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Involvement of microtubule integrity in memory impairment caused by colchicine

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Abstract

In order to fully evaluate the effects of colchicine treatment on learning ability in rats, colchicine was administered, and both Morris water maze (MWM) and step-through type passive avoidance (PA) learning tests were conducted. In both learning tests, infusion of colchicine into the rat dentate gyrus, at two distinct bilateral rostrocaudal locations, potently impaired memory function in a dose-dependent manner (0.01–2.0 μg/site), whereas systemic injection of colchicine (50–300 μg/kg) did not. In the MWM test, memory impairment was observed even at doses where there was no evidence of any histological changes in the dentate granule cells. This suggests that functional deterioration, that is, learning impairment was induced by the dysfunction of microtubules and/or axons, was caused by colchicine. Moreover, ameliorated learning behavior was observed with chronic treatment of β-estradiol 3-benzoate, which has been suggested to have an important role as an adjuvant treatment for younger Alzheimer's disease (AD), immediately after colchicine infusion (0.3 μg). These results indicate that the animal model accompanying the colchicine-induced functional defect showing early tau pathology, but not neuronal cell degeneration, may well mimic comparatively early stage of AD. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Colchicine; Water maze; Microtubule; Estradiol; Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disease characterized clinically by both severe memory loss and personality changes, and pathologically by both synaptic loss and neuronal death in the vulnerable areas associated with the formation of neurofibrillary tangles (NFTs) and senile plaques (SPs), both pathological hallmarks of AD. The NFTs are composed of intracellular hyperphosphorylated tau, and the extracellular SPs contain A β. Dementia severity is even more significantly correlated with the number of NFTs than that of SPs (Arriagada et al., 1991; McKee et al., 1991; Hyman and Tanzi, 1992; Cummings et al., 1996; Brion, 1998), and the topography of the SPs differs from that of neuronal degeneration in AD. Furthermore, neurofibrillary changes and neuronal death are not observed in transgenic animals with APP mutations (Games et al., 1995; Hsiao et al., 1996), suggesting greater significance of tau pathology in AD but not A β on memory function.

Although the exact role of PHF and A β in the pathogenesis of AD is not established, there is growing evidence from a number of laboratories that the intellectual deterioration in AD patients is associated with neurofibrillary degeneration (Tomlinson et al., 1970; Alafuzoff et al., 1987; Arriagada et al., 1991; Dickson et al., 1991). In tau pathology, hyperphosphorylation of tau in AD or several missense mutations of the *tau* gene in familial front-temporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Hutton, 1999; Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998; Jicha et al., 1999; Mirra et al., 1999) are considered to result in reduced binding ability of tau to microtubules (Hasegawa et al., 1998; Hong et al., 1998), important neuronal cytoskeletal proteins and a lack of microtubule assembly, leading to impairment of axonal transport, then finally neuronal cell death.

We attempted to investigate whether tau pathogenesis can be successfully manipulated pharmacologically by using colchicine, an alkaloid derivative that blocks the axonal transport via depolymerization of microtubules

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(Griffin et al., 1983; Peters et al., 1991) without inhibiting protein synthesis (Suzuki and Terry, 1967). Because the connections made by the entorhinal cortex/perforant path projection to the dentate gyrus are known to become more sparse both in normal aging and, to a greater degree, in AD (Hyman et al., 1986; Geinisman et al., 1992; Rapp et al., 1999), and dystrophic axons which appeared to be prominent and widespread are particularly abundant in the hippocampal fiber systems originating from the subiculum, CA1, and the entorhinal cortex in AD (Gibson, 1987; Su et al., 1993), we infused colchicine in rostrocaudal regions of the dentate gyrus to imitate AD pathogenesis in this study.

Although it has been previously reported that intradentate infusion of colchicine destroyed granule cells in the dentate gyrus of the hippocampus (Goldschmidt and Steward, 1982; Lothman et al., 1982; Jarrard et al., 1984; Walsh et al., 1986; Tilson and Peterson, 1987; Tilson et al., 1987) and induced learning impairment in various learning tasks (Walsh et al., 1986; Tilson et al., 1988; Nanry et al., 1989; Tandon et al., 1991), all of the previous reports show learning impairment only at doses where colchicine can induce distinct destruction of the dentate granule cells, concluding that the integrity of the granule cell layer plays a crucial role for memory function. Moreover, as their previous reports have not scrutinized dose-dependent effects of colchicine on either histological changes or memory function, we report in detail here the dose-responsive effects on them for the first time. As an additional effort, we examined the effect of systemic injection of colchicine.

Finally, we investigated the effect of estradiol, which has been suggested to have an important role as an adjuvant treatment for younger AD, in order to validate this animal model since considerable evidence emerged from neuropathological studies (Luine, 1985; Gould et al., 1990; Toran-Allerand et al., 1992), animal behavioral studies (Joseph et al., 1989; McDermott et al., 1994) and human investigations (Sherwin, 1988; Ditkoff et al., 1991) to suggest that estradiol may be beneficial in improving cognition and mood in AD. We report here that subcutaneous implantation of a 14-day sustained release formulation of β-estradiol 3-benzoate immediately after colchicine infusion into the dentate gyrus ameliorated deranged learning behavior.

These results have been presented in abstract form (Nakayama and Sawada, 2000).

2. Materials and methods

2.1. Materials

Animals used in this experiment (male Sprague–Dawley rats) were supplied by Charles River Japan (Yokohama, Japan). Colchicine and β-estradiol 3-benzoate were obtained from Sigma-Aldrich (Tokyo, Japan), and microperfusion of β-estradiol 3-benzoate was regulated using an ALZET osmotic minipump (ALZA, Palo Alto, CA). Tubulin Ab-4 and AutoProbe II kit for tubulin staining were obtained from Neomarkers (Fremont, CA) and Biomeda (Foster, CA), respectively. The Morris water maze (MWM) and the step-through type passive avoidance (PA) instruments were from Neuroscience (Tokyo, Japan). Other reagents used in this experiment were derived from Wako (Osaka, Japan).

2.2. Treatment

2.2.1. Drug intrahippocampal infusion of colchicine

Male Sprague–Dawley rats weighing 245–319 g were used in this experiment. All experiments were carried out according to the guidelines of the Japanese Community's Council for Animal Experiments. All efforts were made to minimize animal suffering and to reduce the number of animal used. During surgical procedures, the animals were anesthesized with sodium pentobarbital (50 mg/kg) and restrained in a stereotaxic apparatus. Colchicine (0.01, 0.03, 0.1, 0.3, 1.0 or 2.0 μg dissolved in 0.5 μl sterile saline) was infused into the dentate gyrus at two distinct bilateral rostrocaudal locations, the rostral being 1.8 mm lateral to the midline, 3.3 mm posterior to bregma and 3.3 mm ventral to the cortical surface, and the caudal being 4.7 mm lateral, 5.3 mm posterior and 5.5 mm ventral. Colchicine and sterile saline were infused slowly using a 10-μl Hamilton syringe, which was left in place for 3 min to allow diffusion of the perfusate. Animals were exposed to behavioral testing 1 week following the colchicine or saline infusion. Thereafter, 15 days after infusion, the rat brain was removed. The control animals without any treatment (normal control) were evaluated together as behaviorally unchanged controls and animals infused saline were defined as sham-operated controls (saline control).

2.2.2. Systemic injection of colchicine

Male Sprague–Dawley rats weighing 319–397 g were used in this experiment. Colchicine (50, 150 or 300 μg/kg) was dissolved in saline just immediately before use. Saline or colchicine was repetitively injected intraperitoneally 90 min before each trial for behavioral testing.

2.2.3. Chronic treatment with β-estradiol 3-benzoate

Male Sprague–Dawley rats weighing 216–269 g were used in this experiment. β-Estradiol 3-benzoate (0.5 or 2.0 mg/168 μl) suspended with saline containing 5% gum arabic (vehicle) was poured into an ALZET osmotic minipump. Under anesthesia, colchicine (0.3 μg/0.5 μl/site) or saline (0.5 μl) was infused into the rostrocaudal regions of the dentate gyrus followed by subcutaneous implantation of the minipump filled with β-estradiol 3-benzoate or vehicle. Efflux rate of the minipump is defined as 0.5 μl/h. Animals which received saline infusion into the dentate gyrus followed by implantation of the minipump filled with vehicle were defined as the saline control, and the vehicle control was defined as an animal group which received implantation of the minipump filled with vehicle following colchicine

(0.3 μg/site) infusion into the dentate gyrus. Fourteen days after implantation, the vacant pump was taken out by surgical operation under anesthesia. Thereafter, animals were exposed to the MWM learning test. Four weeks after colchicine infusion, the rat brain was removed.

2.3. Behavioral estimation

2.3.1. MWM learning task

2.3.1.1. Experiment on dose dependency of colchicine.

Animals were housed individually with ad libitum food and water and maintained in controlled temperature environment (24±2 °C) with a 12-h light/dark cycle. Behavioral experiments were conducted during the light cycle according to the minor modification of the previously reported method (Morris, 1984). One week after drug infusion, rats were tested on the acquisition of a task in the MWM. Rats were placed into a black circular pool (150 cm in diameter, 45 cm in height) of water (at 22±2 °C) from which they could escape onto a hidden transparent platform (12 cm in diameter, 21 cm in height). The platform, which was located in a constant position in the middle of one quadrant equidistant from the center and edge of the pool, was hidden 2 cm below the water level. The pool was located in a large test room in which many cues external to the maze (e.g., desk, lamp and shelf) were visible from within the pool and could be used by the rat for spatial orientation. The positions of cues were unchanged throughout the experiment. Experimental procedure of the modified MWM was shown in Fig. 1.

For five training trials, the rat was placed in the water so that it faced the wall of the pool. Each rat started at one quadrant, on the side opposite the platform, with the starting point being fixed throughout these training trials. In each training trial, the latency required to escape onto the hidden platform and the swimming path length (distance) were recorded. If the rat found the platform, it was allowed to remain there for 30 s and was then returned to its home cage. If the rat was unable to find the platform within 180 s, after it was made to stay there for 30 s, the training trial was terminated and a maximum score of 180 s was assigned. Training trials were given on 5 consecutive days, and rats underwent one training trial each day. On the day after the final training trial, each rat was exposed to an additive training test after the starting point was changed to the adjacent left quadrant of the platform (the additive training trial). The latency and distance were recorded in the same way as the previous five training trials. On the next day, rats performed a spatial probe test trial with the same starting point as in the five training trials. This trial consisted of removing the platform from the pool and allowing the rat to swim for 60 s in search of it. The time spent in each of the four equal quadrants, into which the pool was divided, of the tank was calculated as a percentage over 60 s. If the rat demonstrated a persistent preference during the trial to navigate in the pool quadrant where the escape platform had previously been placed, this was taken to indicate that the rat had acquired the spatial task and remembered it.

2.3.2. Step-through type PA learning task

Two weeks after drug infusion, rats were tested on the acquisition of a task in a step-through type PA. The apparatus consisted of two compartments, a lighted box and a dark box, connected via a guillotine door. Two hours before the acquisition trial, each rat was placed in the lighted compartment and then allowed to enter the dark compartment. The time taken to do so was recorded in seconds. Rats that had latencies greater than 30 s were discarded as being outside the normal range (the adaptive trial). In the acquisition trial, each rat was placed in the lighted compartment and allowed to enter the dark compartment through the guillotine door. The time required to enter the dark side was recorded. Once the rat entered the dark compartment, the guillotine door was closed and immediately after that, a scrambled footshock (0.6 mA, 3 s, 60 Hz) was delivered through the grid floor. The rat

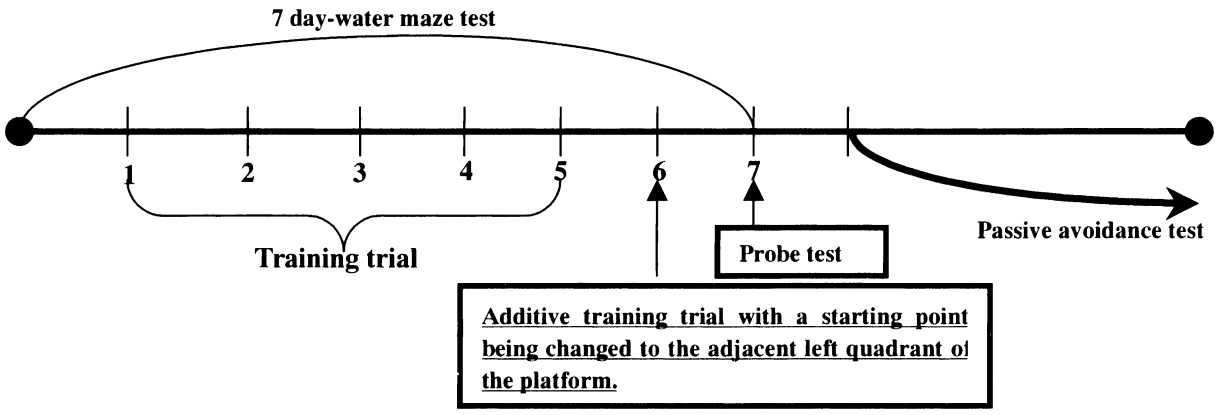

Fig. 1. Schematic representation for experimental procedure.

