International Journal of Neuropsychopharmacology (2014), **17**, 1957–1968. © CINP 2014
doi:10.1017/S1461145714000613

ARTICLE

Galantamine promotes adult hippocampal neurogenesis via M_1 muscarinic and $\alpha 7$ nicotinic receptors in mice

Yuki Kita[1]*, Yukio Ago[1]*, Kosuke Higashino[1], Kazuki Asada[1], Erika Takano[1], Kazuhiro Takuma[1] and Toshio Matsuda[1,2]

[1] *Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan*

[2] *United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University and University of Fukui, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan*

Abstract

Galantamine, an inhibitor of acetylcholinesterase, promotes hippocampal neurogenesis, but the exact mechanism for this is not known. In the present study, we examined the mechanisms underlying the effects of acute galantamine on neurogenesis in the mouse hippocampus. Galantamine (3 mg/kg) increased the number of 5-bromo-2′-deoxyuridine (BrdU)-positive cells in the subgranular zone of the dentate gyrus. This effect was blocked by the muscarinic receptor antagonist scopolamine and the preferential M_1 muscarinic receptor antagonist telenzepine, but not by the nicotinic receptor antagonists mecamylamine and methyllycaconitine. Galantamine did not alter the ratio of neuronal nuclei (NeuN)- or glial fibrillary acidic protein (GFAP)-positive cells to BrdU-labeled cells in the subgranular zone and granule cell layer. Galantamine (1, 3 mg/kg) promoted the survival of 2-wk-old newly divided cells in mice in the granule cell layer of the dentate gyrus, whereas it did not affect the survival of newly divided cells at 1 and 4 wk. Galantamine-induced increases in cell survival were blocked by the $\alpha 7$ nicotinic receptor antagonist methyllycaconitine, but not by scopolamine. Bilateral injection of recombinant IGF2 into the dentate gyrus of the hippocampus mimicked the effects of galantamine. The effects of galantamine were blocked by direct injection of the IGF1 receptor antagonist JB1. These findings suggest that galantamine promotes neurogenesis via activation of the M_1 muscarinic and $\alpha 7$ nicotinic acetylcholine receptors. The present study also suggests that IGF2 is involved in the effects of galantamine on the survival of 2-wk-old immature cells in the granule cell layer.

Received 24 December 2013; Reviewed 3 February 2014; Revised 21 February 2014; Accepted 31 March 2014; First published online 12 May 2014

Key words: Acetylcholine receptor, galantamine, hippocampus, insulin-like growth factor 2 (IGF2), neurogenesis.

Introduction

Galantamine, an acetylcholinesterase inhibitor, is used for the treatment of patients with Alzheimer's disease. The drug interacts with the nicotinic receptor at binding sites separate from those for acetylcholine and acts specifically to enhance the activity (sensitize) of nicotinic receptors in the presence of acetylcholine (Maelicke and Albuquerque, 2000; Maelicke et al., 2001; Dajas-Bailador et al., 2003; Samochocki et al., 2003). These allosteric potentiating effects on the nicotinic receptor appear to play a key role in the clinical effectiveness of galantamine, as the severity of cognitive impairment in Alzheimer's disease correlates with loss of nicotinic receptors (Nordberg et al., 1995; Perry et al., 2000). In addition to nicotinic receptors, the muscarinic receptor is also involved in the pharmacological effects of galantamine. Wadenberg et al. (2011) reported that muscarinic receptors may be involved in the antipsychotic-like effects of galantamine. We have shown that galantamine improves isolation-rearing-induced deficits of prepulse inhibition through a muscarinic-receptor-mediated mechanism (Ago et al., 2011a, b; Koda et al., 2011; Matsuda, 2013). However, the receptor pharmacology of the clinical effectiveness of galantamine is not known. Following receptor activation, galantamine increases extracellular dopamine levels in the prefrontal cortex in rats (Schilström et al., 2007) and mice (Yano et al., 2009). Furthermore, Jin et al. (2006) reported that galantamine promotes neurogenesis in the subgranular zone of the hippocampus

Address for correspondence: T. Matsuda, Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan.
Tel.: +81-6-6879-8161 *Fax*: +81-6-6879-8159
Email: matsuda@phs.osaka-u.ac.jp
* These authors contributed equally to this work.

and the subventricular zone of the lateral ventricles in mice, when it was administered daily for 14 d. Previous studies have shown that brain-derived neurotrophic factor (Lee et al., 2002; Rossi et al., 2006), nerve growth factor (Frielingsdorf et al., 2007), vascular endothelial growth factor (Schänzer et al., 2004), insulin-like growth factor 1 (IGF1) (Anderson et al., 2002; Llorens-Martín et al., 2009) and IGF2 (Agis-Balboa et al., 2011; Bracko et al., 2012) are involved in adult hippocampal neurogenesis. Thus, it is likely that the effects of galantamine on hippocampal neurogenesis are mediated by neurotrophic/growth factors. In this line, we have recently demonstrated that galantamine increased hippocampal IGF2 expression via activation of the $\alpha 7$ nicotinic receptor in mice (Kita et al., 2013).

In the subgranular zone of the hippocampal dentate gyrus of adult mammals, neurogenesis is sustained throughout life (Eriksson et al., 1998). Newborn neurons in the subgranular zone survive, migrate, and differentiate into mature neurons in the granule cell layer, where they are functionally incorporated into the hippocampal circuitry (Aasebø et al., 2011; Ming and Song, 2011). Previous studies show that cholinergic stimulation promotes the proliferation and survival of neural precursor cells (Berger et al., 1998; Coronas et al., 2000; Ma et al., 2000) and that dysfunction of the cholinergic system impairs neurogenesis (Cooper-Kuhn et al., 2004; Harrist et al., 2004; Mohapel et al., 2005). However, it is not known whether IGF2 is involved in neurogenesis induced by cholinergic stimulation.

The present study examined the effects of galantamine on adult mouse hippocampal neurogenesis, focusing on proliferation, differentiation, and survival of newly divided cells. We examined the role of IGF2 in galantamine-induced hippocampal neurogenesis, in view of the recent finding that galantamine increases IGF2 levels in the hippocampus (Kita et al., 2013). We demonstrate that acute treatment with galantamine promotes the proliferation of adult neural progenitor cells via M_1 muscarinic receptor activation and enhances the survival of newly divided cells at 14–16 d old via activation of the $\alpha 7$ nicotinic receptor, and that the latter effects may be mediated by IGF2.

Method

Animals and drugs

The experimental procedures involving the use of animals in this work were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society. Every effort was made to minimize animal suffering and to reduce the number of animals used. Eight-week-old male ddY mice were obtained from Shimizu Laboratory Supplies Co., Ltd (Japan) and housed in cages (24×17×12 cm) of five to six animals under controlled environmental conditions (22±1 °C; 12:12-h light–dark cycle, lights on at 08:00 h, food and water provided ad libitum) for 1 wk before use in the experiments. We used 367 mice in total. The following drugs were used: 5-bromo-2′-deoxyuridine (BrdU), mecamylamine, methyllycaconitine, oxotremorine, physostigmine, scopolamine, and telenzepine (Sigma, USA), and galantamine, donepezil, and PHA-543613 (Tocris Bioscience, UK). These drugs were dissolved in saline (0.9% solution of NaCl). All drugs were administered in a volume of 10 ml/kg intraperitoneally (i.p.) (galantamine, donepezil, BrdU, mecamylamine, methyllycaconitine, oxotremorine, physostigmine) or subcutaneously (s.c.) (PHA-543613, scopolamine, telenzepine). The doses of galantamine and donepezil used here were selected according to previous studies (Csernansky et al., 2005; Schilström et al., 2007; Wang et al., 2007; Koda et al., 2008; Tanaka et al., 2009; Noda et al., 2010). Physostigmine was used at a dose that elevates acetylcholine levels in the hippocampus (Erb et al., 2001), and oxotremorine was used at a dose that improves apomorphine- or isolation rearing-induced deficits in prepulse inhibition (Yano et al., 2009; Koda et al., 2011). Recombinant IGF2 (Sigma, USA), JB1 (Bachem Biosciences, USA), and anti-IGF2 receptor antibodies (R&D Systems, USA) were used.

