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The Alzheimer's Disease Drug Memantine Increases the Number of Radial Glia-like Progenitor Cells in Adult Hippocampus

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KEY WORDS
neurogenesis; NMDA receptor; GFAP; proliferation; nestin; Sox2

ABSTRACT
New neurons are continuously generated in the hippocampus of the adult mammalian brain, and *N*-methyl-D-aspartate receptor (NMDA-R) antagonists have been found to increase the number of newly generated neurons in the dentate gyrus (DG) of the adult hippocampus. In this study, we examined the effect of memantine, an NMDA-R antagonist that is clinically used for the treatment of Alzheimer's disease, on primary progenitor cells exhibiting a radial glia-like (RGL) morphology in the DG. We injected 3-month-old mice with memantine (50 mg/kg body weight, intraperitoneally [i.p.]); 3 days later, we injected the mice with 5-bromo-2-deoxyuridine (BrdU; 75 mg/kg body weight, i.p.). We then counted the number of BrdU-labeled RGL progenitor cells in the DG 1 or 7 days after the BrdU-injection. The number of BrdU-labeled RGL progenitor cells had increased significantly by 5.1-fold on day 1 and by 13.7-fold on day 7 after BrdU-injection. Immunohistochemical staining revealed that the BrdU-labeled RGL progenitor cells expressed two primary progenitor cell marker proteins, nestin and Sox2. These results clearly demonstrated that memantine promotes the proliferation of RGL progenitor cells. We also found that memantine increased the ratio of horizontally aligned RGL progenitor cells, which are probably produced by symmetric division. These findings suggest that memantine increases the proliferation of primary progenitor cells and expands the primary progenitor cell pool in the adult hippocampus by stimulating symmetric division.

INTRODUCTION

The generation of new neurons, so-called neurogenesis, persists throughout life in the hippocampus of mammals, including humans (Altman and Das, 1965; Eriksson et al., 1998; Kuhn et al., 1996; Maekawa et al., 2005; Namba et al., 2005; Seki and Arai, 1993, 1995). Neural progenitor cells divide and give rise to new neurons in at least two regions of the adult brain: the dentate gyrus (DG) of the hippocampus and the subventricular zone of the lateral ventricle (Doetsch et al., 1999; Fukuda et al., 2003; Seki et al., 2007; Seri et al., 2001). Neurogenesis in the hippocampus associated with synaptic plasticity (Schmidt-Hieber et al., 2004) and cognitive functions, including learning and memory (Becker and Wojtowicz, 2007; Kee et al., 2007; Wojtowicz et al., 2008). The initial step in hippocampal neurogenesis is the proliferation of progenitor cells in the subgranular zone (SGZ) of the DG, which contains several types of progenitor cells (Filippov et al., 2003; Fukuda et al., 2003; Seki et al., 2007; Seri et al., 2004; von Bohlen Und Halbach, 2007). Primary progenitor cells, which are characterized by the expression of glial fibrillary acidic protein (GFAP) and nestin, extend their radial processes across the granule cell layer (GCL), exhibit a radial glia-like (RGL) morphology, and then divide to produce intermediate progenitor cells. The intermediate progenitor cells generate immature neurons, which migrate into the GCL where they differentiate into mature granule neurons and ultimately contribute to the local neural network (van Praag et al., 2002).

Neurogenesis in the DG is promoted not only by pathological factors, such as ischemia, epileptic seizures, and traumatic brain injury (Liu et al., 1998; Parent et al., 1997), but also by various physiological factors, including growth factors, neurotransmitters, an enriched environment, learning and exercise (reviewed in Abrous et al., 2005). Glutamate signaling is also involved in hippocampal neurogenesis. Our recent study and a study by Jin et al. (2006) showed that memantine, an uncompetitive *N*-methyl-D-aspartate receptor (NMDA-R) antagonist that is clinically used for the treatment of Alzheimer's disease, increases the cell proliferation and promotes the subsequent production of mature neurons, similar to other NMDA-R antagonists, such as MK-801, D(−)-2-amino-5-phosphonopentanoic acid (D-APV), and CGP-43487 (Cameron et al., 1995; Hirasawa et al., 2003; Nacher et al., 2001). However, because the mechanism

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TABLE 1. Antibodies

Marker	Species, isotype	Label	Working dilution	Vendor
Primary antibodies				
BrdU	Rat IgG	None	1:400	ImmunologicalsDirect.com, UK
GFAP	Mouse IgG	None	1:2000	Sigma, Mo, USA
GFAP	Rabbit IgG	None	1:800	Dako, Denmark
Nestin	Mouse IgG	None	1:400	BD Bioscience, CA, USA
PSA-NCAM	Mouse IgM	None	1:800	Seki and Arai (1991)
Sox2	Rabbit IgG	None	1:2000	Chemicon International, CA, USA
Secondary antibodies				
Anti-mouse IgG	Donkey IgG	Cy5	1:200	Jackson, PA, USA
Anti-mouse IgM	Donkey IgG	Cy5	1:200	Jackson
Anti-rabbit IgG	Donkey IgG	Cy2	1:200	Jackson
Anti-rabbit IgG	Donkey IgG	Cy5	1:200	Jackson
Anti-rat IgG	Donkey IgG	Cy3	1:200	Jackson

responsible for the stimulation of cell proliferation by NMDA-R antagonists remains unknown, in the present study we focused on primary progenitor cells exhibiting a RGL morphology and examined the effect of memantine on their proliferation. The present results clearly demonstrated that memantine promotes the proliferation of RGL progenitor cells and the subsequent expansion of the RGL progenitor cell pool in the GCL by stimulating symmetric division.