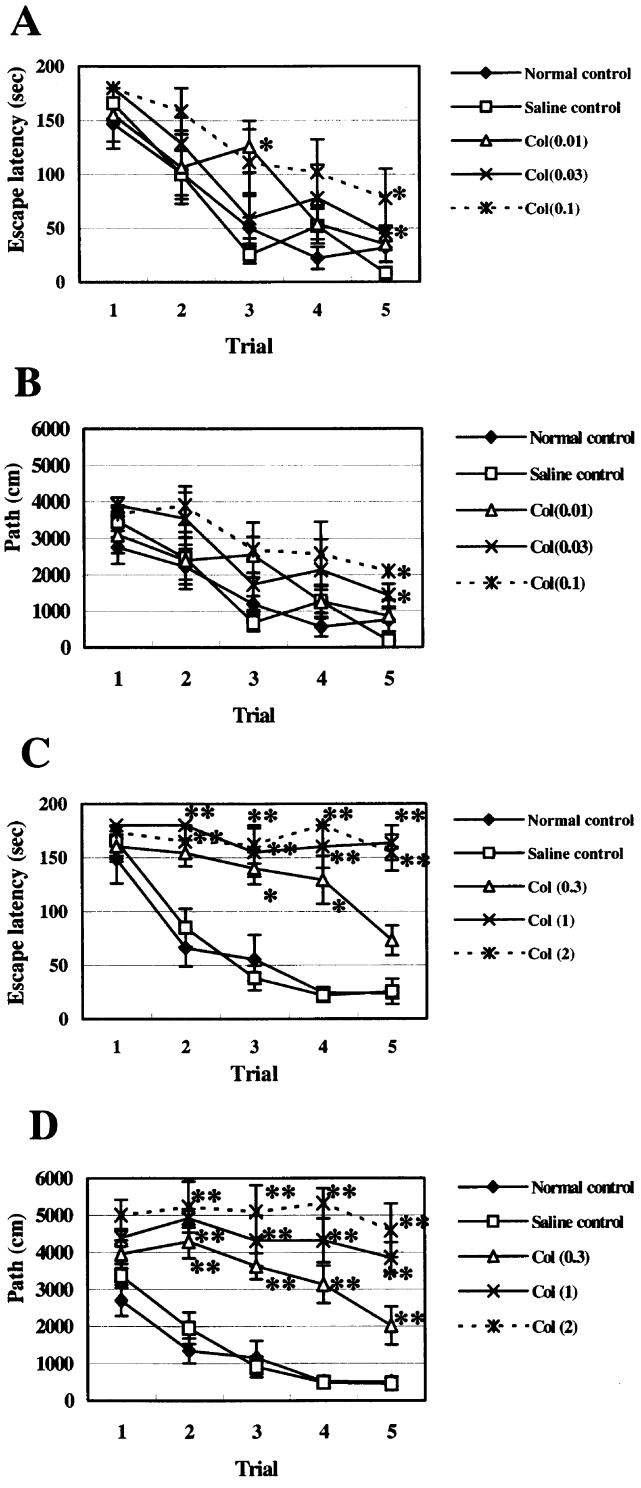

Fig. 2. Effect of intrahippocampal infusion of colchicine on water maze training test in male CRJ:SD rats. The mean escape times (A, C) and swimming distances (B, D) are plotted for (A–D): Normal control, Saline control and (A, B) 0.01 μg of colchicine (Col(0.01)), 0.03 μg of colchicine (Col(0.03)), and 0.1 μg of colchicine (Col(0.1)), and (C, D) 0.3 of μg colchicine (Col(0.3)), 1.0 μg of colchicine (Col(1)) and 2.0 μg colchicine (Col(2)). The vertical bars show the standard errors for five to seven animals. Asterisks indicate significant differences (* $P<.05$, ** $P<.01$ compared with the saline control; Dunnett's test using ranked data or Dunnett's test following two-way ANOVA).

was then put back into the home cage until the retention trial. Retention was measured 24 h after the acquisition. In the retention trial, the rat was again placed in the lighted compartment and the latency to enter the dark side was recorded, with a cutoff point at 300 s.

2.4. Histological evaluation

After completion of the behavioral testing, the rats were anesthesized with sodium pentobarbital (50 mg/kg) and were perfused transcardially with saline, followed by 3.7% paraformaldehyde solution prepared with 10 mM phosphate-buffered saline (PBS). The brains were removed from the skull, postfixed in paraformaldehyde solution and sectioned on a microtome in the coronal plane (5 μm in thickness) from the olfactory bulb to the cerebellum. Cresyl violet (Szczepanik et al., 1996) and Nauta (Grant and Aldskogius, 1967) stainings were performed as described previously. For immunohistochemistry using an antitubulin antibody, performed as described previously (Crowther and

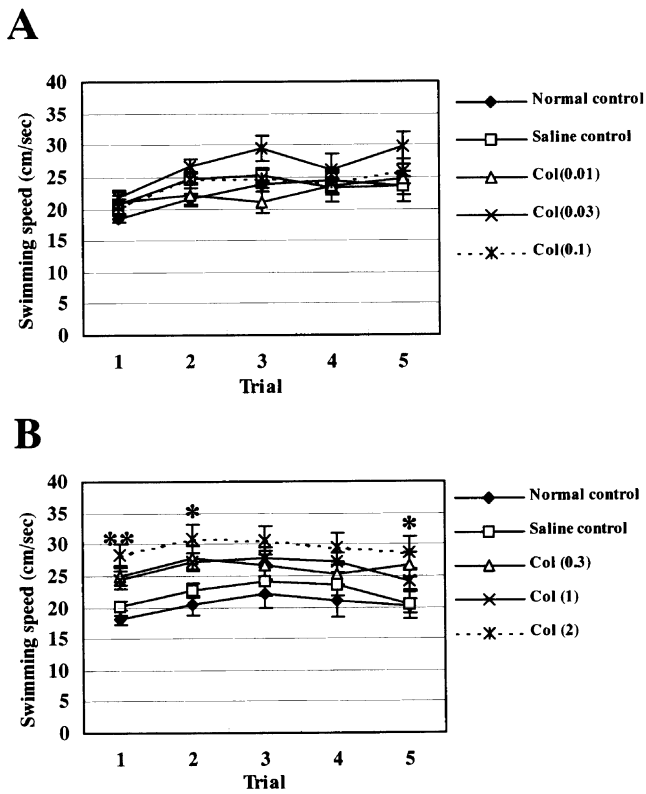

Fig. 3. Effect of intrahippocampal infusion of colchicine (A: 0.01–0.1 μg; B: 0.3–2.0 μg) on motor behavior in water maze training test. The mean swimming speeds are plotted as follows: (A) normal control, saline control, 0.01 μg of colchicine (Col(0.01)), 0.03 μg of colchicine (Col(0.03)) and 0.1 μg of colchicine (Col(0.1)), and (B) normal control, saline control, 0.3 μg of colchicine (Col(0.3)), 1.0 μg of colchicine (Col(1)) and 2.0 μg of colchicine (Col(2)). The vertical bars show the standard errors for five to seven animals. Asterisks indicate significant differences (* $P<.05$, ** $P0.01$ compared with the saline control; Dunnett's test following two-way ANOVA).

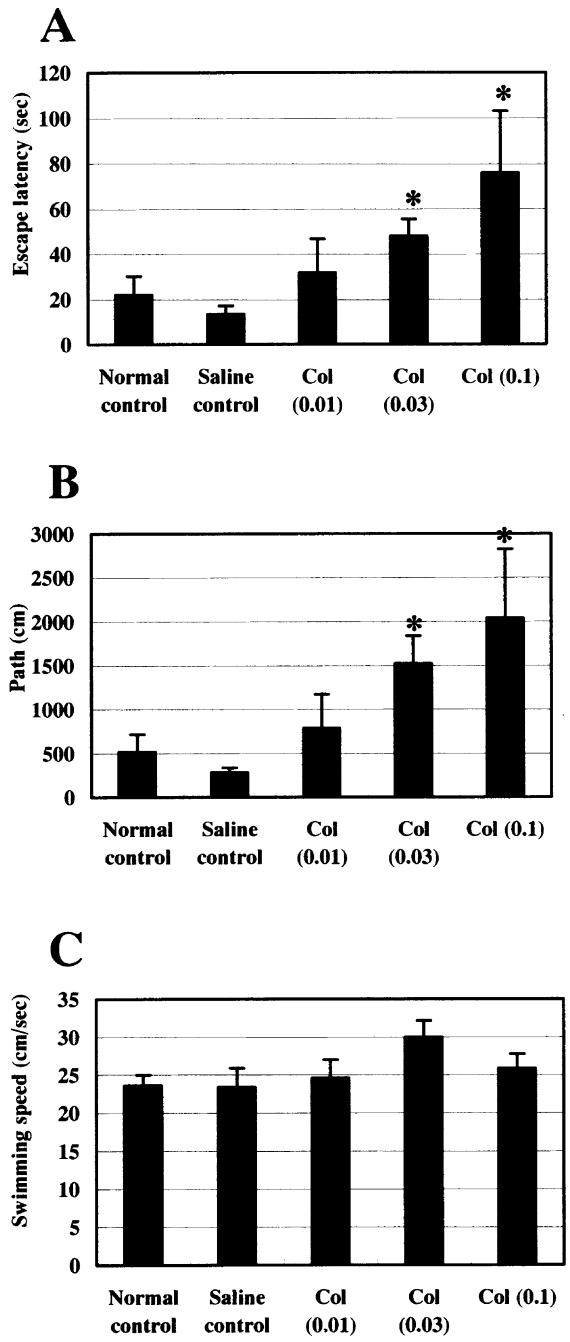

Fig. 4. Effect of intrahippocampal infusion of colchicine on the additive training trial in male CRJ:SD rats. The mean escape times (A), swimming distances (B) and swimming speeds (C) are expressed for (A–C): normal control, saline control, 0.01 μg of colchicine (Col(0.01)), 0.03 μg of colchicine (Col(0.03)) and 0.1 μg of colchicine (Col(0.1)). The vertical bars show the standard errors for five to seven animals. Asterisks indicate significant differences (* $P<.05$ compared with the saline control; Dunnett's test using ranked data or Dunnett's test).