To evaluate the proliferation and differentiation of newly divided cells, mice received two injections at 12 h intervals of BrdU (50 mg/kg) 24 h after the injection of the test drugs. Over 3 consecutive days, mice received three intraperitoneal injections of BrdU (50 mg/kg) per day, every 4 h, to assess the survival of newly divided cells. Animals were sacrificed either 12 h after the last BrdU injection to evaluate the proliferation of newly divided cells, or 28 d after the last BrdU injection to evaluate the differentiation of newly divided cells, or 42 d after the last BrdU injection to assess the survival of newly divided cells.

Injection of drugs into the dentate gyrus of the hippocampus

Fifteen days after the last injection of BrdU, mice were deeply anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and positioned in a stereotaxic apparatus. Bilateral injections of drugs into the dentate gyrus of the hippocampus were performed according to the method of Agis-Balboa et al. (2011). A burr hole was made using the following coordinates (anteroposterior −1.7 mm; lateral ±1 mm; ventral 2 mm, relative to bregma and skull) and stock solutions of IGF2 (1 μg/μl in 0.1% BSA (bovine serum albumin) in sterile phosphate-buffered saline (PBS; pH 7.4)), the IGF1 receptor antagonist JB1 (80 ng/μl in sterile PBS), and an anti-IGF2 receptor antibody (20 ng/μl in sterile PBS) were prepared. The injections were carried out using a 26-gauge needle connected via polyethylene tubing to a Hamilton syringe at 0.125 μl per side and at a rate of 0.125 μl/min. The total

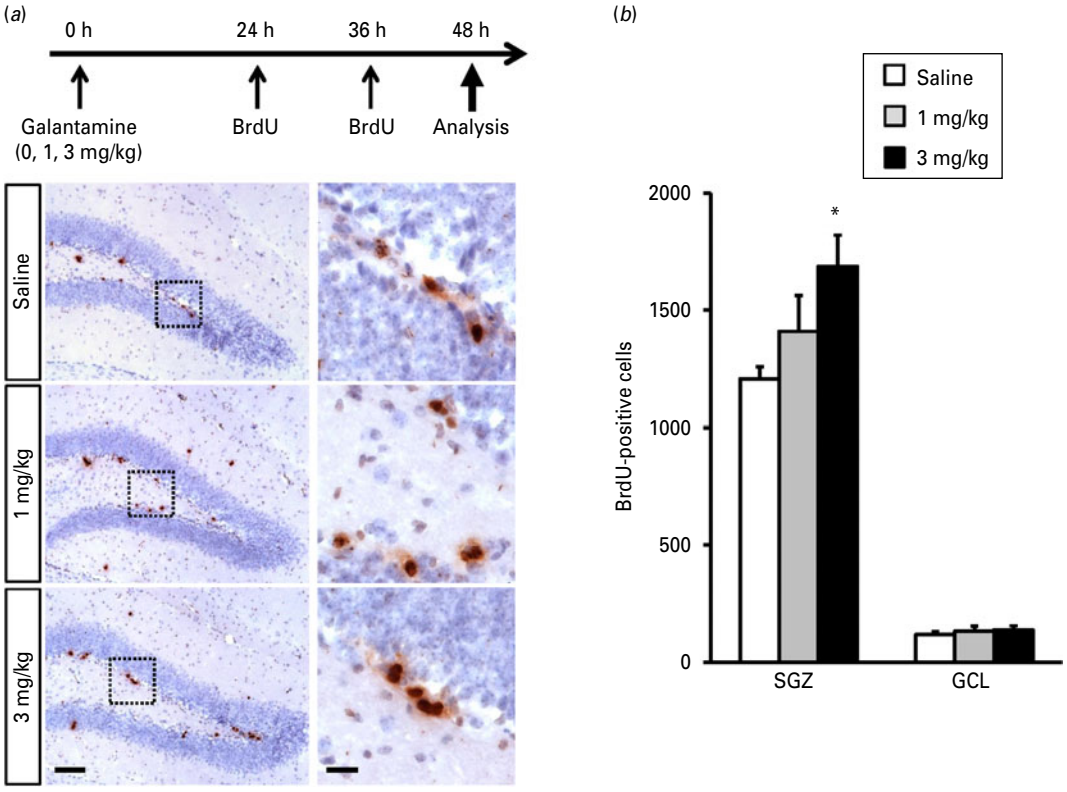

Fig. 1. Effects of galantamine on newly divided cell proliferation in the dentate gyrus of the adult mouse hippocampus. Mice were injected intraperitoneally (i.p.) with galantamine (1 and 3 mg/kg) or saline. Twenty-four hours after the injection of galantamine or saline, 5-bromo-2′-deoxyuridine (BrdU) (50 mg/kg, i.p.) was injected twice at 12 h intervals. Animals were sacrificed 12 h after the last injection of BrdU, and BrdU-positive cells in the subgranular zone (SGZ) and granule cell layer (GCL) were counted. (*a*) The experimental schedule and representative photomicrographs showing localization of BrdU-positive cells in saline- or galantamine-treated (1–3 mg/kg) mice. Scale bars of low and high magnifications show 100 and 20 μm, respectively. (*b*) Quantitative analysis of the number of BrdU-positive cells in the SGZ and GCL. The data are expressed as the mean±S.E.M of 6–7 mice/group. *$p<0.05$, compared with the saline-treated group.

amounts of the drugs injected in the brain were 250 ng (IGF2), 20 ng (JB1) and 5 ng (Anti-IGF2 receptor antibody). The injection needle was left in place for 2 min after the injection to allow complete dispersion of the solution. Postoperative analgesia was induced with a single injection of buprenorphine (0.1 mg/kg, i.p.) (Ago et al., 2013).

BrdU immunohistochemistry in the dentate gyrus

Mice were deeply anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and perfused transcardially with saline, followed by 4% paraformaldehyde in PBS. Their brains were removed, post-fixed in the same fixative, and then cryoprotected. Thick coronal brain sections of 20 μm were cut on a cryostat at −20 °C. Every sixth section was collected between the stereotaxic coordinates −1.40 to −2.48 mm according to a brain atlas. The free-floating sections were first preincubated for 30 min in 0.3% hydrogen peroxide in PBS containing 0.3% Triton-X100 (PBST) to remove endogenous peroxidase activity, and then mounted on slides. Sections were heated in a microwave oven in a 0.01 M sodium citrate buffer (pH 6.0) for 10 min and then treated with 2 M HCl at 35 °C for 30 min. After being rinsed for 10 min at room temperature in 0.1 M boric acid/NaOH (pH 8.5), they were blocked by 5% normal goat serum in PBST for 1 h at room temperature.

