* **3:** 75, 1961.
12. Kuribara, H. and S. Tadokoro. Schedule dependent change of punished responding after diazepam in rats. *Jap. J. Pharmac.* **30:** 264–268, 1980.
13. Kuribara, H. and S. Tadokoro. Correlation between antiavoidance activities of antipsychotic drugs in rats and daily clinical doses. *Pharmac. Biochem. Behav.* **14:** 181–192, 1981.
14. Kuribara, H. and S. Tadokoro. Enhancement of the avoidance-suppressing effects of neuroleptic agents after repeated administration: Changes in the discriminated avoidance response in rats. *Psychopharmacology* **75:** 148–151, 1981.
15. Muller, P. and P. Seeman. Dopaminergic supersensitivity after neuroleptics: Time-course and specificity. *Psychopharmacology* **60:** 1–11, 1978.
16. Nielsen, I. M., B. Fjalland, V. Pedersen and M. Nymark. Pharmacology of neuroleptics upon repeated administration. *Psychopharmacologia* **34:** 95–104, 1974.
17. Ohashi, K. and S. Tadokoro. Effects of thyroidectomy on amphetamine-induced acceleration of locomotor activity in mice. *Jap. J. Pharmac.* **26:** 621–624, 1976.
18. Post, R. M. and F. K. Goodwin. Time-dependent effects of phenothiazines on dopamine turnover in psychiatric patients. *Science* **190:** 488, 1975.
19. Post, R. M., R. T. Kopanda and K. E. Black. Progressive effects of cocaine on behavior and central amine metabolism in rhesus monkeys: Relationship to kindling and psychosis. *Biol. Psychiat.* **11:** 403–419, 1976.

20. van Praag, H. M. *Psychotropic Drugs*. Assen: Van Corcum, 1978.
21. Rushton, R., H. Steinberg and M. Tomkiewicz. Equivalence and persistence of the effects of psychoactive drugs and past experience. *Nature* **220:** 885–889, 1968.
22. Schreiber, H. L., W. G. Wood and R. H. Carlson. The role of locomotion in conditioning methylphenidate-induced locomotor activity. *Pharmac. Biochem. Behav.* **4:** 393–395, 1976.
23. Schuster, C. R. Theoretical basis of behavioral tolerance: Implications of the phenomenon for problems of drug abuse. In: *Behavioral Tolerance: Research and Treatment Implications*, edited by N. A. Krasnegor. NIDA Research Monograph 18. Rockville, MD: National Institute of Drug Abuse, 1978, pp. 4–17.
24. Seevers, M. H. and G. A. Deneau. Physiological aspects of tolerance and physical dependence. In: *Physiological Pharmacology*, vol. 1, edited by W. S. Root and E. G. Hofmann. New York: Academic Press, 1963, p. 629.
25. Segal, D. and A. J. Mandell. Long-term administration of d-amphetamine: Progressive augmentation of motor activity and stereotypy. *Pharmac. Biochem. Behav.* **2:** 249–255, 1974.
26. Shuster, L., G. Yu and A. Bates. Sensitization to cocaine stimulation in mice. *Psychopharmacology* **52:** 185–190, 1977.
27. Sparber, S. B., H. A. Tilson and D. W. Peterson. Environmental influences upon morphine or d-amphetamine induced suppression of operant behavior. *Pharmac. Biochem. Behav.* **1:** 133–136, 1973.
28. Suzuki, H., Y. Minaki, M. Iwaisaki, Y. Sekine, A. Kagemoto, Y. Utsui, M. Hashimoto, G. Yagi and H. Itoh. Determination of haloperidol in human serum by radioimmunoassay. *J. Pharm. Dyn.* **3:** 250–257, 1980.
29. Tadokoro, S. *Drugs and Behaviors*. Tokyo: Soft Science Inc., 1980, pp. 113–130. (In Japanese)
30. Tatum, A. L. and M. H. Seevers. Experimental cocaine addiction. *J. Pharmac. exp. Ther.* **36:** 401–410, 1929.
31. Tilson, H. A. and R. H. Rech. Prior drug experience and effects of amphetamine on schedule controlled behavior. *Pharmac. Biochem. Behav.* **1:** 129–132, 1973.
32. Tilson, H. A. and R. H. Rech. Conditioned drug effects and absence of tolerance to d-amphetamine induced motor activity. *Pharmac. Biochem. Behav.* **1:** 149–153, 1973.