Whittaker, 1992; Barry et al., 1995; Yagi et al., 1995), sections were deparaffinized for 5 min in xylenes, rehydrated through a descending alcohol series, rinsed three times with 10 mM PBS and placed for 30 min in 3% H_2O_2 to quench endogenous peroxidases. After three additional washes in PBS, sections were incubated with 3% BSA (dissolved in distilled water) and normal goat serum at room temperature for 30 min. After blocking, sections were incubated at 37 °C for 1 h with antitubulin antibody (Tubulin Ab-4; a dilution of 1:200). Thereafter, sections

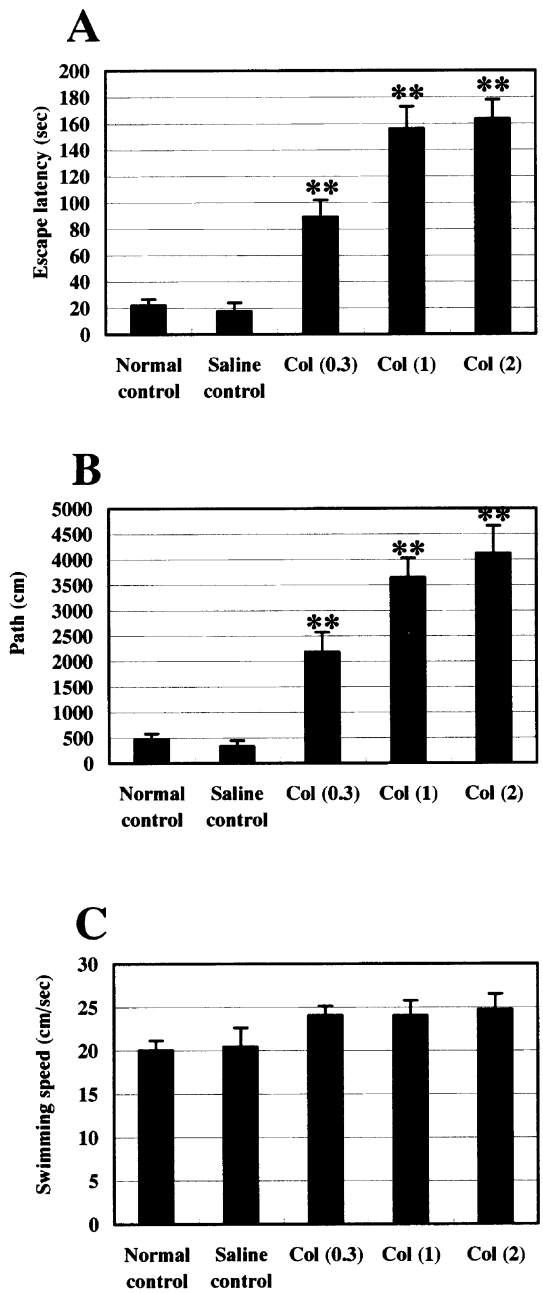

Fig. 5. Effect of intrahippocampal infusion of colchicine on the additive training trial in male CRJ:SD rats. The mean escape times (A), swimming distances (B) and swimming speeds (C) are expressed for (A–C): normal control, saline control, 0.3 μg of colchicine (Col(0.3)), 1.0 μg of colchicine (Col(1)) and 2.0 μg of colchicine (Col(2)). The vertical bars show the standard errors for six to seven animals. Asterisks indicate significant differences (** $P<.01$ compared with the saline control; Dunnett's test using ranked data or Dunnett's test).

were rinsed thoroughly and incubated with biotinylated goat antirabbit antibody and subsequently with Avidin D-HRP (AutoProbe II kit) at room temperature for 30 min. After incubation, the sections were washed three times with 10 mM PBS. Finally, immunoreactive products were detected by incubation in a mixture of 0.06% diaminobenzidine tetrahydrochloride (DAB), 0.01% H_2O_2 in 50 mM Tris–HCl buffer (pH 7.6) at room temperature for 1 min. Each viable cell in bilateral sides of the dentate gyrus obtained by the cresyl violet staining was counted under a microscopic examination and data were expressed as the average counts per one side.

2.5. Statistical analysis

Student's *t* test (parametric) was used for the statistical analysis between the normal and saline controls in both the swimming speed and the swimming distance required to reach the platform in the MWM training task following two-way analysis of variance (ANOVA). Also in an experiment using animals treated with estradiol, Student's *t* test (parametric) was used between the saline and vehicle controls in the MWM training task following two-way ANOVA. Three effects (Dose, Trial and Dose × Trial) were examined by two-way ANOVA, and interactions between

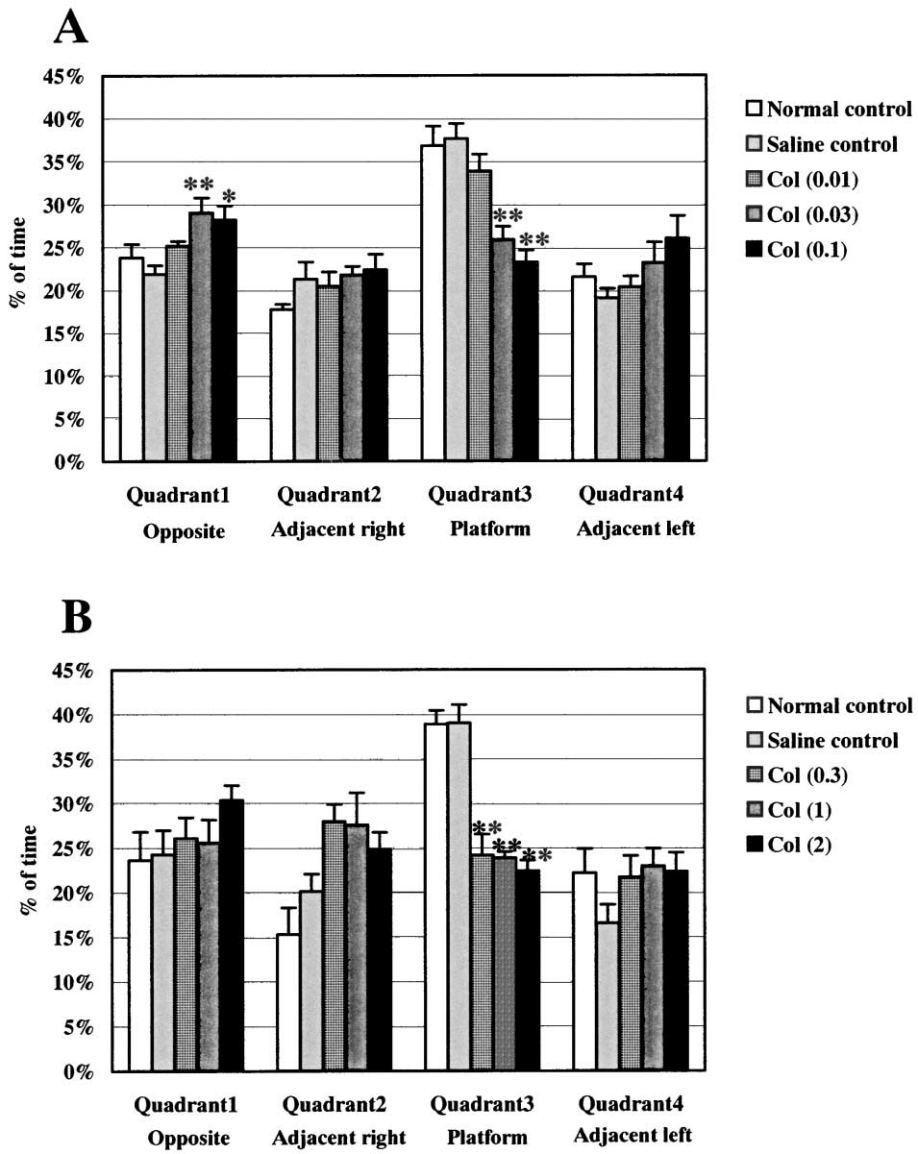

Fig. 6. Effect of intrahippocampal infusion of colchicine on water maze probe test trial in male CRJ:SD rats. The mean percentages of total time spent in each pool quadrant by rats treated with as follows are expressed as (A) normal control, saline control, 0.01 μg of colchicine (Col(0.01)), 0.03 μg of colchicine (Col(0.03)) and 0.1 μg of colchicine (Col(0.1)), and (B) normal control, saline control, 0.3 μg of colchicine (Col(0.3)), 1.0 μg of colchicine (Col(1)) and 2.0 μg of colchicine (Col(2)). The vertical bars show the standard errors for five to seven animals. Asterisks indicate significant differences (* $P<.05$, ** $P<.01$ compared with the saline control; Dunnett's test).

experimental groups were considered as significant only when either the Dose or Dose × Trial effect demonstrated statistical significances. The interactions were not taken as significant even though only the trial effect showed a statistically significant level. The Wilcoxon rank-sum test (nonparametric) was used for the statistical analysis between the normal and saline controls or the saline and vehicle controls in the escape latency in the MWM following two-way ANOVA and PA task. Student's *t* test or Wilcoxon rank-sum test and Dunnett's test or Dunnett's test using ranked data at each trial in the MWM five training trials was carried out only when there were significant interactions between groups. Dunnett's test (parametric) was used for the statistical analysis between the saline control and colchicine-treated groups or the vehicle-treated and estradiol-treated groups in the swimming speed and the swimming distance required to reach the platform in the MWM training and additive training tests. Dunnett's test using ranked data (nonparametric) was used for the statistical analysis between the saline control and colchicine-treated groups or the vehicle-treated and the estradiol-treated groups in the escape latency required to reach the platform in the MWM training and additive training tests. Also, Dunnett's test using ranked data (nonparametric) was used for the statistical analysis between the saline control and colchicine-treated groups in the escape latency in the PA task. In the probe test, Student's *t* test was used between the normal and saline controls or the saline and vehicle control, and Dunnett's test was used between the saline control and colchicine-treated groups or the vehicle-treated and estradiol-treated groups at each quadrant. Statistical significance was assumed at $P<.05$.

3. Results

3.1. Effects of various doses of intradentate colchicine

3.1.1. Low doses (0.01–0.1 μg)

In the MWM training test, ANOVA showed no statistical differences in both Dose and Dose × Trial effects on escape latencies, lengths of the swimming path and swimming speeds between the normal and saline controls [Dose effects: $F(1,10)=0.001$, for latencies; $F(1,10)=0.151$, for swimming distances; $F(1,10)=0.854$, for swimming speeds; Dose × Trial effects: $F(4,40)=1.163$, for latencies; $F(4,40)=1.441$, for swimming distances; $F(4,40)=1.069$, for swimming speeds]. It, however, demonstrated a significant trial (time) effects on escape latencies, lengths of the swimming path and swimming speeds between the normal and saline controls [$F(4,40)=24.922$, $P<.01$, for latencies; $F(4,40)=17.767$, $P<.01$, for swimming distances; $F(4,40)=5.683$, $P<.01$, for swimming speeds]. By contrast, ANOVA demonstrated significant dose effects on escape latencies and lengths of the swimming path displayed by colchicine-treated rats as compared with the saline-treated controls [$F(3,19)=3.211$, $P<.05$, for latencies; $F(3,19)=3.131$, $P<.05$, for swimming distances (Fig. 2A and B)], although it did not show a significant dose effect on swimming speeds [$F(3,19)=2.694$, for swimming speeds (Fig. 3A)]. Also, it showed significant trial effects on escape latencies, lengths of the swimming path and swimming speed displayed by colchicine-treated rats as compared with the saline-treated controls [$F(4,76)=30.721$, $P<.01$, for latencies; $F(4,76)=18.454$, $P<.01$, for swimming distances; $F(4,76)=7.074$, $P<.01$, for swimming speeds]. But there were no Dose × Trial effects on escape latencies, lengths of the swimming path and swimming speed between the colchicine- and saline-treated rats [$F(12,76)=1.368$, for latencies; $F(12,76)=0.990$, for swimming distances; $F(12,76)=0.997$, for swimming speeds].