For BrdU staining, sections were incubated with a rat anti-BrdU monoclonal primary antibody (1:100, Abcam, UK) at 4 °C overnight, followed by a biotin-conjugated goat anti-rat IgG (1:250, Abcam, UK) for 30 min at room temperature. Biotinylated secondary antibodies were detected using an ABC immunoperoxidase kit (Vector Laboratories, USA). Brown cytosolic products were obtained by a reaction with 3,3′-diaminobenzidine (Sigma, USA). The sections were counterstained with hematoxylin. For double staining of neuronal nuclei (NeuN) (a mature neuronal marker) and BrdU, sections were incubated with a mouse anti-NeuN monoclonal primary antibody (1:200, millipore, USA) and a rat anti-BrdU monoclonal primary antibody (1:100, Abcam, UK) at 4 °C overnight, followed by an Alexa Fluor 594 anti-mouse IgG (1:200, Life Technologies, USA) and an

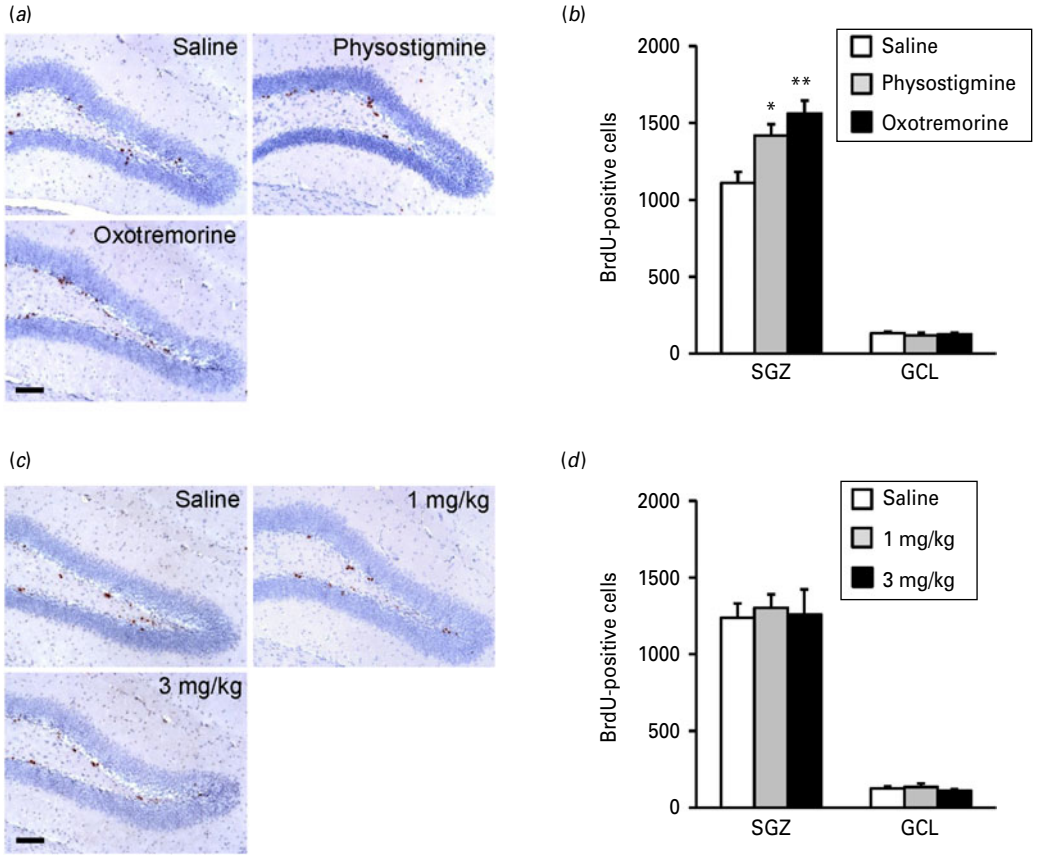

Fig. 2. Effects of physostigmine, oxotremorine, and donepezil on newly divided cell proliferation in the dentate gyrus of the adult mouse hippocampus. Mice were injected intraperitoneally (i.p.) with physostigmine (0.1 mg/kg), oxotremorine (0.1 mg/kg) (*a, b*), donepezil (1 and 3 mg/kg) (*c, d*), or saline. Twenty-four hours after the injections, 5-bromo-2′-deoxyuridine (BrdU) (50 mg/kg, i.p.) was injected twice at 12 h intervals. Animals were sacrificed 12 h after the last injection of BrdU, and BrdU-positive cells in the subgranular zone (SGZ) and granule cell layer (GCL) were counted. (*a, c*) Representative photomicrographs showing localization of BrdU-positive cells. Scale bar: 100 μm. (*b, d*) Quantitative analysis of the number of BrdU-positive cells in the SGZ and GCL. The data are expressed as the mean±S.E.M of 8–10 mice/group. *$p<0.05$, **$p<0.01$, compared with the saline-treated group.

Alexa Fluor 488 anti-rat IgG (1:200, Life Technologies, USA) for 2 h at room temperature. For double staining of glial fibrillary acidic protein (GFAP) (the glial marker) and BrdU, sections were incubated with rabbit anti-GFAP polyclonal primary antibody (1:200, millipore, USA) and rat anti-BrdU monoclonal primary antibody (1:100, Abcam, UK) at 4 °C overnight, followed by an Alexa Fluor 594 anti-rabbit IgG (1:200, Life Technologies, USA) and an Alexa Fluor 488 anti-rat IgG (1:200, Life Technologies, USA) for 2 h at room temperature.

Quantification of cell immunostaining

Every sixth section throughout the hippocampus (for a total of nine sections from each mouse) was processed for BrdU immunohistochemistry. All BrdU-labeled cells in the subgranular zone and granule cell layer were assessed using an Axio Imager upright microscope (Zeiss, Germany) and counted by an experimenter blinded to the condition. To distinguish single cells within clusters, all counts were performed at 400× magnification (objective; 40×). To obtain the total number of BrdU-labeled cells in the subgranular zone and granule cell layer, we multiplied the counted number of positive cells by six. Double stained cells were assessed using the Axio Imager upright microscope, scanned through the z axis, and analyzed at intervals of 1.0 μm, to exclude false double-positive cells due to the overlay of signals from different cells.

Data analysis

All data are expressed as the mean±S.E.M. The data were analyzed using one-way (Figs. 1*b*, 2*b, d* and 6*b*) or two-way (Figs. 3 and 5*b*) analysis of variance (ANOVA) followed by either Dunnett's or Tukey–Kramer's *post-hoc* test as appropriate. Two-tailed Student's unpaired *t* test was used for two-group comparisons (Figs. 4*b, c*, 5*a, c* and 7*b, d*). Statistical analyses were performed using the software package Statview 5.0J for Apple Macintosh (SAS Institute Inc., USA). A value of $p<0.05$ was considered statistically significant.

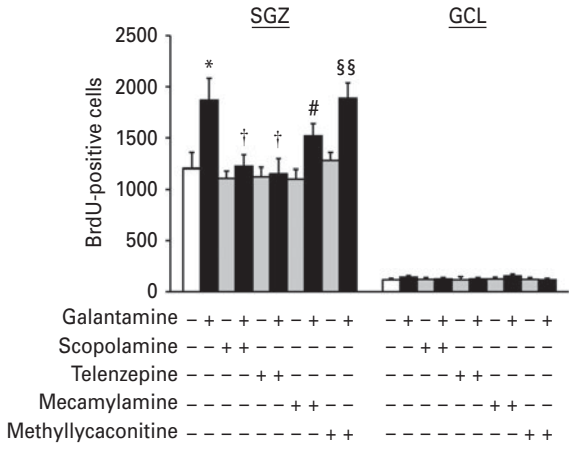

Fig. 3. Effects of scopolamine, telenzepine, mecamylamine, and methyllcaconitine on the galantamine-induced increase in newly divided cell proliferation in the dentate gyrus of the adult mouse hippocampus. Mice were injected intraperitoneally (i.p.) with galantamine (3 mg/kg) or saline. Scopolamine (0.1 mg/kg, i.p.), telenzepine (3 mg/kg, s.c.), mecamylamine (3 mg/kg, i.p.), methyllycaconitine (6 mg/kg, i.p.) or saline (i.p.) was administered 30 min before the galantamine or saline treatment. Twenty-four hours after the injection of galantamine or saline, 5-bromo-2′-deoxyuridine (BrdU) (50 mg/kg, i.p.) was injected twice at 12 h intervals. Animals were sacrificed 12 h after the last injection of BrdU, and BrdU-positive cells in the subgranular zone (SGZ) and granule cell layer (GCL) were counted. The data are expressed as the mean±S.E.M of 6–9 mice/group. *$p<0.05$, compared with the saline/saline-treated group; †$p<0.05$, compared with the saline/galantamine-treated group; #$p<0.05$, compared with the mecamylamine/saline-treated group; §§$p<0.01$, compared with the methyllycaconitine/saline-treated group.