MATERIALS AND METHODS

Three-month-old male C57BL6/J mice (Clea Japan, Tokyo, Japan) were used in this study. All experimental procedures were approved by The Animal Care and Use Committee of the National Institute of Neuroscience.

Animals and Drug Administration

Mice were injected with memantine (Sigma, St. Louis, MO) at a dose of 50 mg/kg body weight i.p. or with the same volume of 0.9% saline (Ohtsuka Pharmaceuticals, Tokyo, Japan), as a control. The dose of memantine was determined as that sufficient to promote the proliferation of RGL progenitor cells in pilot studies. Three days later, the mice were injected with BrdU (Sigma) at a dose of 75 mg/kg body weight i.p. on three separate occasions at an interval of 2 h. The mice were then sacrificed on day 1, 2 or day 7 after the BrdU-injection.

Tissue Preparation

Mice were deeply anesthetized with sodium pentobarbital (Kyoritsu Pharmaceuticals, Tokyo, Japan) and then transcardially perfused with 4% paraformaldehyde in 0.1-M phosphate buffer. Their brains were removed and immersion-fixed for 24 h at 4°C in the same fixative. After washing in phosphate-buffered saline (PBS), the brains were successively equilibrated in 10% and 20% sucrose in PBS. The cerebral cortices containing the hippocampal formation were dissected away from the remaining brain structure. Next, 1- to 2-mm-thick slices were cut from the medial part of the hippocampus in a plane perpendicular to the septo-temporal axis of the hippocampal formation, embedded in Tissue-Tek optimal cutting temperature compound (Sakura, Tokyo, Japan), and frozen with liquid nitrogen (Seki et al., 2007).

Immunohistochemistry

Immunohistochemistry was performed using a floating method, as described previously (Namba et al., 2005, 2007). Frozen brains were sliced into 40-μm sections with a cryostat (CM-3000; Leica, Nussloch, Germany). After washing the sections with PBS, they were incubated at 4°C for 72 h in PBS containing 1% bovine serum albumin (BSA), 1% normal donkey serum, and 0.1% triton X-100 plus one of the primary antibodies shown in Table 1. To stain poly-sialylated neural cell adhesion molecule (PSA-NCAM), the sections were pretreated with 100% methanol (Seki and Arai, 1991). After washing in PBS, the sections were then incubated at room temperature for 1–2 h in PBS containing 1% BSA plus an appropriate secondary antibody shown in Table 1. For immunostaining with anti-BrdU antibody, the sections were incubated in 2 N HCl at 37°C for 35 min after staining with the other antibodies (anti-GFAP and anti-Nestin antibodies or anti-GFAP and anti-Sox2 antibodies and the appropriate secondary antibodies), neutralized with 0.1-M borate buffer (pH 8.5), and then incubated with anti-BrdU antibody (Table 1) at 4°C for 24 h in PBS containing 1% BSA. After washing in PBS, the sections were incubated at room temperature for 1–2 h in PBS containing 1% BSA plus Cy3-conjugated anti-rat IgG antibody (Table 1). The sections were mounted on a glass slide (SUPERFROST; Matsunami, Osaka, Japan), and examined for fluorescent signals using a confocal laser-scanning microscope (CLSM) with 20×, 40×, and 60× objectives (FV1000; Olympus, Tokyo, Japan).

Cell Counting

To measure the number of GFAP- or Nestin-positive RGL progenitor cells or PSA-NCAM-positive cells in the GCL including the SGZ, an average of five sections per mouse was analyzed (see Fig. 1). We defined RGL progenitor cells as GFAP- or nestin-positive cells that extend a single process from SGZ toward the molecular layer. To measure the number of BrdU-labeled RGL