Intradentate infusion of colchicine at doses of 0.01–0.1 μg increased escape latencies and swimming distances required for colchicine-treated animals to find the platform versus the saline-treated group in each training trial. Following

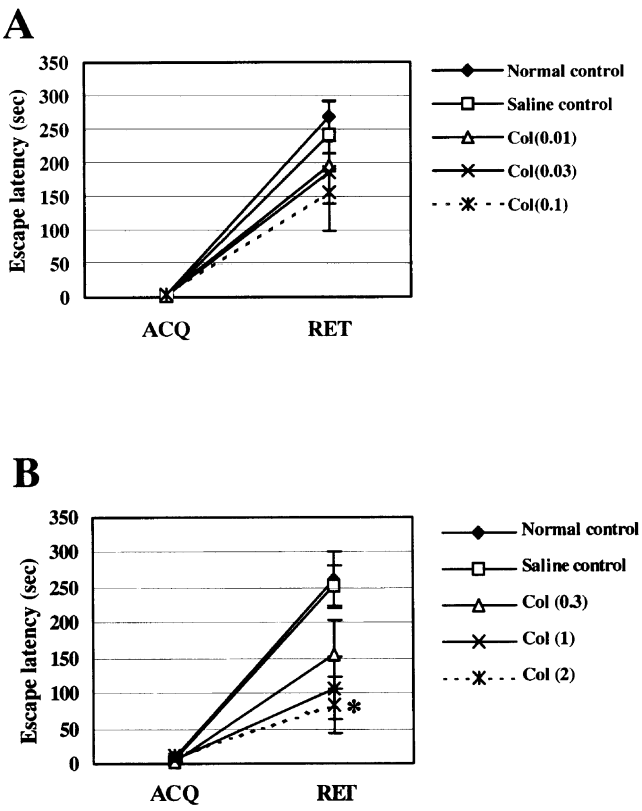

Fig. 7. Effect of intrahippocampal infusion of colchicine on a step-through type PA learning test in male CRJ:SD rats. The results of acquisition trials (ACQ) and retention trials (RET) are plotted as follows: (A) normal control, saline control, 0.01 μg of colchicine (Col(0.01)), 0.03 μg of colchicine (Col(0.03)), 0.1 μg of colchicine (Col(0.1)), and (B) normal control, saline control, 0.3 μg of colchicine (Col(0.3)), 1.0 μg of colchicine (Col(1)), 2.0 μg of colchicine (Col(2)). Each data point represents the mean values and the standard errors for five to seven animals. Asterisks indicate significant differences (* $P<.05$ compared with the saline control; Dunnett's test using ranked data).

two-way ANOVA, a significant difference in latency was observed in Trial 3 ($P < .05$, Dunnett's test using ranked data; Fig. 2A) at a dose of 0.01 μg, and significant differences in latencies and distances were observed in Trial 5 ($P < .05$, respectively, Dunnett's test or Dunnett's test using ranked data) at doses of 0.03 and 0.1 μg (Fig. 2A and B). The escape latencies, swimming distances and speeds in the saline-treated animals did not show any change versus those in the normal controls throughout the experiment (Figs. 2A,B and 3A). In the subsequent additive training trial in which the starting point was changed to the adjacent left quadrant of the platform, escape latencies

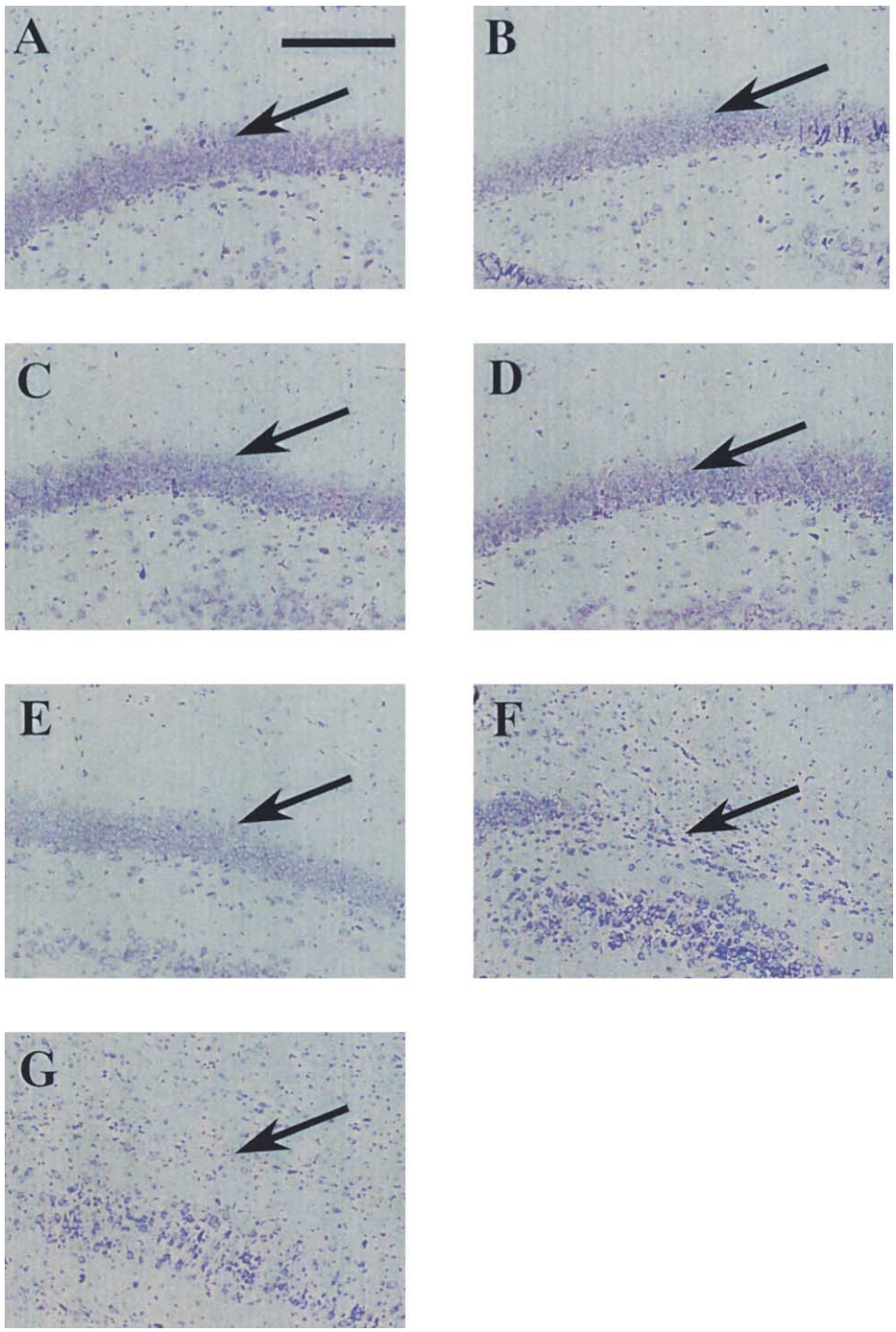

Fig. 8. Cresyl violet staining of Nissl substance in the dentate gyrus of the hippocampal formation from male CRJ:SD rats at 15 days after intrahippocampal infusion of saline (A), colchicine (0.01 μg/site) (B), (0.03 μg/site) (C), (0.1 μg/site) (D), (0.3 μg/site) (E), (1.0 μg/site) (F) or (2.0 μg/site) (G). Arrows (→) indicate the dentate granule cell layers (5 μm sections). Scale bar = 200 μm.

and swimming distances displayed by colchicine (0.03 and 0.1 μg)-treated rats were significantly prolonged as compared with the saline-treated controls ($P<.05$, respectively, Fig. 4A and B), whereas swimming speeds were not significantly different between the colchicine- and saline-treated groups (Fig. 4C). The escape latencies, swimming distances and speeds in the saline-treated animals did not show any change versus those in the normal controls. The results of the probe test trial revealed that the colchicine (0.03 and 0.1 μg)-treated rats spent significantly less time in the previously reinforced quadrant (Quadrant 3) than the saline-treated rats ($P<.01$, respectively) and that they spent significantly more time in Quadrant 1 than controls (0.03 μg, $P<0.05$; 0.1 μg, $P<.01$; Fig. 6A). The times spent in each quadrant of the tank by the saline-treated animals did not show any significant differences as compared with those by the normal controls (Fig. 6A). In the PA test, no differences were found in the escape latencies between the saline- and colchicine-treated groups within the dose range of 0.01–0.1 μg (Fig. 7A). The escape latencies in the saline-treated animals did not exhibit any change versus those in the normal controls (Fig. 7A).

Fifteen days after colchicine infusion, treatment at doses of 0.01–0.3 μg/site left the dentate granule cells visibly unaffected (Fig. 8). On the other hand, as revealed by tubulin and axon stainings in Figs. 10 and 11, it was found that microtubules and axons were damaged beginning around a dose of 0.1 μg/site of colchicine. Histological changes similar to those observed in this rostral side of the dentate gyrus were also recognized in the caudal side of that but not in the other brain regions (data not shown).

3.1.2. High doses (0.3–2.0 μg)

In the MWM training test, ANOVA showed no statistical differences in both Dose and Dose × Trial effects on escape latencies, lengths of the swimming path and swimming speeds between the normal and saline controls [Dose effects: $F(1,11)=0.074$, for latencies; $F(1,11)=0.579$, for swimming distances; $F(1,11)=1.093$, for swimming speeds; Dose × Trial effects $F(4,44)=0.578$, for latencies; $F(4,44)=1.054$, for swimming distances; $F(4,44)=0.232$, for swimming speeds]. It, however, showed significant trial effects on escape latencies, lengths of the swimming path and swimming speeds between the normal and saline controls [$F(4,44)=31.952$, $P<.01$, for latencies; $F(4,44)=26.671$, $P<.01$, for swimming distances; $F(4,44)=2.909$, $P<.05$, for swimming speeds]. By contrast, ANOVA demonstrated both significant dose and trial effects on escape latencies, lengths of the swimming path and swimming speeds displayed by colchicine-treated rats as compared with the saline-treated controls [Dose effects: $F(3,23)=24.365$, $P<.01$, for latencies; $F(3,23)=18.447$, $P<.01$, for swimming distances; $F(3,23)=3.614$, $P<.05$, for swimming speeds (Figs. 2C,D and 3B); Trial effects: $F(4,92)=14.942$, $P<.01$, for latencies; $F(4,92)=10.388$, $P<.01$, for swimming distances; $F(4,92)=4.524$, $P<.01$, for swimming speeds]. Also it showed significant Dose × Trial effects on escape latencies and lengths of the swimming path displayed by colchicine-treated rats as compared with the saline-treated controls [$F(12,92)=5.229$, $P<.01$, for latencies; $F(12,92)=2.716$, $P<.01$, for swimming distances], although it did not demonstrate a significant Dose × Trial effect on swimming speeds [$F(12,92)=0.572$, for swimming speeds]. Intradentate colchicine at doses of (0.3–2.0 μg) dose-dependently increased the escape latencies and the swimming distances compared to the saline-treated group. Significant differences in latencies were found in Trials 2, 3, 4 and 5 at doses of 1.0 and 2.0 μg ($P<.01$, respectively; Dunnett's test using ranked data) and in Trials 3 and 4 at a dose of 0.3 μg ($P<.05$, respectively; Dunnett's test using ranked data) (Fig. 2C). Significant differences in distances were found in Trials 2, 3, 4 and 5 at all of three doses ($P<.01$, respectively; Dunnett's test) (Fig. 2D). The colchicine-treated groups showed dose-dependent increases in the swimming speeds at several trials with significant differences at a dose of 2.0 μg versus the saline-treated group ($P<.01$ at Trial 1; $P<.05$ at Trials 2 and 5; Fig. 3B). In the subsequent additive training trial in which the starting point was changed to the adjacent left quadrant of the platform, escape latencies and swimming distances displayed by colchicine-treated rats were significantly prolonged at all of three doses as compared with the saline-treated controls ($P<.01$, respectively, Fig. 5A and B), whereas swimming speeds were not significantly different between the colchicine- and saline-treated groups (Fig. 5C). The escape latencies, swimming distances and speeds in the saline-treated animals did not show any change versus those in the normal controls. Likewise, the results of the probe test trial revealed that the colchicine-treated rats spent significantly less time in the previously reinforced quadrant (Quadrant 3) than the saline-treated rats ($P<.01$, respectively), although there were no significant differences in the other quadrants between the colchicine- and saline-treated groups (Fig. 6B). The times spent in each quadrant of the tank by the saline-treated animals did not show any significant differences as compared

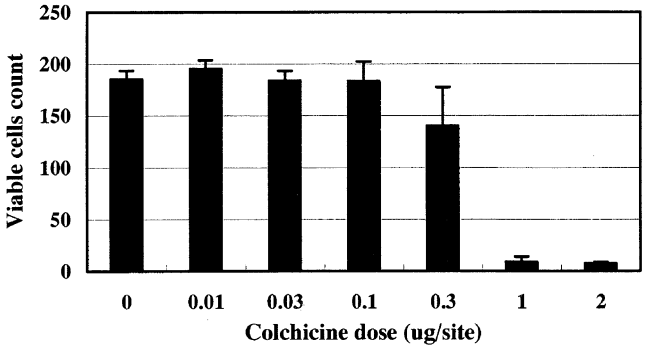

Fig. 9. Effects of infused doses of colchicine on neuronal cell viability in the dentate gyrus. The plotted values are the mean viable cells count in cresyl violet stained sections and the vertical bars show the standard errors for three animals.

with those by the normal controls (Fig. 6B). In the PA test, while no differences were found in the escape latencies between the saline- and colchicine-treated groups within the dose range of 0.3–1.0 μg (Fig. 7B), a significant decrease in the colchicine (2.0 μg)-treated group was observed compared with the saline-treated group ($P < .05$, Dunnett's test using ranked data; Fig. 7B). The escape latencies in the saline-treated animals did not exhibit any change versus those in the normal controls (Fig. 7B).