Results

Effects of galantamine and other cholinergic drugs on proliferation of newly divided cells in the dentate gyrus of the adult mouse hippocampus

The effects of a single injection of galantamine on proliferation of newly divided cells were examined in the dentate gyrus of the hippocampus (Fig. 1). Figure 1*a, b* show representative photomicrographs of the localization and quantitative analysis of BrdU-positive cells, respectively. Most of the BrdU-labeled cells were found in the subgranular zone of the dentate gyrus in all groups, and galantamine (3 mg/kg, i.p.) significantly increased the number of BrdU-positive cells in the subgranular zone of the dentate gyrus ($F_{2,16}=4.4$, $p<0.05$). It did not affect the number of BrdU-positive cells in the granule cell layer ($F_{2,16}=0.3$, $p>0.05$).

Figure 2 shows the effects of acute treatment with the acetylcholinesterase inhibitors physostigmine and donepezil and the nonselective muscarinic receptor agonist oxotremorine on newly divided cell proliferation. Physostigmine (0.1 mg/kg, i.p.) and oxotremorine (0.1 mg/kg, i.p.) significantly increased the number of BrdU-positive cells in the subgranular zone ($F_{2,23}=9.9$, $p<0.001$). These drugs did not affect the number of BrdU-positive cells in the granule cell layer ($F_{2,23}=0.3$, $p>0.05$). On the other hand, donepezil (1 and 3 mg/kg, i.p.) did not affect proliferation of newly divided cells ($F_{2,21}=0.1$, $p>0.05$).

The effects of receptor antagonists against galantamine-induced increases in the number of BrdU-positive cells in the subgranular zone of the dentate gyrus were examined to clarify the receptor mechanisms for these effects of galantamine (Fig. 3). Galantamine-induced increases in the number of BrdU-positive cells in the subgranular zone were blocked by the nonselective muscarinic receptor antagonist scopolamine (0.1 mg/kg, i.p.) and the preferential M_1 muscarinic receptor antagonist telenzepine (3 mg/kg, s.c.), but not by the nonselective nicotinic receptor antagonist mecamylamine (3 mg/kg, i.p.) or the selective $\alpha 7$ nicotinic receptor antagonist methyllycaconitine (6 mg/kg, i.p.). The dose of mechamylamine used here was enough to block nicotine-induced hypothermia and hypolocomotion in mice (Supplementary Figure S1). Two-way ANOVA revealed a significant main effect of galantamine ($F_{1,62}=16.9$, $p<0.001$) and these antagonists ($F_{4,62}=4.4$, $p<0.01$), but there was no significant interaction between galantamine and treatment with these antagonists ($F_{4,62}=2.1$, $p>0.05$). Scopolamine, telenzepine, mecamylamine, and methyllycaconitine did not affect the proliferation of newly divided cells when given alone.

Effects of acute galantamine treatment on the differentiation of newly divided cells in the dentate gyrus

Newly divided cells in the dentate gyrus display typical features of mature neurons within 4 wk (Cameron and McKay, 2001). It is not known whether galantamine affects the differentiation of newly divided cells. Using NeuN-positive and GFAP-positive cells, this study investigated the effects of acute galantamine treatment on the differentiation of newly divided cells in the dentate gyrus of the adult mouse hippocampus (Fig. 4). Mice were sacrificed 28 d after the last BrdU injection and we counted the number of NeuN- and GFAP-positive cells among BrdU-labeled cells in the dentate gyrus. Galantamine (3 mg/kg, i.p.) significantly increased the number of NeuN/BrdU-positive cells in the subgranular zone and granule cell layer, and also significantly increased the number of GFAP/BrdU-positive cells in the subgranular zone. Galantamine did not alter the ratio of NeuN- or GFAP-positive cells to BrdU-labeled cells in the subgranular zone and granule cell layer, suggesting that the drug did not affect cell differentiation (Fig. 4). The determination of the NeuN-positive and GFAP-positive cells was also confirmed by a confocal laser scanning microscope (Supplementary Figure S2).

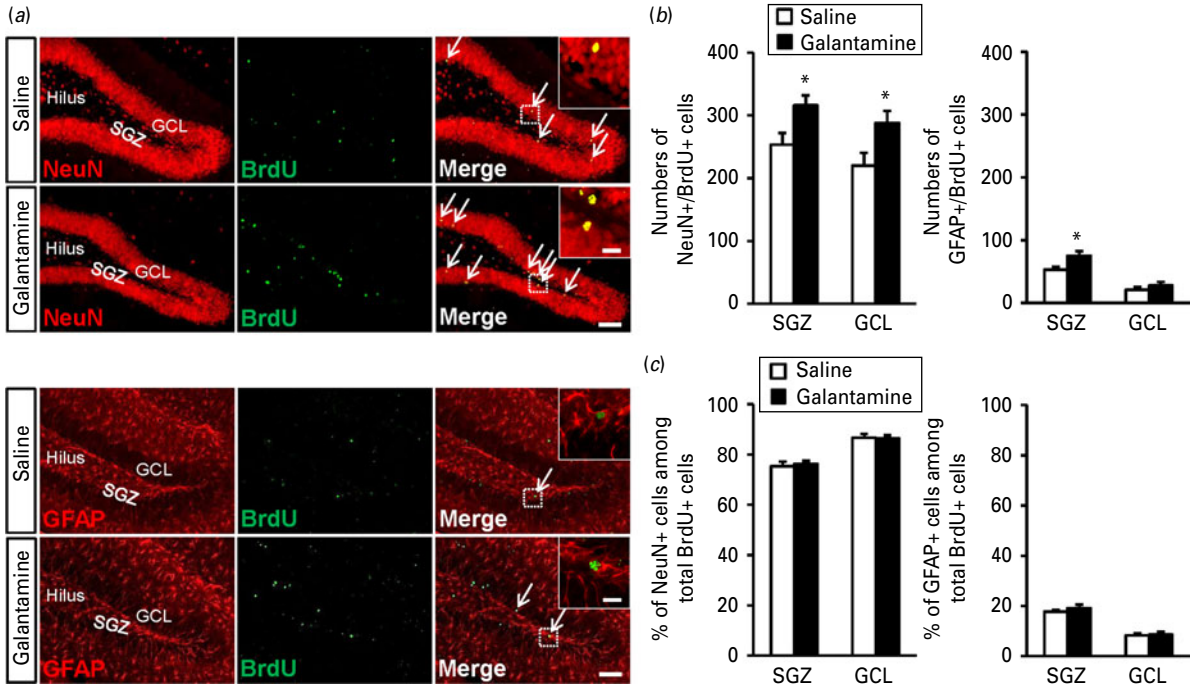

Fig. 4. Effects of galantamine on the differentiation of newly divided cells in the dentate gyrus of the adult mouse hippocampus. Mice were injected intraperitoneally (i.p.) with galantamine (3 mg/kg) or saline. Twenty-four hours after the injection of galantamine or saline, 5-bromo-2′-deoxyuridine (BrdU) (50 mg/kg, i.p.) was injected twice at 12 h intervals. Animals were sacrificed 28 d after the last injection of BrdU, and BrdU-positive cells in the subgranular zone (SGZ) and granule cell layer (GCL) were counted. (*a*) Representative photomicrographs showing localization of neuronal nuclei (NeuN)/BrdU- and glial fibrillary acidic protein (GFAP)/BrdU-double positive cells in saline- or galantamine-treated mice, respectively. Arrows indicate NeuN/BrdU- and GFAP/BrdU-double positive cells. Scale bars low magnification images: 100 μm; high magnification: 20 μm. (*b*) Quantitative analysis of the number of NeuN/BrdU- and GFAP/BrdU-double positive cells in the SGZ and GCL. The data are expressed as the mean±S.E.M of 6 mice/group. *$p<0.05$, compared with the saline-treated group. (*c*) Percentage of NeuN- and GFAP-positive cells among total BrdU-positive cells in the SGZ and GCL. The data are expressed as the mean±S.E.M of 6 mice/group.