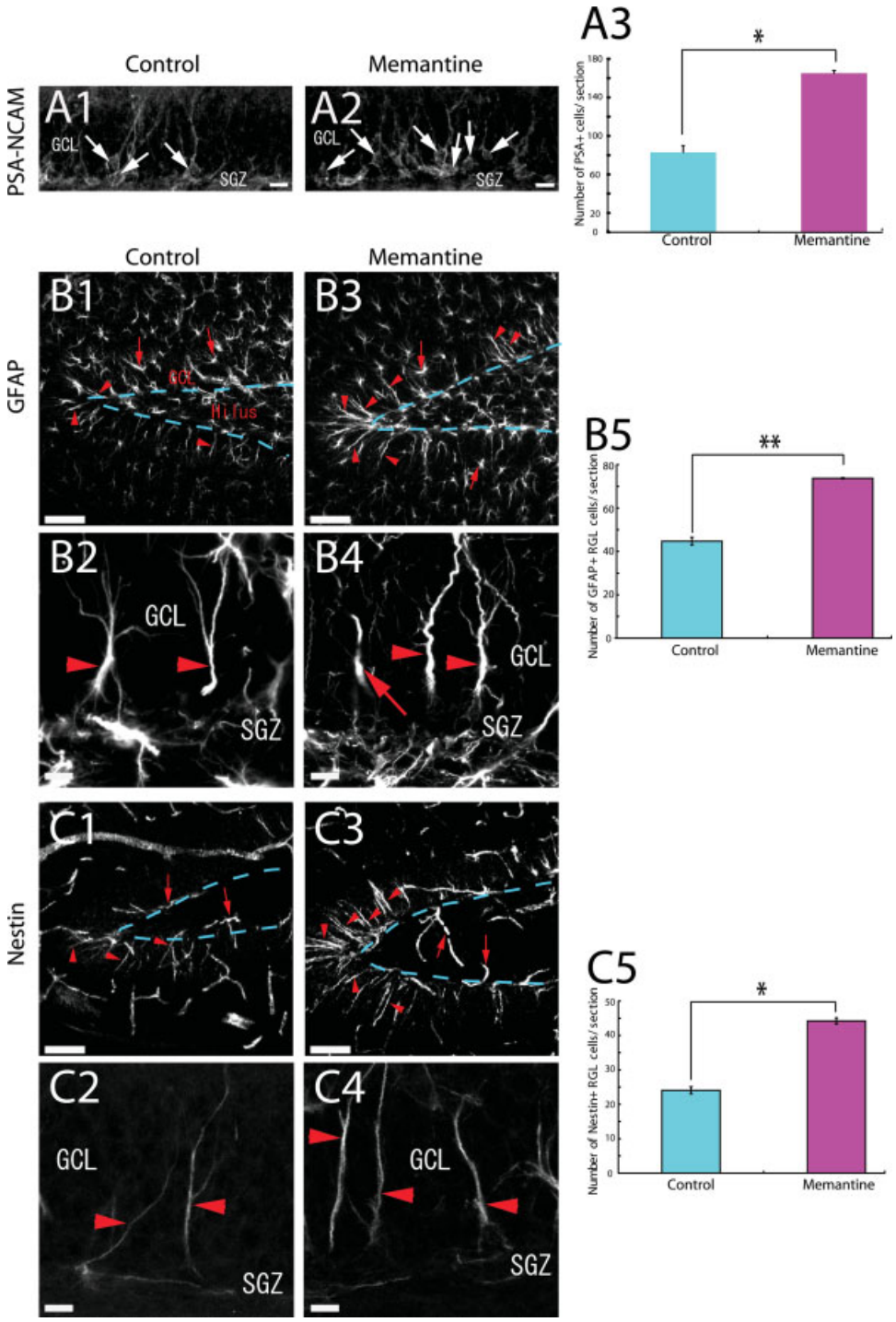

Fig. 1. Effect of memantine on the number of immature neurons and RGL progenitor cells in the DG. Example of immunohistochemical staining with anti-PSA-NCAM antibody (**A**), anti-GFAP antibody (**B**), and anti-nestin antibody (**C**) 10 days after the injection of saline as a control (A1, B1-2, and C1-2) or the injection of memantine (A2, B3-4, and C3-4). The arrows in panel A indicate the PSA-NCAM-positive cells. The arrowheads in panels B and C indicate the RGL progenitor cells. The arrows in panels B and C indicate polygonal astrocytes and cell fragments and blood vessels, respectively. The blue dotted lines in panels B and C indicate the border between the GCL and hilus. Scale bars = 10 μm in panel A, B2, B4, C2, and C4, and 50 μm in panels B1, B3, C1, and C3. Quantitative analysis of the number of immature neurons (A3), GFAP-positive RGL progenitor cells (B5), and nestin-positive RGL progenitor cells (C5) in the GCL. $*P < 0.001$ and $**P < 0.0001$, respectively, when compared with the control group.

progenitor cells in the GCL including the SGZ, an average of nine sections per mouse was analyzed (Figs. 2–5). Cell counting was carried out under a CLSM (FV1000).

Cell Alignment Analysis

The mode of cell division was analyzed as described in previous reports (Chenn and McConnell, 1995; Haydar et al., 2003). Briefly, the position of daughter cells was classified as vertical (daughter cells aligned vertically to the axis of the radial glial fiber) or horizontal (daughter cells aligned horizontally to the axis of the radial glial fiber). Cells were counted while viewing the sections through the 60× objective of a CLSM (FV1000).

Statistical Analysis

Data were evaluated using a one-way analysis of variance followed by the post-hoc Scheffe's *F*-test; some of