High doses of colchicine (1.0 and 2.0 μg/site) destroyed the dentate granule cells in the dorsal hippocampal region (the rostral side of the dentate gyrus) 15 days after colchicine infusion, as shown in the sections stained by cresyl violet (Fig. 9). On the other hand, as revealed by tubulin and axon stainings in Figs. 10 and 11, it was found that microtubules and axons were damaged in the dose range of 0.1–1.0 μg/site of colchicine. To be brief, doses of 0.1 and 0.3 μg/site among those examined here induced

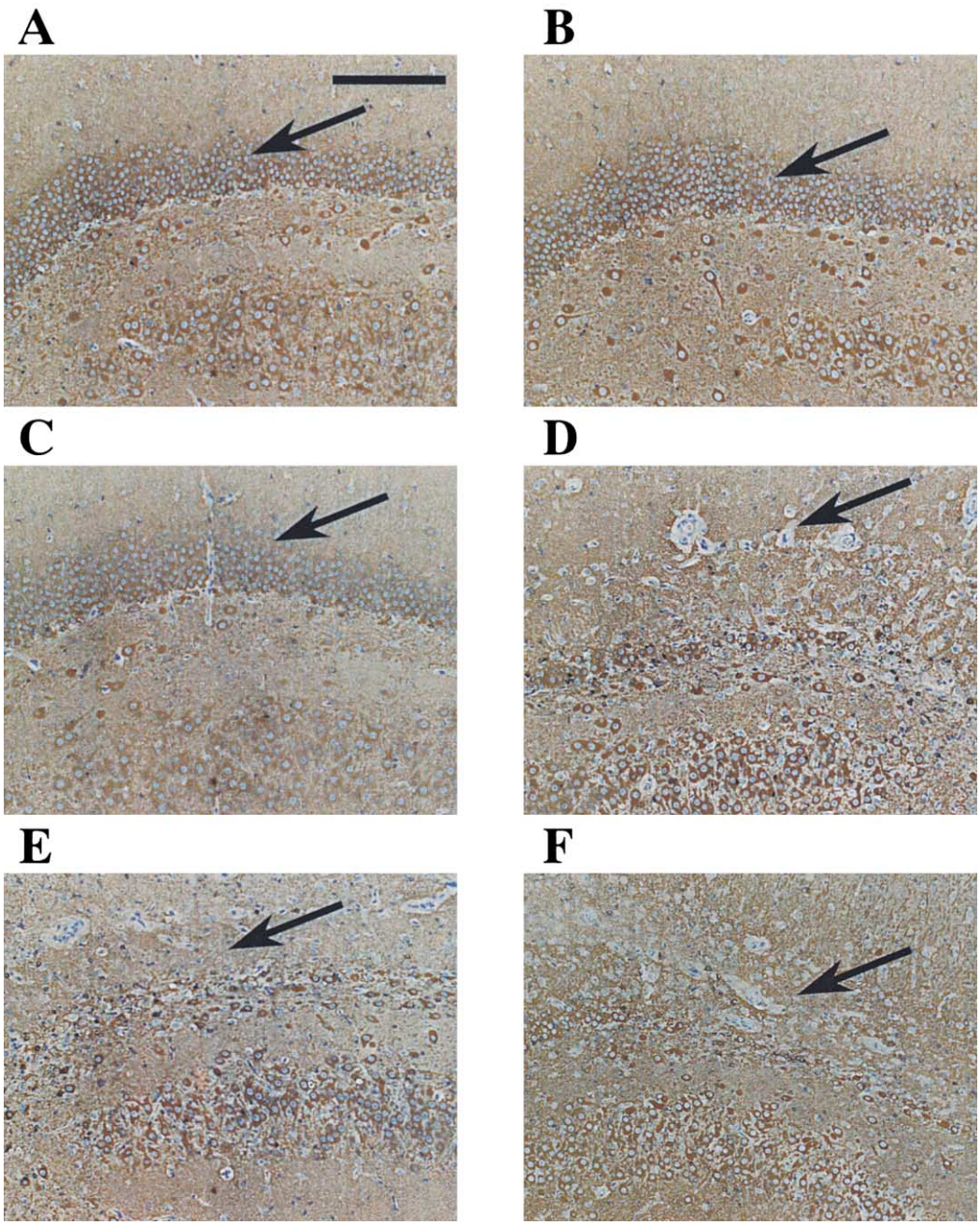

Fig. 10. Antitubulin staining of the dentate gyrus of the hippocampal formation from male CRJ:SD rats at 15 days after intrahippocampal infusion of saline (A), colchicine (0.01 μg/site) (B), (0.03 μg/site) (C), (0.1 μg/site) (D), (0.3 μg/site) (E) or (1.0 μg/site) (F). Arrows (→) indicate the dentate granule cell layers (5 μm sections). Scale bar = 200 μm.

microtubule depolymerization without producing any neuronal death of the dentate granule cells.

3.2. Effects of systemic administration of colchicine

As revealed by ANOVA, systemic injection of colchicine at doses of 50–300 μg showed no effects on each parameter in the MWM test as compared with the saline-treated rats [Dose effects: $F(3,38) = 0.271$, for latencies; $F(3,38) = 1.083$, for swimming distances; $F(3,38) = 2.132$, for swimming speeds; Dose × trial effects: $F(12,152) = 1.590$, for latencies; $F(12,152) = 1.400$, for swimming distances; $F(12,152) = 1.067$, for swimming speeds] (Fig. 12A, B and C). It was demonstrated that there were significant trial effects on each parameter between the colchicine- and saline-treated groups [$F(4,152) = 23.474$, $P < .01$, for latencies; $F(4,152) = 26.864$, $P < .01$, for swimming distances; $F(4,152) = 3.312$, $P < .05$, for swimming speeds]. Also in the PA learning task, the colchicine-treated group did not show any significant changes as compared with the saline-treated controls (Fig. 13).

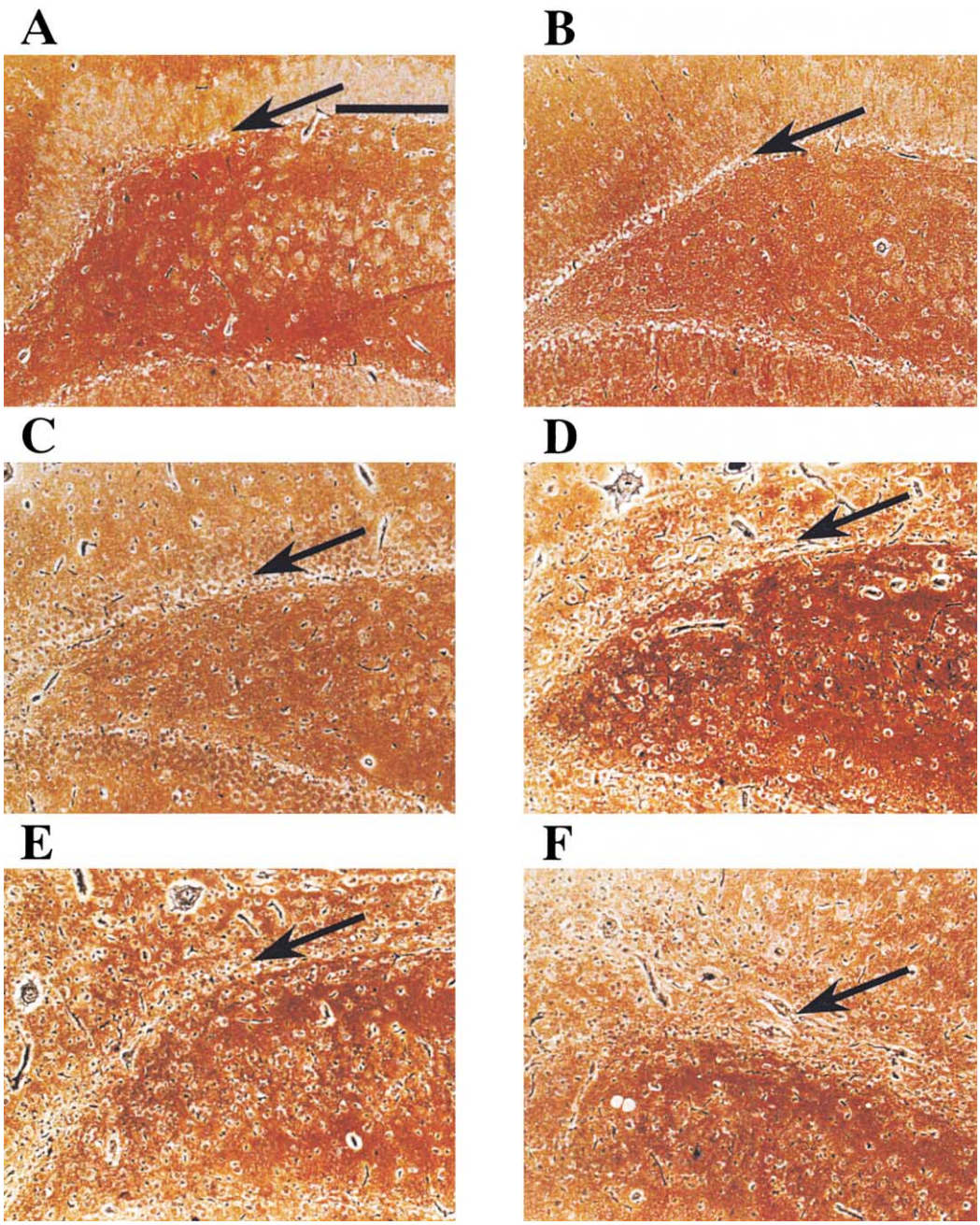

Fig. 11. Nauta staining of the dentate gyrus of the hippocampal formation from male CRJ:SD rats at 15 days after intrahippocampal infusion of saline (A), colchicine (0.01 μg/site) (B), (0.03 μg/site) (C), (0.1 μg/site) (D), (0.3 μg/site) (E) or (1.0 μg/site) (F). Arrows (→) indicate the dentate granule cell layers (5 μm sections). Scale bar = 200 μm.

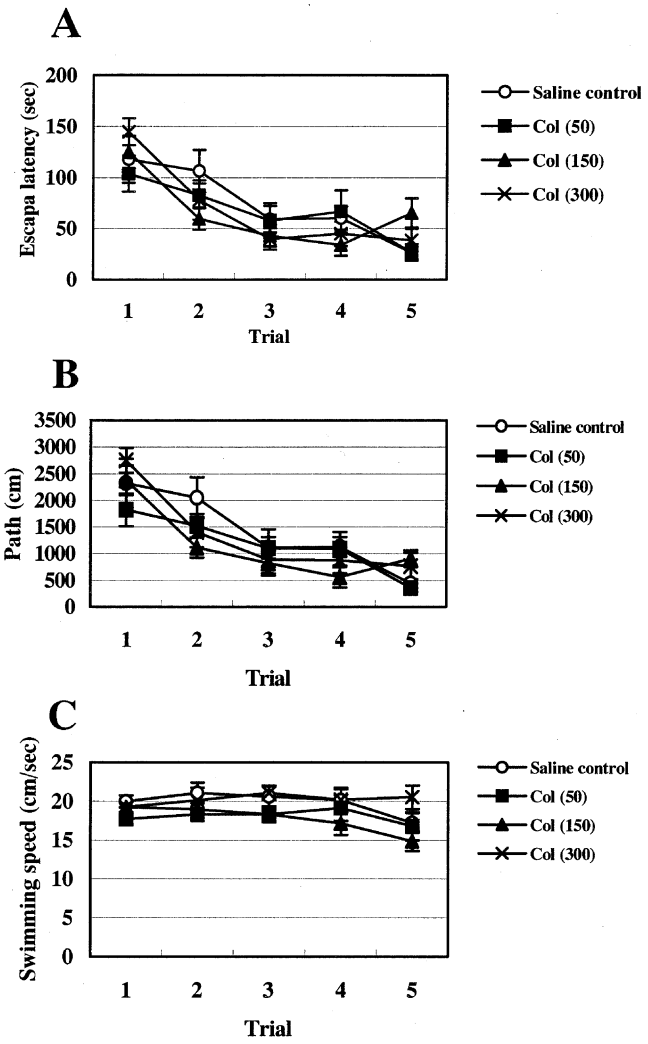

Fig. 12. Effect of intraperitoneal injection of colchicine on water maze training test and motor behavior in male CRJ:SD rats. The mean escape times (A), swimming distances (B) or swimming speeds (C) are plotted for the saline control and three colchicine dose levels, 50 μg (Col(50)), 150 μg (Col(150)) and 300 μg (Col(300)). The vertical bars show the standard errors for 10–11 animals.