Effects of acute galantamine treatment on the survival of newly divided cells at different time points in the dentate gyrus

The effects of acute galantamine treatment on the survival of newly divided cells were examined at different time points in the dentate gyrus of the adult mouse hippocampus (Fig. 5). Galantamine (3 mg/kg) was administered, when BrdU-labeled cells were 1, 2, and 4 wk old. Galantamine significantly promoted the survival of newly divided cells by 2 wk in the granule cell layer of the dentate gyrus. Galantamine-induced increases in cell survival were blocked by methyllycaconitine (6 mg/kg, i.p.) (Fig. 5*b*) but not by scopolamine (0.1 mg/kg, i.p.) (data not shown). Two-way ANOVA revealed a significant main effect of galantamine ($F_{1,28}=7.7$, $p<0.01$), but not methyllycaconitine ($F_{1,28}=3.6$, $p>0.05$), and there was a significant interaction between treatments ($F_{1,28}=4.6$, $p<0.05$). On the other hand, galantamine did not affect the survival of newly divided cells at 1 and 4 wk (Fig. 5*a, c*). Galantamine did not affect cell survival in the subgranular zone.

To study further the involvement of $\alpha 7$ nicotinic receptors in the survival of newly divided cells by 2 wk in the granule cell layer of the dentate gyrus, we examined the effects of acute treatment with PHA-543613, a selective $\alpha 7$ nicotinic receptor agonist (Wishka et al., 2006) (Fig. 6). PHA-543613 (3 mg/kg, s.c.) significantly promoted the survival of 14–16-d-old newly divided cells in the granule cell layer of the dentate gyrus ($F_{2,20}=13.0$, $p<0.001$). It did not affect cell survival in the subgranular zone. Furthermore, donepezil (3 mg/kg, i.p.) did not promote the survival of 14–16-d-old newly divided cells.

Effects of IGF2 on the survival of newly divided cells in the dentate gyrus of the adult mouse hippocampus

In previous studies, we showed that acute galantamine, but not donepezil, increased hippocampal IGF2 protein levels via $\alpha 7$ nicotinic receptor activation 24 h after the injection (Kita et al., 2013). Figure 7*a, b* show the effects of hippocampal bilateral injection of recombinant IGF2 on the survival of newly divided cells in the granule cell layer of the dentate gyrus. A single bilateral injection of recombinant IGF2 (250 ng) 15 d after the last administration of BrdU promoted the survival of newly divided cells in the granule cell layer of the dentate gyrus. It did not affect the survival in the subgranular zone. The effects

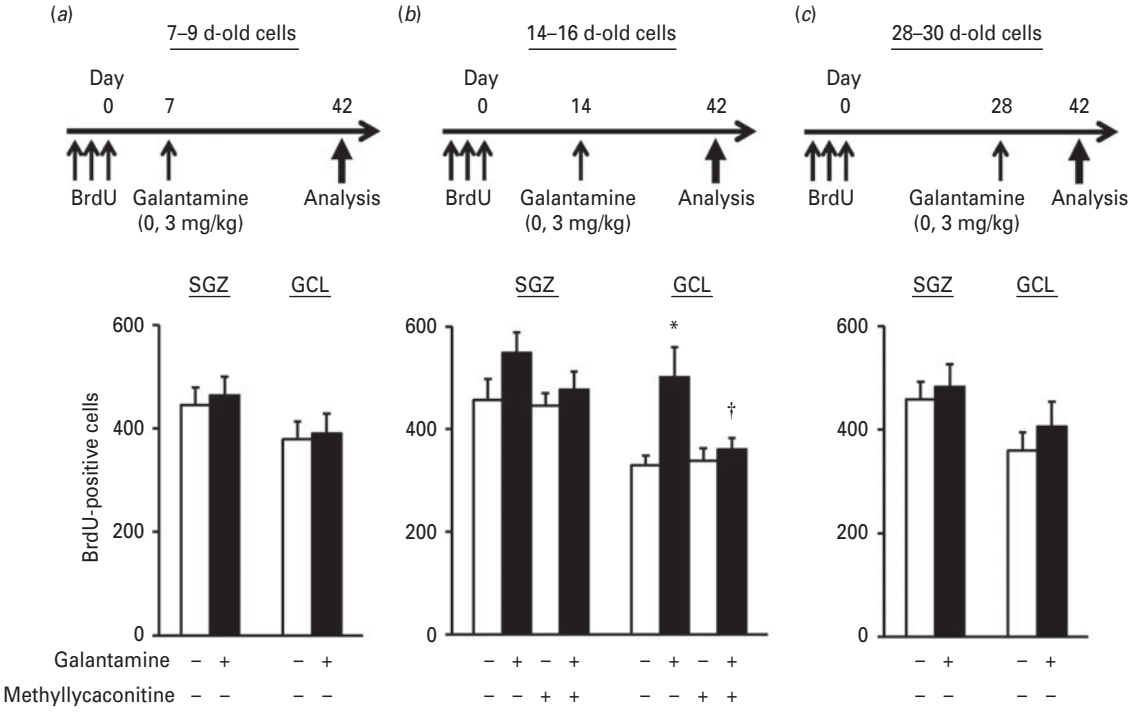

Fig. 5. Effects of galantamine on the survival of newly divided cells at different time points in the dentate gyrus of the adult mouse hippocampus. Over three consecutive days, mice received three injections of 5-bromo-2′-deoxyuridine (BrdU) (50 mg/kg, intraperitoneally (i.p.)) per day, every 4 h. Galantamine (3 mg/kg) or saline was injected i.p. either 7 (*a*), 14 (*b*), or 28 d (*c*) after the last BrdU injection. Methyllycaconitine (6 mg/kg, i.p.), or saline (i.p.) was administered 30 min before the galantamine or saline treatment. Animals were sacrificed 42 d after the last injection of BrdU, and BrdU-positive cells in the subgranular zone (SGZ) and granule cell layer (GCL) were counted. The experimental schedule and quantitative analysis of the number of BrdU-positive cells in the SGZ and GCL are shown. The data are expressed as the mean±S.E.M of 8 mice/group. *$p<0.05$, compared with the saline/saline-treated group; †$p<0.05$, compared with the saline/galantamine-treated group.

of recombinant IGF2 were similar to those of galantamine.

Since IGF2 interacts with the IGF1 receptor and IGF2 receptor (Alberini and Chen, 2012; Fernandez and Torres-Alemán, 2012), we examine which receptor is involved in the effects of galantamine on the survival of newly divided cells in the granule cell layer (Fig. 7*c*, *d*). The specific inhibitor of the IGF1 receptor JB1 (20 ng) abolished galantamine-induced enhancement of cell survival in the granule cell layer. On the other hand, 5 ng of an anti-IGF2 receptor antibody, which completely abolished the memory enhancement of IGF2 injection (Chen et al., 2011), did not affect galantamine-induced enhancement of cell survival in the granule cell layer. Both inhibitors did not affect cell survival in the subgranular zone.