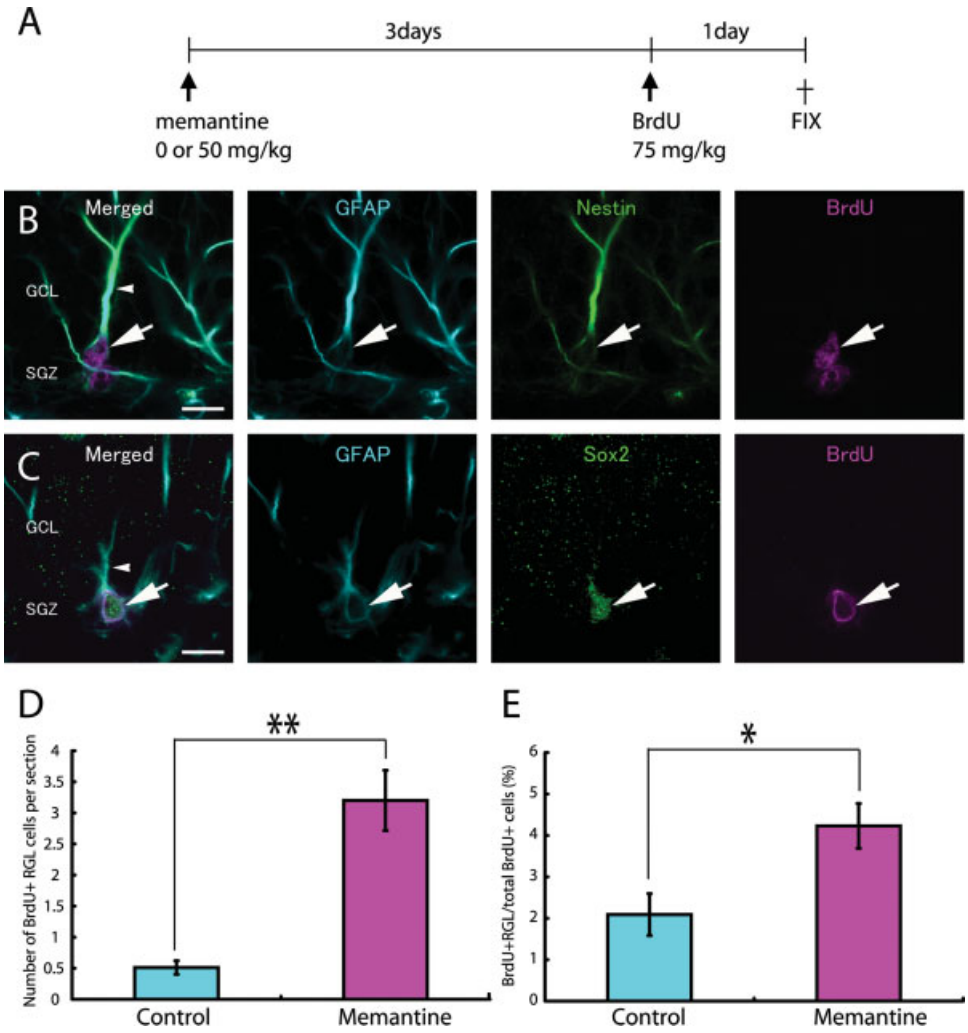

Fig. 2. Immunohistochemical and quantitative analysis of RGL progenitor cells in the GCL 1 day after BrdU-injection. (**A**) Schematic illustration of the experimental design. (**B, C**) Example of BrdU-labeled RGL progenitor cells (arrows) expressing nestin (green) (B) or Sox2 (green) (C) in the memantine-injected group. The arrowheads point to a radial fiber. Scale bar = 10 μm. (**D, E**) Quantitative analysis of the number of BrdU-labeled RGL progenitor cells (D) and the percentage of BrdU-labeled RGL progenitor cells (E) in the GCL. $^{*}P < 0.05$, and $^{**}P < 0.001$, respectively, when compared with the control group.

the data were analyzed using the Mann–Whitney *U*-test. All values were expressed as the mean ± SEM, and *P*-values less than 0.05 were considered significant.

RESULTS

Increase in the Number of RGL Progenitor Cells in Memantine-Injected Mice

To investigate the effect of memantine on the number of RGL progenitor cells in the adult hippocampus, we intraperitoneally injected 3-month-old mice with a dose of 50 mg/kg memantine, fixed their brains 10 days later, and then prepared brain sections. We initially stained the sections with anti-PSA-NCAM antibody, a marker antibody for immature neurons. The number of PSA-NCAM-positive cells in the memantine-injected group was 2.0-fold higher than that in the control group (Fig. 1A; control, 80.3 ± 11.9 cells/section, $n = 3$; memantine, 162.4 ± 25.5 cells/section, $n = 3$), suggesting that memantine promoted neurogenesis in adult mouse hippocampus. We next stained the sections with anti-GFAP antibody and counted the number of GFAP-positive cells with a RGL morphology. The number of GFAP-positive RGL progenitor cells in the memantine-injected group was 1.6-fold higher than that in the control group (Fig. 1B; control, 44.7 ± 9.6 cells/section, $n = 3$; memantine, 73.7 ± 0.25 cells/section, n = 3). In contrast, memantine had no effect on the number or morphology of stellate fibrous GFAP-positive cells in the hilus or molecular layer (data not shown). Similarly, the number of RGL progenitor cells expressing nestin in the memantine-injected group increased by 1.8-fold (Fig. 1C; control, 24.1 ± 3.2 cells/section, $n = 3$; memantine, 44.2 ± 2.52 cells/section, $n = 3$). The greatest increase was observed in the tip of the GCL, where large numbers of progenitor cells are present (Babu et al., 2007). These results indicate that memantine increased the number of RGL progenitor cells in the hippocampus but had no effect on the number or morphology of the stellate fibrous astrocytes.