Furthermore, each parameter of each experimental group (e.g., escape latencies, distances and swimming speeds) in the additive training trial in which the starting point was changed to the adjacent left quadrant of the platform was not different from the results of the final training trial (data not shown). Subsequent spatial probe test for 60 s revealed that there were no differences in time spent in the previously reinforced quadrant between the saline- and colchicine-treated groups (data not shown), suggesting that systemic administration of colchicine did not induce spatial memory impairment.

3.3. Effects of β-estradiol 3-benzoate

ANOVA showed statistical significant dose (group) effects on latencies and swimming distances between the saline and vehicle controls [$F(1,12)=7.03$, $P<.05$, for latencies; $F(1,12)=10.161$, $P<.01$, for swimming distances], while no significant difference in swimming speeds was observed [$F(1,12)=0.966$, for swimming speeds]. There were significant trial effects on the three parameters in the MWM test [$F(4,48)=8.801$, $P<.01$, for latencies; $F(4,48)=5.410$, $P<.01$, for swimming distances; $F(4,48)=5.837$, $P<.01$, for swimming speeds], whereas there were no significant Dose × Trial effects on them between the saline and vehicle controls [$F(4,48)=0.343$, for latencies; $F(4,48)=1.219$, for swimming distances; $F(4,48)=0.524$, for swimming speeds]. Subsequent ANOVA demonstrated both significant Trial and Dose × Trial effects on escape latencies, lengths of the swimming path and swimming speeds displayed by the estradiol-treated rats as compared with the vehicle-treated controls [Trial effects: $F(4,60)=12.499$, $P<.01$, for latencies; $F(4,60)=6.164$, $P<.01$, for swimming distances; $F(4,60)=8.452$, $P<.01$, for swimming speeds Dose × Trial effects: $F(8,60)=2.392$, $P<.05$, for latencies; $F(8,60)=2.101$, $P<.05$, for swimming distances; $F(8,60)=2.538$, $P<.05$, for swimming speeds], although it did not show any significant dose effects on those three parameters [$F(2,15)=1.398$, for latencies; $F(2,15)=0.789$, for swimming distances; $F(2,15)=0.471$, for swimming speeds]. The vehicle group spent significantly longer time (latency) to reach the platform than the saline group ($P<.05$ at Trials 4 and 5, respectively, Wilcoxon rank-sum test following two-way ANOVA; Fig. 14A), and swam significantly longer distances to find the platform as compared with the saline group ($P<.05$ at Trials 2, 4 and 5, respectively, Student's t test following two-way ANOVA; Fig. 14B). The swimming speeds in the vehicle group did not exhibit any significant changes as compared with the saline group throughout the experiment (Student's t test following two-way ANOVA; Fig. 14C). The β-estradiol 3-benzoate-treated group had a tendency to shorten the escape latencies and swimming distances required to find the platform as training trials progressed, and the rats treated with 2.0 mg of β-estradiol 3-benzoate showed a significant effect at Trial 5

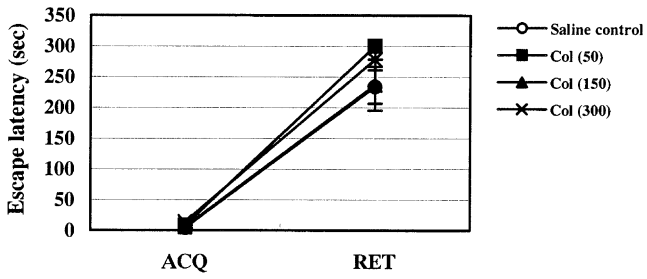

Fig. 13. Effect of intraperitoneal injection of colchicine on a step-through type PA learning test in male CRJ:SD rats. The results of acquisition trials (ACQ) and retention trials (RET) are plotted for the saline control, and 50 μg (Col(50)), 150 μg (Col(150)) and 300 μg (Col(300)) of colchicine dose levels. Each data point represents the mean values and the standard errors for 7–11 animals.

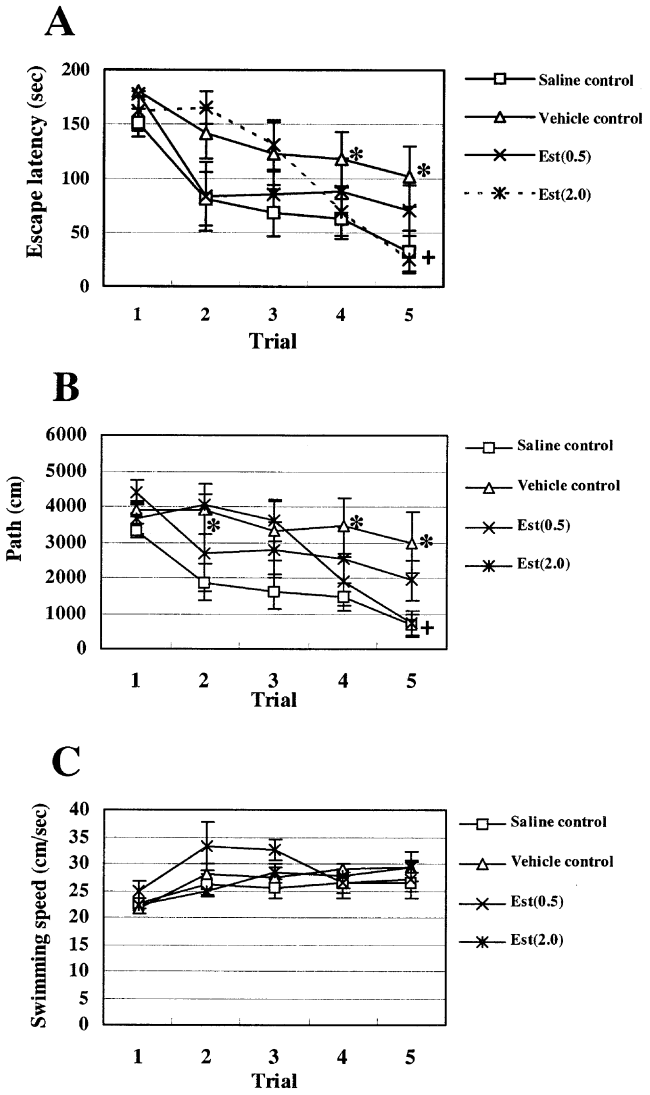

Fig. 14. Effect of treatment with estradiol on colchicine (0.3 μg)-induced learning deficits and motor behavior in water maze training test. The mean escape times (A), swimming distances (B) or swimming speeds (C) for the saline control, vehicle control, Est (0.5) (0.5 mg/168 μl β-estradiol 3-benzoate) and Est (2.0) (2.0 mg/168 μl β-estradiol 3-benzoate) are plotted. The vertical bars show the standard errors for six to eight animals. Marks indicate significant differences (* $P<.05$ compared with the saline-treated group; Wilcoxon rank-sum test or Student's t test following two-way ANOVA, † $P<.05$ compared with the vehicle-treated group; Dunnett's test using ranked data or Dunnett's test following two-way ANOVA).

($P<.05$, respectively) in comparison with the vehicle-treated ones (Fig. 14A and B). No significant differences in swimming speeds were found between the vehicle- and estradiol-treated groups throughout the experiment (Dunnett's test following two-way ANOVA; Fig. 14C). In the subsequent additive training trial, escape latencies and swimming distances displayed by the vehicle-treated rats were significantly prolonged as compared with the saline-treated controls ($P<.01$, respectively, Student's t test or Wilcoxon rank-sum test; Fig. 15A and B), whereas swimming speeds were not different between the vehicle- and saline-treated groups (Fig. 15C). On the other hand, escape latencies and swimming distances displayed by the β-estradiol 3-benzoate-treated rats were significantly shortened as compared with

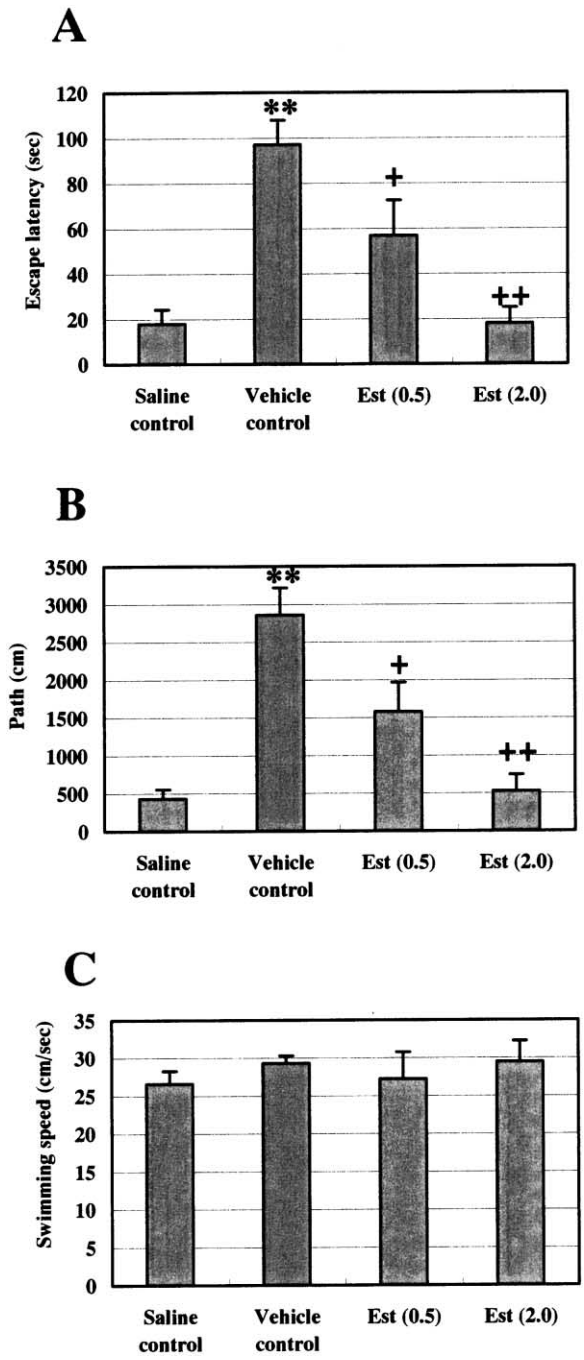

Fig. 15. Effect of treatment with estradiol on colchicine (0.3 μg)-induced learning deficits and motor behavior in the additive training trial. The mean escape times (A), swimming distances (B) or swimming speeds (C) for the saline control, vehicle control, Est (0.5) (0.5 mg/168 μl β-estradiol 3-benzoate) and Est (2.0) (2.0 mg/168 μl β-estradiol 3-benzoate) are expressed. The vertical bars show the standard errors for six to eight animals. Marks indicate significant differences (** $P<.01$ compared with the saline-treated group; Wilcoxon rank-sum test or Student's t test, † $P<.05$, †† $P<.01$ compared with the vehicle-treated group; Dunnett's test using ranked data or Dunnett's test).