Discussion

The present study shows that acute treatment with galantamine promotes the proliferation of neural progenitor cells and the production of mature granule neurons in the subgranular zone of the hippocampal dentate gyrus. This effect is in agreement with the previous observation that daily galantamine for 14 d increases BrdU-labeled cells in the subgranular zone of the dentate gyrus in mice (Jin et al., 2006). The effects of galantamine on cell proliferation were blocked by the nonselective muscarinic receptor antagonist scopolamine and the preferential M_1 muscarinic receptor antagonist telenzepine, but not by the nonselective nicotinic receptor antagonist mecamylamine and the selective $\alpha 7$ nicotinic receptor antagonist methyllycaconitine. Furthermore, we found that the nonselective muscarinic receptor agonist oxotremorine and the cholinesterase inhibitor physostigmine, like galantamine, promoted the proliferation of the newly divided cells. These findings suggest that the effects of galantamine on the proliferation of neural progenitor cells are mediated by activation of the M_1 muscarinic receptor. Indeed, the M_1 muscarinic receptor is expressed in newly divided cells in the subgranular zone of the dentate gyrus (Mohapel et al., 2005; Kaneko et al., 2006). Furthermore, an *in vitro* study showed that muscarine increased proliferation of P19 progenitor cells and this effect was blocked by pirenzepine (Resende et al., 2008).

Many newborn neurons in the dentate gyrus of the adult hippocampus die during maturation within 4 wk after birth (Cameron and McKay, 2001), and only a small proportion of newborn neurons are functionally integrated into the existing hippocampal circuitry (Zhao et al., 2008). Especially during 1–3 wk after birth,

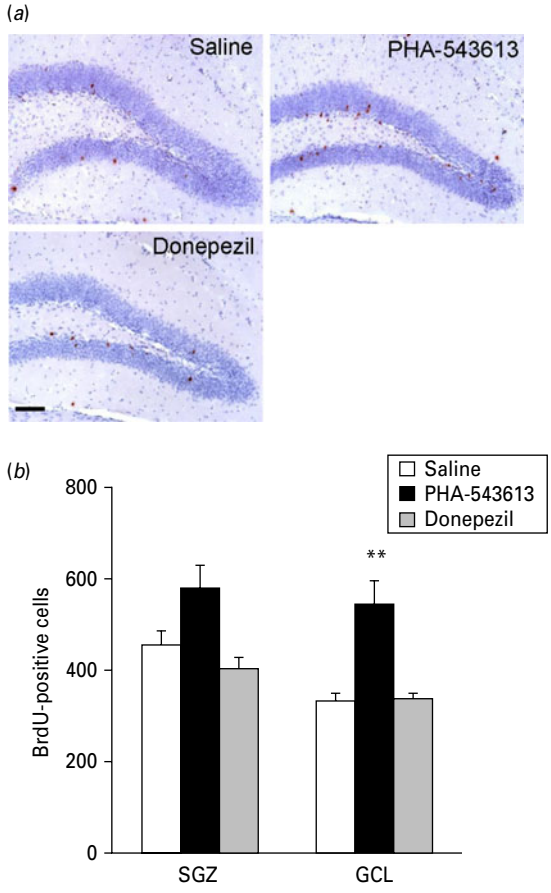

Fig. 6. Effects of PHA-543613 and donepezil on the survival of newly divided cells in the dentate gyrus of the adult mouse hippocampus. Over three consecutive days, mice received three injections of 5-bromo-2′-deoxyuridine (BrdU) (50 mg/kg, intraperitoneally (i.p.)) per day, every 4 h. PHA-543613 (3 mg/kg, s.c.), donepezil (3 mg/kg, i.p.), or saline (i.p.) was injected 14 d after the last BrdU injection. Animals were sacrificed 42 d after the last injection of BrdU, and BrdU-positive cells in the subgranular zone (SGZ) and granule cell layer (GCL) were counted. (*a*) Representative photomicrographs showing localization of BrdU-positive cells. Scale bar: 100 μm. (*b*) Quantitative analysis of the number of BrdU-positive cells in the SGZ and GCL. The data are expressed as the mean ± S.E.M of 7–8 mice/group. $^{**}p<0.01$, compared with the saline-treated group.

newborn neurons are immature and display unique physiological properties (e.g. morphological and synaptic changes) (Espósito et al., 2005; Ge et al., 2006; Zhao et al., 2006), and thus, the survival of these neurons probably contributes to hippocampal-dependent memory formation (Tashiro et al., 2007; Veyrac et al., 2013). The present study shows that acute treatment with galantamine specifically promotes the survival of newly divided cells at 14–16 d in the granule cell layer of the hippocampal dentate gyrus and this effect is blocked by the selective $\alpha7$ nicotinic receptor antagonist methyllycaconitine. We also found that the selective $\alpha7$ nicotinic receptor agonist PHA-543613, but not donepezil, promoted the survival of newly divided cells in the granule cell layer. These observations suggest that galantamine promotes the survival of newly divided cells at 14–16 d via activation of the $\alpha7$ nicotinic receptor. It should be noted that the effects of galantamine on the survival of newly divided cells at 14–16 d was observed at 1 and 3 mg/kg, whereas that of galantamine on the proliferation of newly divided cells was observed only at 3 mg/kg. That is, there is a difference in sensitivity to galantamine between muscarinic-receptor-mediated cell proliferation and nicotinic-receptor-mediated cell survival. In line with this, we have recently found that galantamine at doses of 0.3–3 mg/kg increased hippocampal IGF2 levels and this effect was mediated by activation of the $\alpha7$ nicotinic receptor (Kita et al., 2013). These findings suggest that galantamine-induced increases in hippocampal IGF2 are involved in the effects of galantamine on cell survival. Concerning the role of IGF2 in hippocampal neurogenesis, Bracko et al. (2012) showed that IGF2 regulates proliferation of hippocampal neural stem cells through AKT-dependent signaling. AKT appears to be involved in cell survival and anti-apoptotic signaling (Song et al., 2005). Furthermore, Agis-Balboa et al. (2011) reported that hippocampal IGF2 is involved in contextual fear extinction, and that IGF signaling specifically promotes the survival of 17–19-d-old newborn hippocampal neurons. Taken together, it is likely that nicotinic-receptor-mediated cell survival by galantamine is mediated by IGF2 expression.

To clarify the involvement of IGF2 in galantamine-induced survival of hippocampal immature cells, we examined the effects of direct injection of IGF2 and inhibitors of IGF2 signal on the survival of newly divided cells at 14–16 d. The present study shows that bilateral injection of recombinant IGF2 promotes cell survival, which is similar to the effects of galantamine. We did not study whether IGF2 injection affects proliferation of newly divided cells, but Bracko et al. (2012) reported that addition of IGF2 to neural stem cells of normal mice *in vitro* had only a minor effects on their proliferation, and Ouchi et al. (2013) recently showed that injection of IGF2 to normal mice *in vivo* did not affect neural progenitor cell proliferation. We further found that the effects of galantamine on the survival of immature cells were blocked by the IGF1 receptor antagonist JB1, but not an anti-IGF2 receptor antibody. This experiment using a neutralizing antibody and a pharmacological inhibitor suggests that galantamine promotes the survival of immature cells at 14–16 d via an IGF1 receptor-mediated IGF2 signal. It is not known why the effects of IGF2 are specific for the survival of immature cells.

