3. General Method

Subjects

The subjects were male Wistar-Imamichi rats (250 to 350 g), individually housed in a temperature-controlled colony room on a 12:12-hr light-dark cycle. Lights in the colony room were illuminated from 8:00 to 20:00. Animals were given ad-lib access to water.

Apparatus

The apparatus were an elevated (50 cm above the floor) 8-arm radial maze and elevated (50 cm above the floor) plus maze made of gray Plexiglas.

The illustration of the radial arm maze is shown in Fig. 10. In the 8-arm radial maze, each arm measured 59×12 cm, and the diameter of the hexagonal center platform was 35.5 cm. Transparent Plexiglas guillotine doors separated arms from the center platform. The food cups (3.0 cm in diameter, 1.0 cm deep) at the distal end of each arm served as reward-wells. The brightness of the center platform was 210 lx. The maze was surrounded by several extramaze cues fixed throughout the experiment. A rough drawing of the context of experimental room is shown in Fig. 11.

The illustration of the plus maze is shown in Fig. 19. In the plus maze, the side of the square center platform was 11 cm. Gray Plexiglas guillotine doors separated arms from the center platform. The size of each arm, the position of each reward-well, and the brightness of the center platform were identical to those of the 8-arm radial maze. The maze

was surrounded by several extramaze cues fixed throughout the experiment. A rough drawing of the context of experimental room is shown in Fig. 21.

Drug

The drug was ethylcholine mustard aziridinium ion (AF64A). AF64A was prepared by the method of Fisher, Mantione, Abraham, and Hanin (1982) with some modification. The acetylethylcholine mustard hydrochloride (Research Biochemical Inc., MA.) was dissolved in distilled water and was adjusted to pH 11.3-11.7 with 8 N and 1 N NaOH. Then the solution was maintained within the same pH range for 30 min. Subsequently, the pH was reduced to about 5.0 with 6 N and 1 N HCl, and finally adjusted to pH 7.4 with $NaHCO_3$ aqueous solution. Final concentrations of AF64A were 0.20 nmol/ μl, 0.50 nmol/ μl (Exp. 2), and 0.15 nmol/ μl (Exp. 3, Exp. 4 and Exp. 5). The solution was kept at 4℃ until injection conducted within 6 hr after preparation.

Surgery

Before surgery, animals were anesthetized with 40 mg/kg of sodium pentobarbital. AF64A or saline was injected bilaterally into the striatum or the hippocampus using standard stereotaxic techniques together with the stereotaxic atlas of Paxinos and Watson (1986). Coordinates for striatal injection were AP=+0.7 mm from bregma, ML= ± 2.8 mm, DV=-4.6 mm from dura and those for hippocampal injection were AP=-3.8 mm from bregma, ML= ± 2.7 mm, DV=-2.8 mm from dura in Exp. 3. In Exp.

4, Exp. 5, and Exp. 6, coordinates for striatal injection were AP= ± 0.5 mm from bregma, ML= ± 3.0 mm, DV=-4.5 mm from dura and those for hippocampal injection were AP=-3.8 mm, ML= ± 2.2 mm and 3.4 mm from bregma, DV=-3.0 mm (ML= ± 2.2 mm) and -3.4 mm (ML= ± 3.4 mm) from dura. The solutions were injected in a volume of 5 μl over 5 min (Exp. 3) or in a volume of 3 μl over 3 min (Exp. 4. Exp. 5, and Exp. 6) through a Hamilton syringe which was left in place for an additional 5 min before withdrawal to allow for sufficient diffusion of the drug.

Statistical analysis

In evaluating behavioral data, statistical analysis were employed. The days to criterion were evaluated by Kruskal-Wallis test (H test). The statistical significance of differences in the days to criterion among groups was determined by Mann-Whitney test (U test). The number of correct choices were evaluated by 1- or 2-factor ANOVA with repeated measures (Exp. 3, 4, 5: drug × dayblock; Exp. 6: drug × training × dayblock). Either tests of simple main effects or Tukey-Kramer's post hoc tests were employed to determine the significant differences among groups depending on the results of ANOVA with repeated measures. The biochemical data were evaluated by 1- or 2-factor ANOVA (drug; drug × training) followed by Tukey-Kramer's post hoc tests.