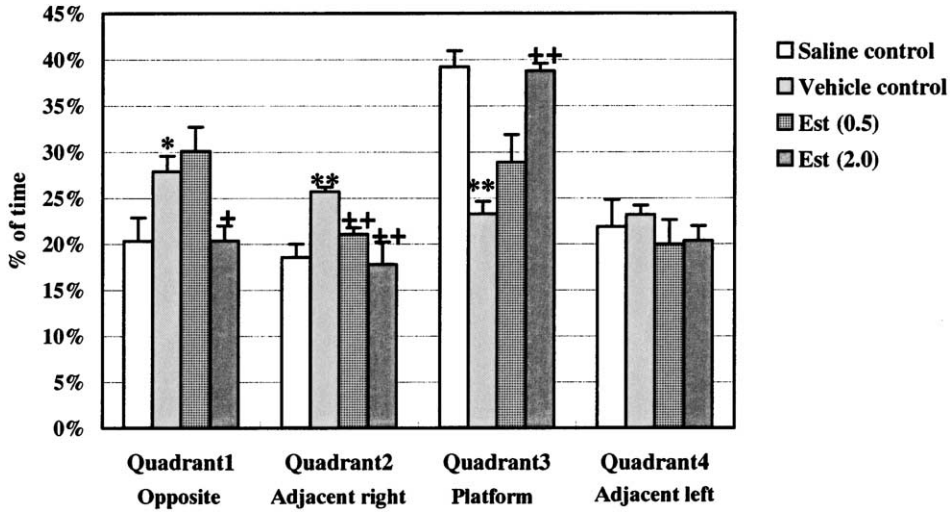

Fig. 16. Effect of treatment with estradiol on colchicine (0.3 μg)-induced learning deficits in water maze probe test. The mean percentages of total time spent in each pool quadrant by rats treated with saline + vehicle (the saline control), colchicine + vehicle (the vehicle control), colchicine + 0.5 mg β-estradiol 3-benzoate (Est (0.5)) and colchicine + 2.0 mg β-estradiol 3-benzoate (Est (2.0)) are expressed. The vertical bars show the standard errors for six to eight animals. Marks indicate significant differences (* $P<.05$, ** $P<.01$ compared with the saline-treated group; Student's t test, † $P<.05$, †† $P<.01$ compared with the vehicle-treated group; Dunnett's test).

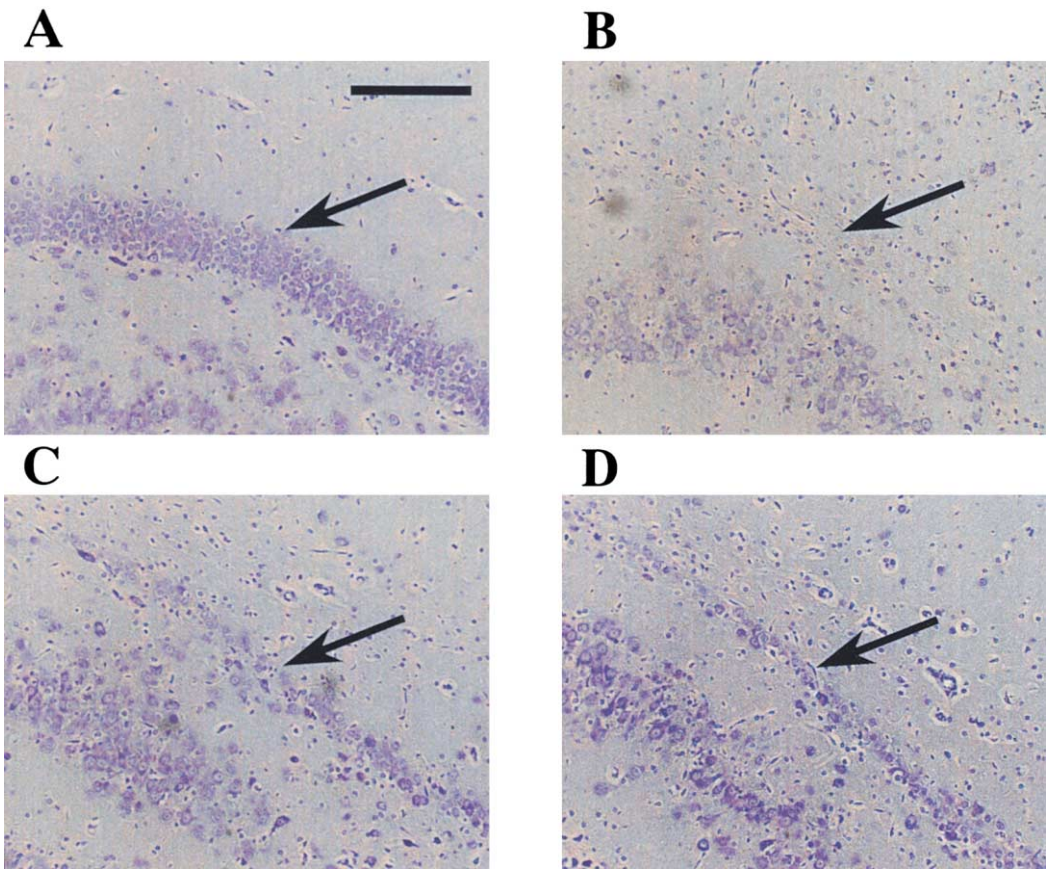

Fig. 17. Cresyl violet staining of Nissl substance in the dentate gyrus of the hippocampal formation from male CRJ:SD rats at 4 weeks after intrahippocampal infusion of saline (A), colchicine (0.3 μg/site) + vehicle (B), colchicine (0.3 μg/site) + β-estradiol 3-benzoate (0.5 mg) (C) and colchicine (0.3 μg/site) + β-estradiol 3-benzoate (2.0 mg) (D). Arrows (→) indicate the dentate granule cell layers (5 μm sections). Scale bar = 200 μm.

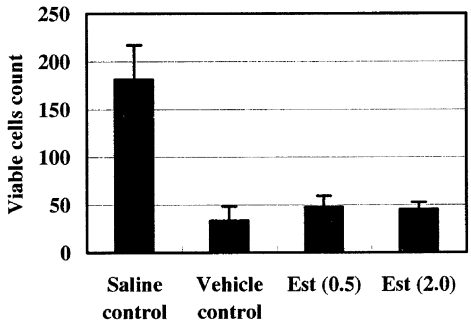

Fig. 18. Effect of treatment with β-estradiol 3-benzoate on colchicine-induced neuronal death in the dentate gyrus. The plotted values are the mean viable cells count in cresyl violet-stained sections and the vertical bars show the standard errors (two to five animals) for the saline control, vehicle control, Est (0.5) (0.5 mg/168 μl β-estradiol 3-benzoate) and Est (2.0) (2.0 mg/168 μl β-estradiol 3-benzoate).

the vehicle-treated controls (0.5 mg, $P<.05$; 2.0 mg, $P<.01$; Dunnett's test or Dunnett's test using ranked data; Fig. 15A and B), whereas swimming speeds were not different between the β-estradiol 3-benzoate- and vehicle-treated groups (Fig. 15C). The results of the probe test trial showed that the vehicle-treated rats stayed for significantly longer time in Quadrants 1 and 2 ($P<.05$ and $P<.01$, respectively) and for significantly shorter time ($P<.01$) in the previously reinforced quadrant (Quadrant 3) in comparison with the saline-treated rats (Fig. 16). The β-estradiol 3-benzoate-treated rats at a dose of 2.0 mg stayed for significantly shorter time in Quadrant 1 ($P<.05$), and at doses of 0.5 and 2.0 mg stayed dose-dependently for significantly shorter time in Quadrant 2 ($P<.01$, respectively) as compared with the vehicle-treated rats (Fig. 16). Treatment with β-estradiol 3-benzoate also dose-dependently and significantly increased time to stay in Quadrant 3 as compared with the vehicle group (2.0 mg group, $P<.01$; Fig. 16), suggesting that β-estradiol 3-benzoate could ameliorate deranged memory function.

The photograph of cresyl violet staining (Fig. 17) revealed that the vehicle-treated rats exhibited severe destruction of the dentate granule cells versus the saline-treated group. By contrast, a quantitative data analysis by counting of viable cells using the brain sections of animals treated with the vehicle and β-estradiol showed an almost overlapping result between the groups (Fig. 18). It was, however, ascertained that degeneration of both microtubules and axons might be faintly mitigated by subchronic

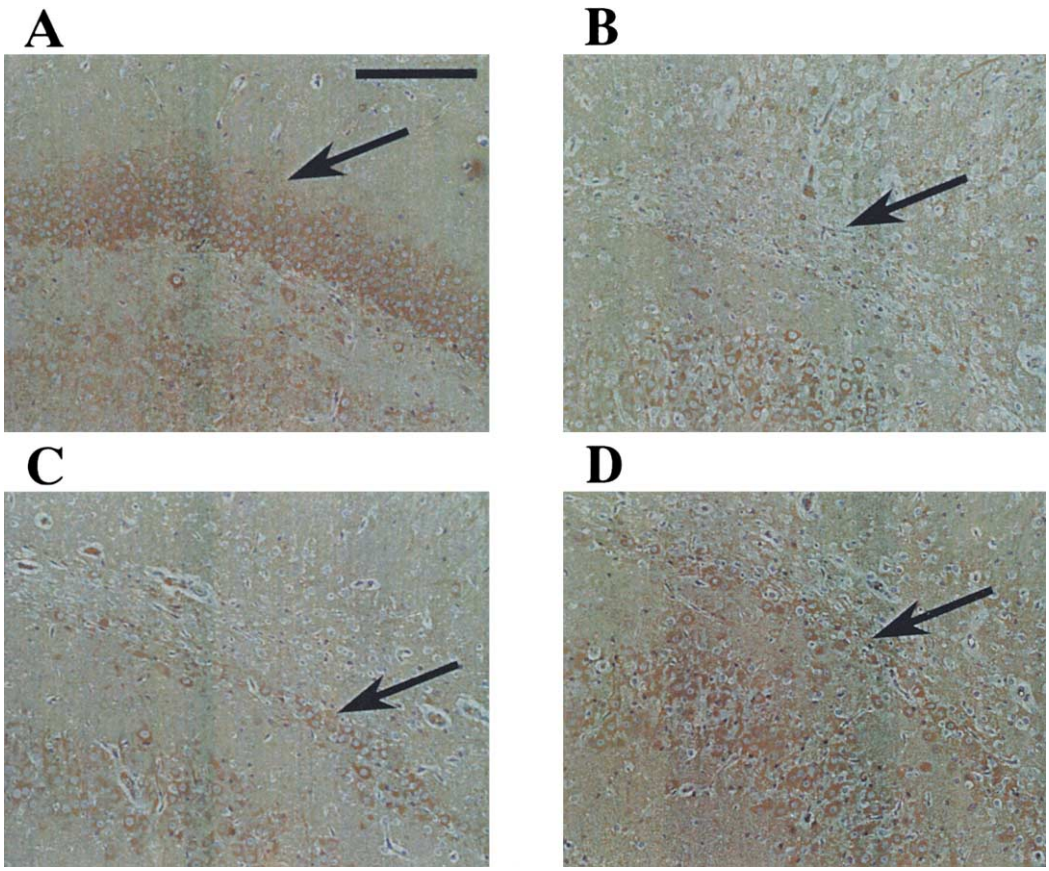

Fig. 19. Antitubulin staining of the dentate gyrus of the hippocampal formation from male CRJ:SD rats at 4 weeks after intrahippocampal infusion of saline (A), colchicine (0.3 μg/site) + vehicle (B), colchicine (0.3 μg/site) + β-estradiol 3-benzoate (0.5 mg) (C) and colchicine (0.3 μg/site) + β-estradiol 3-benzoate (2.0 mg) (D). Arrows (→) indicate the dentate granule cell layers (5 μm sections). Scale bar = 200 μm.