Galantamine is typically used in a chronic fashion to treat Alzheimer's patients. Jin et al. (2006) reported that chronic treatment with galantamine increased BrdU labeling of cells in the hippocampal dentate gyrus and forebrain subventricular zone of the adult mouse brain. The finding suggests that the effect of galantamine on neurogenesis may contribute to the therapeutic effect. In the

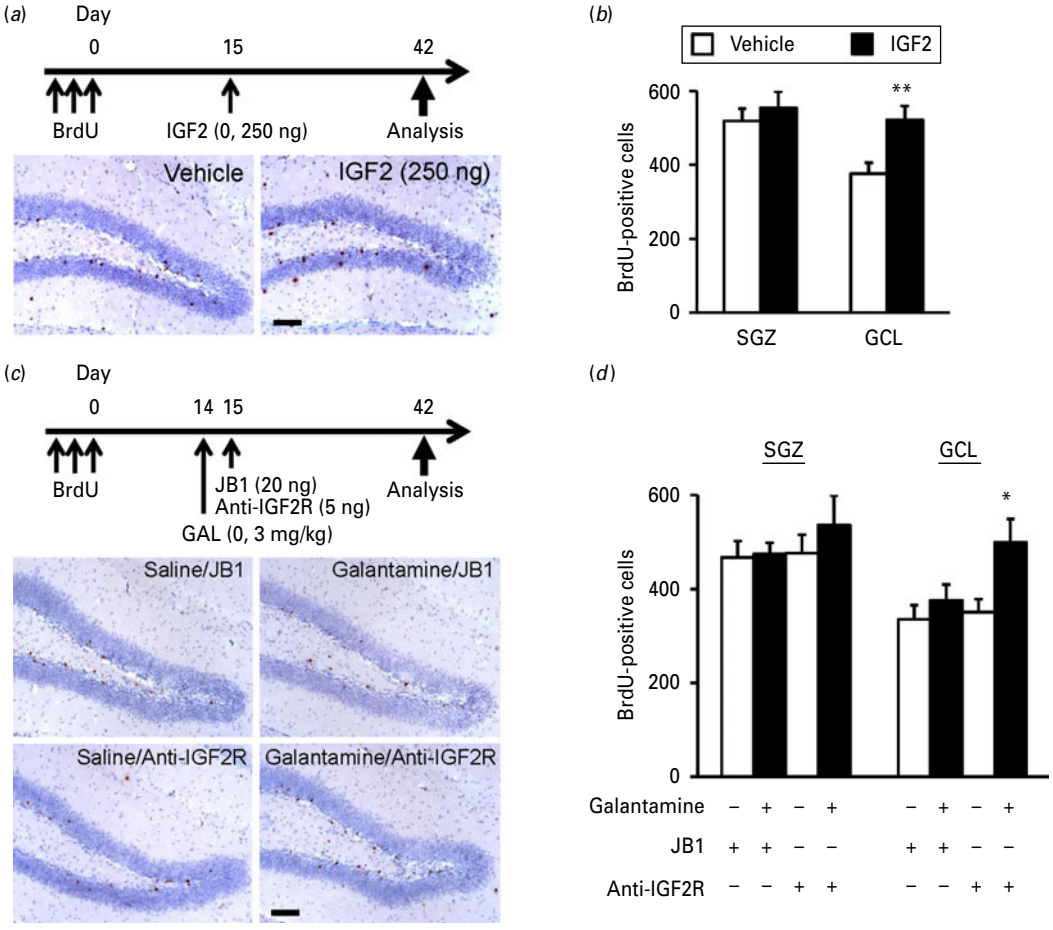

Fig. 7. Effects of IGF2 on the survival of newly divided cells (*a, b*), and the effects of JB1 and an anti-IGF2 receptor antibody on galantamine-enhanced survival of newly divided cells (*c, d*) in the dentate gyrus of the adult mouse hippocampus. Over three consecutive days, mice received three injections of 5-bromo-2′-deoxyuridine (BrdU) (50 mg/kg, intraperitoneally (i.p.)) per day, every 4 h. (*a, b*) Recombinant IGF2 (250 ng) or vehicle was bilaterally administered 15 d after the last administration of BrdU. (*c, d*) Galantamine (3 mg/kg) or saline was injected i.p. 14 d after the last BrdU injection. JB1 (20 ng) or the anti-IGF2 receptor antibody (5 ng; Anti-IGF2R) was bilaterally administered 24 h after the galantamine or saline treatment. Animals were sacrificed 42 d after the last injection of BrdU, and BrdU-positive cells in the subgranular zone (SGZ) and granule cell layer (GCL) were counted. (*a, c*) Experimental schedule and representative photomicrographs showing localization of BrdU-positive cells. Scale bar: 100 μm. (*b, d*) Quantitative analysis of the number of BrdU-positive cells in the SGZ and GCL. The data are expressed as the mean±S.E.M of 8 mice/group. **$p<0.01$, compared with the vehicle-treated group (*b*). *$p<0.05$, compared with the saline/Anti-IGF2R -treated group (*d*).

present study, galantamine was acutely administered to investigate the effect on each process of the neurogenesis, such as proliferation of the newborn cells and survival of the cells. We found that galantamine promotes the proliferation of the newly divided cells and survival of the cells via the activation of the M_1 muscarinic and $\alpha 7$ nicotinic receptors, respectively. The present study also showed that donepezil did not affect the proliferation of newly divided cells. This contrasts to the previous observations using a multiple doses of donepezil (Kaneko et al., 2006; Kotani et al., 2006). The exact reason for the difference in the effect of donepezil between the acute and chronic treatment is not known. Since donepezil antagonizes muscarinic receptor-induced responses *in vivo* and *in vitro* (Ago et al., 2011b), we speculate that

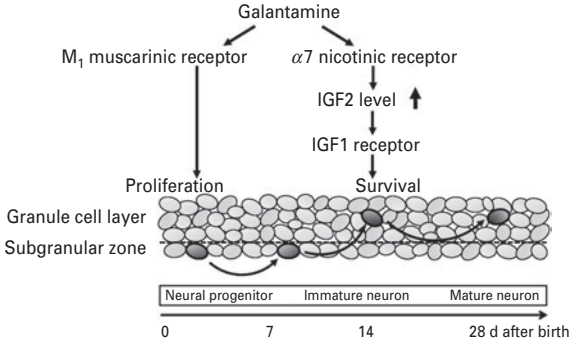

Fig. 8. Possible mechanism for the effects of galantamine on adult hippocampal neurogenesis.

the antagonistic effect of donepezil on the muscarinic receptor may result in the lack of the effect of acute donepezil, but the effect may be desensitized by its chronic administration.

In conclusion, we demonstrate that galantamine promotes the proliferation of neural progenitor cells in the subgranular zone via activation of the M_1 muscarinic receptor and it promotes the survival of the newly divided cells in the granule cell layer via activation of the $\alpha 7$ nicotinic receptor. Furthermore, we found that direct injection of IGF2 promotes the survival of immature cells in the granule cell layer and the effects of galantamine on cell survival were blocked by an inhibitor of IGF2 receptor signaling (the IGF1 receptor antagonist JB1). These findings, taken together with the recent finding that galantamine increases hippocampal IGF2 levels (Kita et al., 2013), suggest that IGF2 signaling plays a key role in galantamine-induced survival of immature cells in the granule cell layer (Fig. 8). Adult hippocampal neurogenesis is impaired in Alzheimer's disease (Crews and Masliah, 2010; Lazarov and Marr, 2010; Curtis et al., 2012) and may be a target for the treatment of mental illness including Alzheimer's disease patients (DeCarolis and Eisch, 2010; Shruster and Offen, 2014). Although it is not known whether galantamine increases hippocampal IGF2 levels and promotes hippocampal neurogenesis in the pathological conditions, we speculate that the increases in hippocampal IGF2 levels and neurogenesis may be involved in the clinical effect of galantamine.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145714000613

Acknowledgment

This study was supported in part by KAKENHI (23390139 and 25460099).

Statement of Interest

None.

References

Aasebø IE, Blankvoort S, Tashiro A (2011) Critical maturational period of new neurons in adult dentate gyrus for their involvement in memory formation. Eur J Neurosci 33:1094–1100.

Agis-Balboa RC, Arcos-Diaz D, Wittnam J, Govindarajan N, Blom K, Burkhardt S, Haladyniak U, Agbemenyah HY, Zovoilis A, Salinas-Riester G, Opitz L, Sananbenesi F, Fischer A (2011) A hippocampal insulin-growth factor 2 pathway regulates the extinction of fear memories. EMBO J 30:4071–4083.