Memantine Promotes the Proliferation of RGL Progenitor Cells in the GCL

Next, to determine whether the increase in the number of RGL progenitor cells shown in Fig. 1 was due to the proliferation of RGL progenitor cells in response to

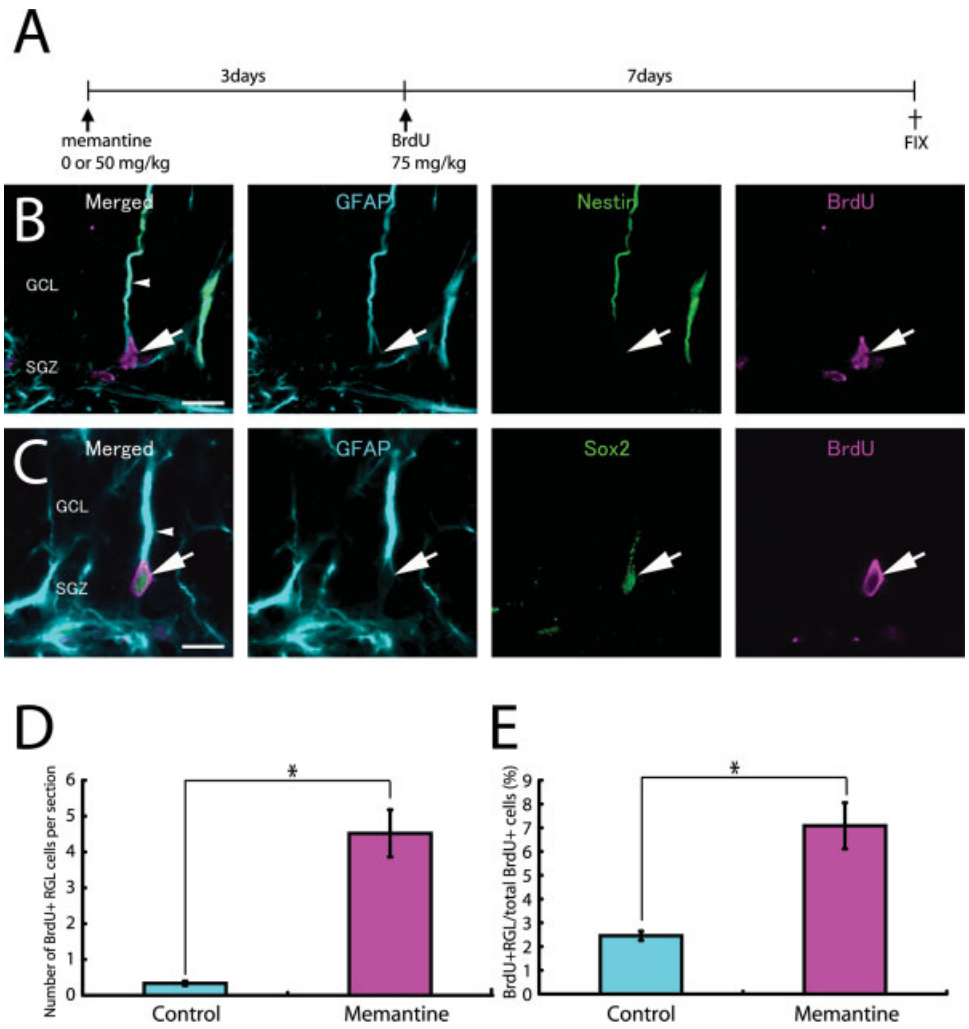

Fig. 3. Immunohistochemical and quantitative analysis of RGL progenitor cells in the GCL 7 days after BrdU-injection. (**A**) Schematic illustration of the experimental design. (**B, C**) Example of BrdU-labeled RGL progenitor cells (arrows) expressing nestin (green) (B) or Sox2 (green) (C) in the memantine-injected group. The arrowheads point to a radial fiber. Scale bar = 10 μm. (**D, E**) Quantitative analysis of the number of BrdU-labeled RGL progenitor cells (D) and the percentage of BrdU-labeled RGL progenitor cells (E) in the GCL. $^{**}P < 0.01$ when compared with the control group.

memantine, we investigated the effect of memantine on the proliferation of RGL progenitor cells. We injected mice with a dose of 50 mg/kg memantine; 3 days later, we injected them with BrdU. Their brains were fixed 1 day after the BrdU-injection (Fig. 2A); after preparing the brain sections, we immunostained them with anti-BrdU antibody and anti-GFAP antibody. In the memantine-injected group, the BrdU-labeled RGL progenitor cells were mainly located in the SGZ and the innermost portion of the GCL (Fig. 2B,C). The staining signals for anti-BrdU antibody were observed in solid or the periphery of the nuclei. Because these staining signals were also observed in previous reports (Nakagawa et al., 2002; Namba et al., 2007), it is unlikely that the different staining signals were caused by the memantine-injection.

The number of BrdU-labeled RGL progenitor cells significantly increased by 5.1-fold in the memantine-injected group (Fig. 2D; control, 0.51 ± 0.11 cells/section, $n = 5$; memantine, 2.59 ± 0.55 cells/section, $n = 5$), and the BrdU-labeled RGL progenitor cells in the memantine-injected group also increased as a percentage of the total BrdU-labeled cells (Fig. 2E; control, 2.09% ± 0.51%, $n = 5$; memantine, 4.23% ± 0.54%, $n = 5$). We then characterized the BrdU-labeled RGL progenitor cells by immunostaining them with two other progenitor cell markers, nestin and Sox2 (Steiner et al., 2006). As shown in Fig. 2B,C, the RGL progenitor cells labeled with BrdU expressed nestin and/or Sox2 as well as GFAP, suggesting that the proliferating RGL progenitor cells possessed the same phenotype as the primary progenitor cells. These findings indicate that memantine significantly increased the proliferation of RGL progenitor cells in the GCL.

