

changes in the neurochemicals during brain sampling (Ikarashi et al., 1985, 2004). The brain was removed from the skull and dissected into seven regions (cerebral cortex, hippocampus, midbrain–thalamus, striatum, hypothalamus, medullas and cerebellum) according to the established method (Glowinski and Iversen, 1966), and then each region was weighed. Each dissected brain region was homogenized with 0.5 ml of 0.1 mol/L perchloric acid containing 0.8 nmol isoproterenol hydrochloride as an internal standard for the determinations of catecholamines, indoleamines and related metabolites, and 5 nmol ethylhomocholine iodide as an internal standard for the determinations of ACh and Ch, using an ultrasonic cell disrupter (US-300T, Nissei, Tokyo, Japan). The homogenate was centrifuged at 17,300 g at 4 °C for 15 min. The supernatant was filtered through a 0.45 µm millipore filter. Aliquots, typically 10 µl of the filtrates, were injected into the liquid chromatographic systems (Eicom HPLC-ECD system, Eicom Co. Ltd., Kyoto, Japan) to determine catecholamines-, indoleamines-, and acetylcholine-related substances (Ikarashi et al., 1992). The sediment was re-homogenized with 2 ml of 1 mol/L NaOH for a protein assay, which was performed using the method of Lowry et al. (1951). The concentrations of neurochemicals in the brain were expressed as the values per milligram of protein.

4.5. Behavioral experiments

4.5.1. Rotarod test

Tg α SYN ($n=10$) and age-matched control mice ($n=10$) at one month to 10.5 months old were examined by the rotarod performance test according to a previous method (Kuribara et al., 1977; Ikarashi et al., 2004). Mice were placed on the rotating rod of the apparatus (Ugo basile, biological research apparatus, Milan, Italy) at a speed of 16 rpm for 300 s, and the time they stayed on the rotating rod was measured. Each set of three trials was performed at 10 minute intervals each day for every mouse.

4.6. Statistical analyses

Two-way repeated measures ANOVA followed by the Mann-Whitney *U* test for the rotarod test and open field test, and two-way ANOVA followed by Student's *t* test to analyze neurochemicals were performed using GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA) and SPSS 10.0 (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL). The means of all data are presented with their standard errors (mean \pm S.E.).

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