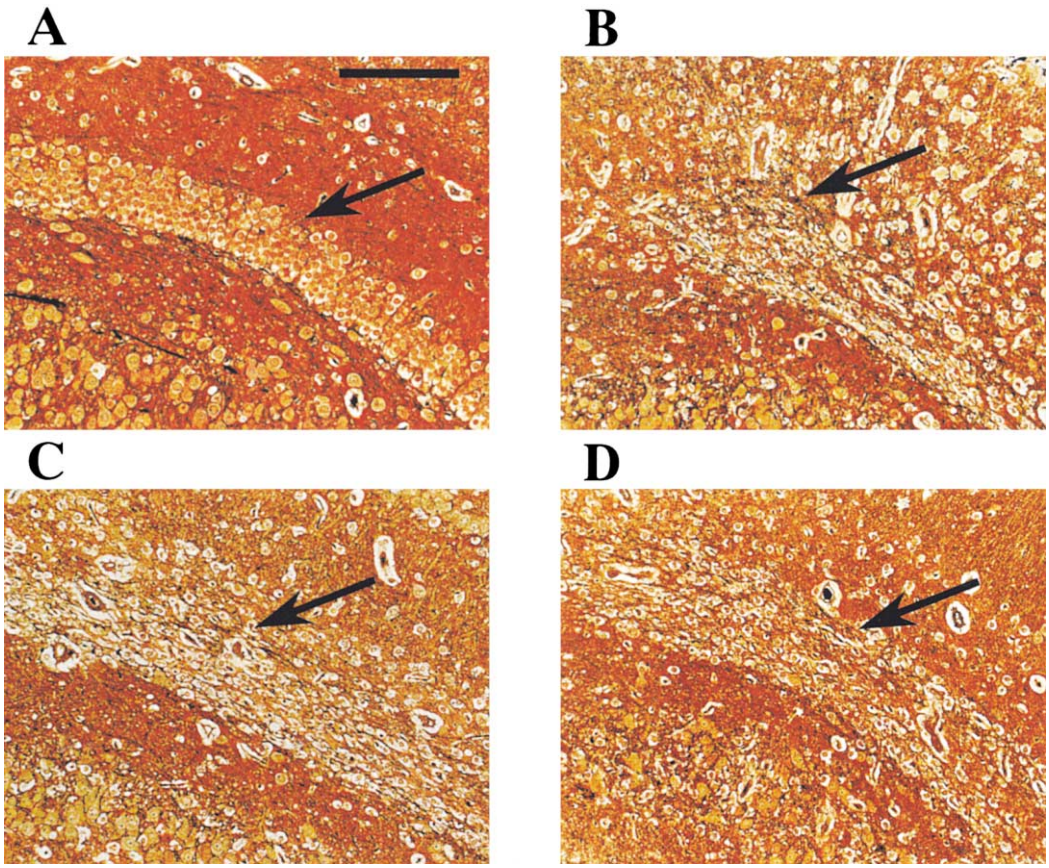

Fig. 20. Nauta staining of the dentate gyrus of the hippocampal formation from male CRJ:SD rats at 4 weeks after intrahippocampal infusion of saline (A), colchicine (0.3 μg/site) + vehicle (B), colchicine (0.3 μg/site) + β-estradiol 3-benzoate (0.5 mg) (C) and colchicine (0.3 μg/site) + β-estradiol 3-benzoate (2.0 mg) (D). Arrows (→) indicate the dentate granule cell layers (5 μm sections). Scale bar = 200μm.

treatment with β-estradiol, as revealed by tubulin and axon stainings shown in Figs. 19 and 20, respectively.

4. Discussion

Although it has been previously reported that intradentate infusion of colchicine destroyed granule cells in the dentate gyrus of the hippocampus (Goldschmidt and Steward, 1982; Lothman et al., 1982; Jarrard et al., 1984; Walsh et al., 1986; Tilson and Peterson, 1987; Tilson et al., 1987) and induced learning impairment in various learning tasks (Walsh et al., 1986; Tilson et al., 1988; Nanry et al., 1989; Tandon et al., 1991), all of the previous reports show learning impairment only at doses where colchicine is able to induce distinct destruction of the dentate granule cells in the hippocampus, concluding that the integrity of the granule cell layer plays a crucial role for memory function. In searching for producing an animal model for early AD, we found that colchicine-induced learning impairment occurs at lower doses without any apparent neuronal degeneration in the dentate gyrus. Moreover, we showed that microtubule depolymerization or axonal injury, which appeared to precede neuronal degeneration, might be implicated in memory function. Several neurodegenerative diseases accompanying memory malfunction such as AD and FTDP-17, representative of tauopathy as a disease group, have been known to have tau pathology, namely, the inability to bind tau to microtubules (Hasegawa et al., 1998; Hong et al., 1998), important neuronal cytoskeletal proteins and a lack of microtubule assembly, finally leading to impairment of axonal transport followed by neuronal cell death. It has been also reported that the extent of tau pathology is even more significantly correlated with dementia severity than that of SPs containing A β (Arriagada et al., 1991; McKee et al., 1991; Hyman and Tanzi, 1992; Cummings et al., 1996; Brion, 1998).

Tau pathogenesis is suggested to play a final common pathway in various neurodegenerative diseases including sporadic AD, but not familial AD. Therefore, we presumed that microtubule destabilization might be a key factor inducing memory impairment.

The postmortem brain examination of demented patients has shown the presence of NFTs, SPs, loss of cortical neurons in temporal and frontal lobes and the reduction of the acetylcholine markers (Mann et al., 1988). Since these changes are observed in the hippocampus of patients with early diagnosis of AD, AD has been defined as 'hippocampal dementia' (Ball et al., 1985).

Colchicine, which has been used clinically in the treatment of goats (Hastie, 1991), is a plant-derived alkaloid that binds to tubulin and depolymerizes microtubules (Osborn and Weber, 1976; Walker and Whitfield, 1985), thereby disrupting axonal transport (Karlsson and Sjostrand, 1969; Fink et al., 1973; Wooten et al., 1975). Several studies have also demonstrated that colchicine can significantly inhibit the function of various ion channels, including voltage-gated sodium (Matsumoto et al., 1984) and calcium (Johnson and Byerly, 1993) channels as well as ligand-gated nicotinic (Hardwick and Parsons, 1995) and $GABA_A$ receptor-gated ion channels (Whatley and Harris, 1996; Whatley et al., 1994). The mechanism(s) underlying these actions is unclear, but has been hypothesized to involve the microtubule-depolymerizing actions of this drug.

Colchicine, infused into the dentate gyrus, seemed to primarily act on microtubules because microtubule disruption emerged at apparently lower doses than those at which the dentate granule cell was destroyed. Memory function, evaluated by MWM, was found to have a better correlation with the integrity of microtubules than with that of neuronal cell death. We showed that microtubule depolymerization or axonal degeneration preceded the granule cell death as revealed by the two observations that neuronal cell death was not detected but microtubule/axon dysfunction was evident 15 days after the infusion of colchicine (0.1 or 0.3 μg/site) and that both dentate granule cell death and microtubule/axon dysfunction were clearly detected 4 weeks after colchicine (0.3 μg/site) treatment. Thus, we consider that an animal model accompanying microtubule dysfunction but not neuronal cell death is suitable for mimicking early-stage AD and is useful to search for novel compounds for treatment, slowing of progression or prevention of the disease. In this experiment, we presumed that intradentate treatment with 0.1 or 0.3 μg of colchicine could be appropriate to mimic early AD pathology.

In the present study, systemic injection of colchicine did not affect memory function, suggesting poor blood–brain barrier permeability of colchicine. Our observation is inconsistent with a previous report (Bensimon and Chermat, 1991). In comparison with doses (15–120 μg/kg) used in their report, we used higher doses, a maximum dose of up to 300 μg/kg at which some peripheral side effects, such as gradual reduction in body weight, were observed (data not shown). The reasons for the different results between our study and their report are unclear, but the differences in the rat strains and behavioral tasks (the food-rewarded operant task in their report) used may lead to different results. Even though systemic administration of colchicine could induce learning impairment, it could not be concluded that hippocampus-dependent hypofunction led to memory dysfunction since colchicine can be widely distributed throughout the brain by systemic circulation. Accordingly, such an animal model does not apparently mimic early AD in which NFTs, that is, tau pathology, are always confined to limbic and paralimbic structures such as hippocampus and entorhinal–transentorhinal cortex (Braak and Braak, 1996). This early stage of NFT distribution may be associated with the states of mild cognitive impairment, senescent forgetfulness and preclinical AD (Delacourte et al., 1999). The neuropathological criterion for definitive diagnosis of AD is reached only when NFT clusters emerge in association neocortex (The National Institute on Aging and the Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease, 1997). Thus, a hippocampal-specific degenerative animal model may be a very significant tool for studying the functional deterioration observed in AD.

As it has been reported that estradiol has various beneficial effects on the central nervous system in neurodegenerative diseases (e.g., the effect on synaptic density (Wolley and McEwen, 1993), neurotrophic action (Toran-Allerand, 1984), the effect on interaction with apoE4 and AD (Paganini-Hill and Henderson, 1994; Stone et al., 1998), the effect on APP metabolism (Jaffe et al., 1994), the effect on cerebral blood flow (Ohkura et al., 1994), the effect on memory function (Sherwin, 1988; Sherwin and Phillips, 1990; Barrett-Connor and Kritz-Silverstein, 1993; Robinson et al., 1994; Shaywitz et al., 1999), and the effect on acetylcholine esterase inhibition (Schneider et al., 1995)), it is interesting to examine whether treatment of our animals with estradiol is effective. Clinical studies have suggested that the abrupt decline of estradiol production in postmenopausal women may be associated with a vulnerability of women to develop AD, although men, in contrast, have an intrinsic supply of estrogen by having the ability to aromatize testosterone (aromatase) into estrogen in the brain. Considerable evidence has emerged from neuropathological studies (Luine, 1985; Gould et al., 1990; Toran-Allerand et al., 1992), animal behavioral studies (Joseph et al., 1989; McDermott et al., 1994) and human investigations (Sherwin, 1988; Ditkoff et al., 1991) to suggest that estradiol may be beneficial in improving cognition and mood in AD. In the present study, subchronic treatment with a 14-day sustained release formulation of β-estradiol 3-benzoate after colchicine administration was found to significantly ameliorate learning deficits. It, however, remains unclear whether the deficit with colchicine and the improvement with estradiol are specific to hippocampal-dependent memory because no tests of hippocampal-independent memory were included. Our quantitative investigation by counting viable cells, however, showed an almost overlapping result between groups. Although we cannot explain the poor correlation between recovery of memory function by estradiol treatment and histological state, possible involvement of a difference in time between the behavioral test (2 weeks after colchicine infusion) and histological examination (4 weeks after that) is considered. Secondly, we suppose that the doses of estradiol were too small to reverse histological changes caused by colchicine, but doses of estradiol used here could barely significantly

ameliorate memory impairment caused by colchicine. Doses of estradiol used in this experiment were calculated as 0.036 and 0.14 mg/day, respectively, and kept lower in order to avoid producing any nonspecific effects such as a cancer-inducing action of estradiol, and systemic administration was adopted. Further investigation using higher doses of estradiol may be necessary in future. Thirdly, it is suggested that the beneficial effects of estradiol on memory function, shown here, may result from one or more effects among the above-mentioned actions of estradiol other than more or less protective effects reported here on cytoskeletal proteins (e.g., microtubule). For example, it is possible that improvement of learning behavior was due to either gene-dependent regulation of neurotransmitter systems, such as the up-regulation of cholinergic activity (Simpkins et al., 1997) or the involvement of surface receptor-associated signaling via ion channels modulating electrical properties of neurons and transmitter release processes (Paul and Purdy, 1992; Razandi et al., 1999). As a consequence, estradiol treatment might potently enhance neuronal function in the remaining viable cells. At present, details in the underlying mechanism(s) of estradiol are obscure. It is interesting, however, that a compound, estradiol, displaying clinically beneficial effects on AD may be also effective in this animal.

In conclusion, we found that memory and learning deficits, as well as destabilization of microtubules and/or axons, were observed at lower doses than those in which colchicine evidently induced dentate granule cell death. This suggests that dysfunction of microtubules and/or axons could be involved in learning behavior but dentate neuronal cell death could not. These results indicate that the animal model accompanying the colchicine-induced functional defect, namely, axoplasmic flow, but not apparent neuronal cell degeneration, may well mimic early-stage AD, and could give a clue to seek an avenue to successful treatment for early AD.

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