Properties of RGL Progenitor Cells Are Maintained Following Memantine-Injection

To further examine the effect of memantine on the fate of the dividing RGL progenitor cells, we injected mice with a dose of 50 mg/kg memantine and 3 days later, we injected them with BrdU; we then fixed their brains 7 days after the BrdU-injection (Fig. 3A). Next, we prepared brain sections and immunostained them with anti-BrdU antibody and anti-GFAP antibody. The number of BrdU-labeled cells with an RGL morphology

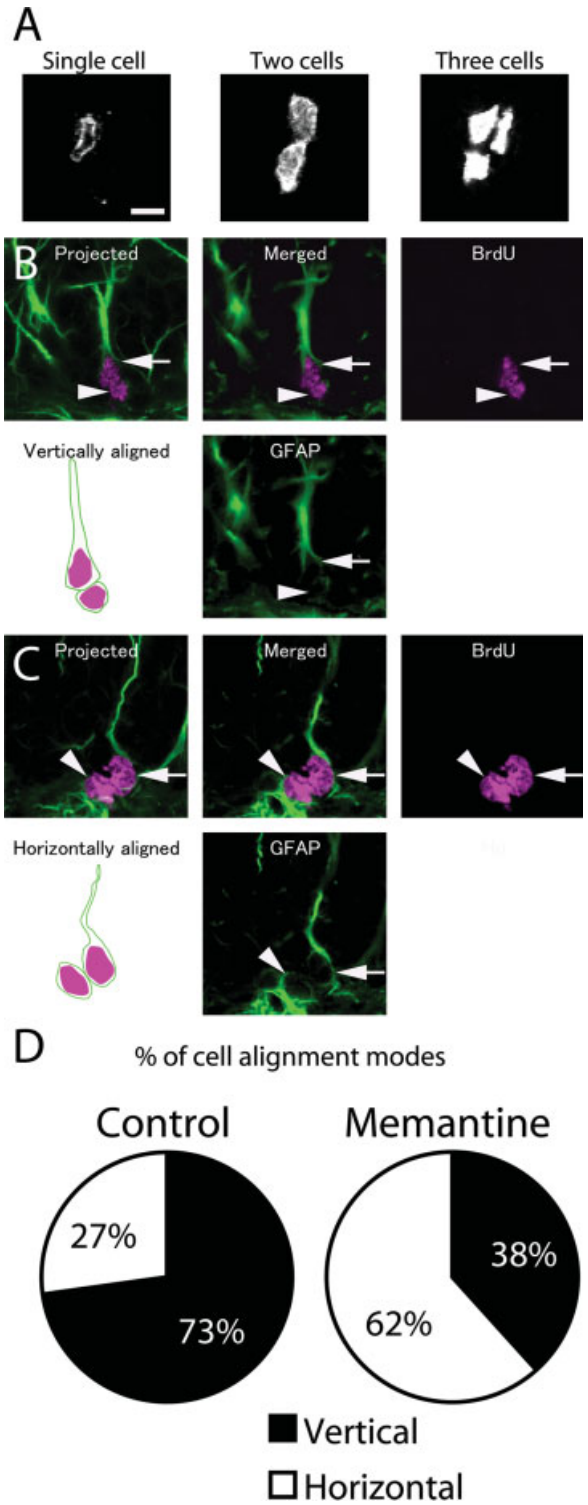

Fig. 4. Cell alignment analysis of BrdU-labeled RGL progenitor cells in the GCL. (**A**) Example of cell clusters one day after BrdU-injection. Scale bar = 10 μm. (**B**) Example of vertically aligned BrdU-labeled RGL progenitor cells (arrow) and non-RGL cells (arrowhead). Both BrdU-labeled cells (red) expressed GFAP (green). (**C**) Example of horizontally aligned BrdU-labeled RGL progenitor cells (arrow) and non-RGL cells (arrowhead). Both BrdU-labeled cells (red) expressed GFAP (green). (**D**) Quantitative analysis of the cell alignment modes in the control group and the memantine-injected group. There was a significant difference ($P < 0.01$) in the cell alignment modes between the control group and the memantine-injected group.

in the GCL dramatically increased by 13.7-fold in the memantine-injected group (Fig. 3D; control, 0.33 ± 0.06 cells/section, $n = 3$; memantine, 4.52 ± 0.66 cells/section, $n = 3$), and the percentage of BrdU-labeled RGL progenitor cells among the total BrdU-labeled cells also increased in the memantine-treated group (Fig. 3E; control, 2.45% ± 0.19%, $n = 3$; memantine, 7.08% ± 0.97%, $n = 3$). Moreover, these BrdU-labeled RGL progenitor cells located in the GCL also expressed the progenitor markers nestin and/or Sox2 (Fig. 3B,C). These findings indicate that a certain number of the divided RGL progenitor cells in the memantine-injected group retained their progenitor properties at least 1 week after cell division.

Memantine Stimulates Symmetric Division of RGL Progenitor Cells in the GCL

Neurogenesis is thought to occur as a result of several modes of cell division. Two examples of these modes are asymmetric cell division, which results in a single daughter neuron and a mother cell that remains a progenitor cell, and symmetric cell division, which expands the pool of progenitor cells (Kriegstein et al., 2006). Recent reports have shown that the mother cell of vertically aligned cells had undergone asymmetric cell division, whereas the mother cell of horizontally aligned cells had undergone symmetric cell division (Encinas et al., 2006; Haydar et al., 2003). To investigate the effect of memantine on the mode of cell division, we examined the alignment of the newly generated pairs of RGL progenitor cells in the GCL 1 day after BrdU-injection. At 1 day after the BrdU-injection, some of the BrdU-labeled cells had formed cell clusters. We divided the cell clusters into three groups based on the number of BrdU-labeled cells in the cell clusters (Fig. 4A) and examined the percentages of these three groups: single cell (control, 40.3% ± 1.6%, $n = 3$; memantine, 35.3% ± 2.5%, $n = 3$), two-cell cluster (control, 29.9% ± 2.0%, $n = 3$; memantine, 29.8% ± 1.8%, $n = 3$), and cluster of three or more cells (control, 29.4% ± 3.1%, $n = 3$; memantine, 34.9% ± 1.9%, $n = 3$). These results indicate that there were no significant differences in the percentages of the three groups between the control and the memantine-injected groups. To investigate the cell alignment modes, we focused on the two-cell clusters with BrdU- and GFAP-positive RGL cells (Fig. 4B,C). More than half of the BrdU-labeled RGL progenitor cells were horizontally aligned in the memantine-injected group (vertical: 38.3%, horizontal: 61.7%), whereas most of the BrdU-labeled RGL progenitor cells in the control group were vertically aligned (vertical: 72.7%, horizontal: 27.3%) (Fig. 4D), suggesting that memantine predominantly stimulates the symmetric division of RGL progenitor cells in the GCL. To further confirm the fate of the RGL progenitor cells, we examined the phenotype of the BrdU-labeled RGL cells in two-cell clusters 2 days after the BrdU-injection in the memantine-injected mice. More than half of the horizontally aligned two-cell

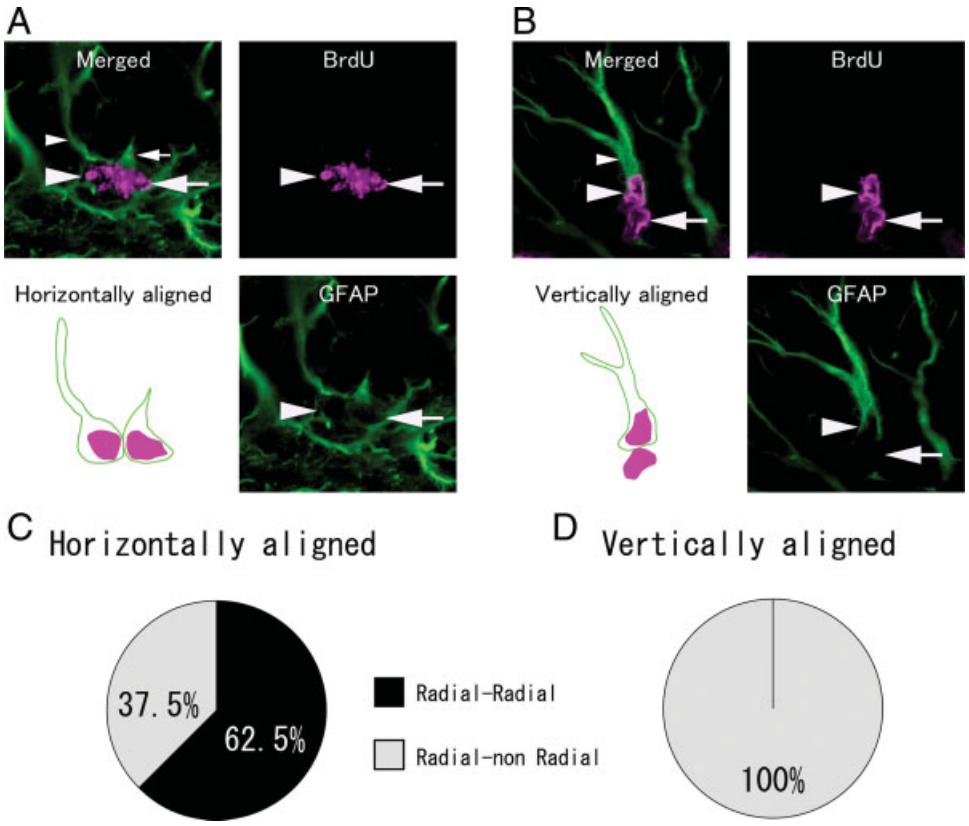

Fig. 5. Phenotype of BrdU-labeled cells in two-cell clusters in memantine-injected mice. (**A**) Example of horizontally aligned BrdU-labeled RGL progenitor cells. One RGL progenitor cell (arrowhead) possesses a long radial process (small arrowhead) and another RGL progenitor cell (arrow) has extended a short process (small arrow). Both BrdU-labeled cells (red) expressed GFAP (green). (**B**) Example of vertically aligned BrdU-labeled RGL progenitor cell (arrowhead) and non-RGL progenitor cell (arrow). The small arrowhead points to the radial process. The BrdU-labeled (red) RGL progenitor cells expressed GFAP (green), but the non-RGL progenitor cells only weakly expressed or did not express GFAP. (**C, D**) Quantitative analysis of the phenotype of BrdU-labeled cells in two-cell clusters.

clusters were formed by two RGL progenitor cells that strongly expressed GFAP (Fig. 5A,C, 62.5%, $n = 3$), suggesting a symmetric fate. In this case, one cell possessed a long radial process and another cell had a short process toward the GCL. This observation suggests that the cells had started to extend their radial processes and to become RGL cells. In contrast, almost all the vertically aligned, two-cell clusters were formed by the RGL progenitor cells and the non-RGL progenitor cells (Fig. 5B,D). The RGL progenitor cell strongly expressed GFAP, whereas the non-RGL progenitor cells weakly expressed or did not express GFAP, suggesting an asymmetric fate. No vertically aligned two-cell clusters formed by two RGL progenitor cells were observed in the present study. Taken together with the findings shown in Figs. 4 and 5, memantine probably enhances the symmetric division of RGL progenitor cells.

DISCUSSION

Neurogenesis in the hippocampus persists throughout life, and the principal source of newly generated neurons is RGL progenitor cells (primary progenitor cells) that express GFAP and/or nestin (Garcia et al., 2004; Lagace et al., 2007; Seki et al., 2007; Seri et al., 2001, 2004). Previous studies have shown that neurogenesis in the adult hippocampus is increased by NMDA-R antagonists, including MK-801 and CGP-43487 (Cameron et al., 1995; Nacher et al., 2001), but the effect of NMDA-R antagonists on RGL progenitor cells was not thoroughly investigated. In the present study, we showed that memantine, an uncompetitive NMDA-R antagonist, had increased the number of BrdU-labeled RGL progenitor cells 1 day after BrdU-injection, indicating that memantine promoted the proliferation of the RGL progenitor cells. Moreover, both the RGL morphology and the expressions of the progenitor cell markers nestin and Sox2 were sustained in the memantine-injected group for as long as 7 days after the BrdU-injection, suggesting that the properties of the progenitor cells were maintained in the newly generated cells following memantine-injection. In consequence, the number of the RGL cells in DG was increased by memantine-injection. This result is supported by the previous study, showing that a competitive NMDA-R antagonist CGP-43487 increased the number of nestin-expressing cells in the adult DG (Nacher et al., 2001). Memantine also affected the mode of cell division by the RGL progenitor cells, because the ratio of horizontally aligned RGL progenitor cells in the memantine-injected group increased significantly in comparison with that in the control group. Recent studies have shown that the alignment of daughter cells is important for determining their fate in the embryonic neocortex (Chenn and McConnell, 1995; Haydar et al., 2003), with horizontally aligned cells appearing to have a symmetric fate and vertically aligned cells appearing to have an asymmetric fate. If this is also the case in the adult hippocampus (Encinas et al., 2006; Kempermann et al., 2004), memantine appears to increase the proportion of

symmetric division in RGL progenitor cells. Taken together with the results of the present study, memantine appears to increase the population of RGL progenitor cells by promoting proliferation through the symmetric division of RGL progenitor cells. This effect of memantine on the primary progenitor cells is unique: memantine induces the expansion of the primary progenitor cell pool, because other stimulations of enhancing neurogenesis such as antidepressants, running, and enriched environment could not increase the number of the primary progenitor cells (Encinas et al., 2006; Kronenberg et al., 2003).

Despite cumulative evidence that NMDA-R antagonists promote neurogenesis, whether functional NMDA receptors are expressed in RGL progenitor cells remains controversial. Although immunohistochemical analyses in recent studies have demonstrated the expression of the NR1 and NR2B subunits of the NMDA-R in primary progenitor cells (Nacher et al., 2007), an electrophysiological study showed that the primary progenitor cells failed to respond to NMDA (Tozuka et al., 2005), indicating that the NR1 and NR2B subunits expressed in the RGL progenitor cells do not form functional NMDA-R. Consequently, it was speculated that NMDA antagonists may inhibit the neuronal activities evoked by glutamate in mature neurons and that the subsequent effects may promote the proliferation of RGL progenitor cells and neurogenesis. One potential candidate affecting the cell proliferation enhanced by memantine is FGF2, a mitogen for neural stem cells (Reynolds and Weiss, 1992). Using quantitative RT-PCR, we found that the expression of FGF2 in the GCL transiently increased by 3.7-fold on 1 day after the memantine-injection but decreased to the basal level on 2 days after the memantine-injection, whereas the expression of EGF, another major mitogen, was not affected by memantine-injection (Supp. Info. Fig. 1). These findings suggest that memantine stimulates the proliferation of RGL progenitor cells via FGF2 signaling. Further studies are needed to elucidate the mechanisms of the up-regulation of FGF2 expression by memantine-injection.

Memantine has been used clinically as a neuroprotective agent for moderate-to-severe Alzheimer's disease but has not been associated with the adverse effects, such as psychotomimetic and cardiovascular effects, shown by other NMDA-R antagonists (Johnson and Kotermanski, 2006; Reisberg et al., 2003). Jin et al. (2006) found that the administration of a clinical dose of memantine in mice (7.5 mg/kg via an intragastric route daily for 2 weeks) increased cell proliferation, and we recently confirmed that most of the newly generated cells in a group of mice injected with memantine ultimately differentiated into mature granule neurons (Maekawa et al., unpublished observations). These results suggest that the promotion of neurogenesis contributes to the therapeutic efficacy of memantine.

In conclusion, we have demonstrated that memantine significantly increases the number of RGL progenitor cells by stimulating symmetric division and that memantine enables the properties of RGL progenitor cells to be maintained in a certain number of newly generated cells, resulting in the expansion of the primary progenitor cell pool in the adult hippocampus.

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