Ago Y, Koda K, Takuma K, Matsuda T (2011a) Pharmacological aspects of the acetylcholinesterase inhibitor galantamine. J Pharmacol Sci 116:6–17.

Ago Y, Koda K, Ota Y, Kita Y, Fukada A, Takuma K, Matsuda T (2011b) Donepezil, but not galantamine, blocks muscarinic receptor-mediated *in vitro* and *in vivo* responses. Synapse 65:1373–1377.

Ago Y, Araki R, Tanaka T, Sasaga A, Nishiyama S, Takuma K, Matsuda T (2013) Role of social encounter-induced activation of prefrontal serotonergic systems in the abnormal behaviors of isolation-reared mice. Neuropsychopharmacology 38:1535–1547.

Alberini CM, Chen DY (2012) Memory enhancement: consolidation, reconsolidation and insulin-like growth factor 2. Trends Neurosci 35:274–283.

Anderson MF, Aberg MA, Nilsson M, Eriksson PS (2002) Insulin-like growth factor-I and neurogenesis in the adult mammalian brain. Dev Brain Res 134:115–122.

Berger F, Gage FH, Vijayaraghavan S (1998) Nicotinic receptor-induced apoptotic cell death of hippocampal progenitor cells. J Neurosci 18:6871–6881.

Bracko O, Singer T, Aigner S, Knobloch M, Winner B, Ray J, Clemenson GD Jr, Suh H, Couilland-Despres S, Aigner L, Gage FH, Jessberger S (2012) Gene expression profiling of neural stem cells and their neuronal progeny reveals IGF2 as a regulator of adult hippocampal neurogenesis. J Neurosci 32:3376–3387.

Cameron HA, McKay RD (2001) Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 435:406–417.

Chen DY, Stern SA, Garcia-Osta A, Saunier-Rebori B, Pollonini G, Bambah-Mukku D, Blitzer RD, Alberini CM (2011) A critical role for IGF-II in memory consolidation and enhancement. Nature 469:491–497.

Cooper-Kuhn CM, Winkler J, Kuhn HG (2004) Decreased neurogenesis after cholinergic forebrain lesion in the adult rat. J Neurosci Res 77:155–165.

Coronas V, Durand M, Chabot JG, Jourdan F, Quirion R (2000) Acetylcholine induces neuritic outgrowth in rat primary olfactory bulb cultures. Neuroscience 98:213–219.

Crews L, Masliah E (2010) Molecular mechanisms of neurodegeneration in Alzheimer's disease. Hum Mol Genet 19(R1):R12–R20.

Csernansky JG, Martin M, Shah R, Bertchume A, Colvin J, Dong H (2005) Cholinesterase inhibitors ameliorate behavioral deficits induced by MK-801 in mice. Neuropsychopharmacology 30:2135–2143.

Curtis MA, Low VF, Faull RL (2012) Neurogenesis and progenitor cells in the adult human brain: a comparison between hippocampal and subventricular progenitor proliferation. Dev Neurobiol 72:990–1005.

Dajas-Bailador FA, Heimala K, Wonnacott S (2003) The allosteric potentiation of nicotinic acetylcholine receptors by galantamine is transduced into cellular responses in neurons: Ca^{2+} signals and neurotransmitter release. Mol Pharmacol 64:1217–1226.

DeCarolis NA, Eisch AJ (2010) Hippocampal neurogenesis as a target for the treatment of mental illness: a critical evaluation. Neuropharmacology 58:884–893.

Erb C, Troost J, Kopf S, Schmitt U, Löffelholz K, Soreq H, Klein J (2001) Compensatory mechanisms enhance hippocampal

acetylcholine release in transgenic mice expressing human acetylcholinesterase. J Neurochem 77:638–646.

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4:1313–1317.

Espósito MS, Piatti VC, Laplagne DA, Morgenstern NA, Ferrari CC, Pitossi FJ, Schinder AF (2005) Neuronal differentiation in the adult hippocampus recapitulates embryonic development. J Neurosci 25:10074–10086.

Fernandez AM, Torres-Alemán I (2012) The many faces of insulin-like peptide signalling in the brain. Nat Rev Neurosci 13:225–239.

Frielingsdorf H, Simpson DR, Thal LJ, Pizzo DP (2007) Nerve growth factor promotes survival of new neurons in the adult hippocampus. Neurobiol Dis 26:47–55.

Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature 439:589–593.

Harrist A, Beech RD, King SL, Zanardi A, Cleary MA, Caldarone BJ, Eisch A, Zoli M, Picciotto MR (2004) Alteration of hippocampal cell proliferation in mice lacking the $\beta 2$ subunit of the neuronal nicotinic acetylcholine receptor. Synapse 54:200–206.

Jin K, Xie L, Mao XO, Greenberg DA (2006) Alzheimer's disease drugs promote neurogenesis. Brain Res 1085:183–188.

Kaneko N, Okano H, Sawamoto K (2006) Role of the cholinergic system in regulating survival of newborn neurons in the adult mouse dentate gyrus and olfactory bulb. Genes Cells 11:1145–1159.

Kita Y, Ago Y, Takano E, Fukada A, Takuma K, Matsuda T (2013) Galantamine increases hippocampal insulin-like growth factor 2 expression via $\alpha 7$ nicotinic acetylcholine receptors in mice. Psychopharmacology 225:543–551.

Koda K, Ago Y, Kawasaki T, Hashimoto H, Baba A, Matsuda T (2008) Galantamine and donepezil differently affect isolation rearing-induced deficits of prepulse inhibition in mice. Psychopharmacology 196:293–301.

Koda K, Ago Y, Yano K, Nishimura M, Kobayashi H, Fukada A, Takuma K, Matsuda T (2011) Involvement of decreased muscarinic receptor function in prepulse inhibition deficits in mice reared in social isolation. Br J Pharmacol 162:763–772.

Kotani S, Yamauchi T, Teramoto T, Ogura H (2006) Pharmacological evidence of cholinergic involvement in adult hippocampal neurogenesis in rats. Neuroscience 142:505–514.

Lazarov O, Marr RA (2010) Neurogenesis and Alzheimer's disease: at the crossroads. Exp Neurol 223:267–281.

Lee J, Duan W, Mattson MP (2002) Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. J Neurochem 82:1367–1375.

Llorens-Martín M, Torres-Alemán I, Trejo JL (2009) Mechanisms mediating brain plasticity: IGF1 and adult hippocampal neurogenesis. Neuroscientist 15:134–148.

Ma W, Maric D, Li BS, Hu Q, Andreadis JD, Grant GM, Liu QY, Shaffer KM, Chang YH, Zhang L, Pancrazio JJ, Pant HC, Stenger DA, Barker JL (2000) Acetylcholine stimulates cortical precursor cell proliferation *in vitro* via muscarinic receptor activation and MAP kinase phosphorylation. Eur J Neurosci 12:1227–1240.

Maelicke A, Albuquerque EX (2000) Allosteric modulation of nicotinic acetylcholine receptors as a treatment strategy for Alzheimer's disease. Eur J Pharmacol 393:165–170.

Maelicke A, Samochocki M, Jostock R, Fehrenbacher A, Ludwig J, Albuquerque EX, Zerlin M (2001) Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease. Biol Psychiatry 49:279–288.

Matsuda T (2013) Neuropharmacological studies on the brain serotonin$_{1A}$ receptor using the selective agonist osemozotan. Biol Pharm Bull 36:1871–1882.

Ming GL, Song H (2011) Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70:687–702.

Mohapel P, Leanza G, Kokaia M, Lindvall O (2005) Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning. Neurobiol Aging 26:939–946.

Noda Y, Mouri A, Ando Y, Waki Y, Yamada SN, Yoshimi A, Yamada K, Ozaki N, Wang D, Nabeshima T (2010) Galantamine ameliorates the impairment of recognition memory in mice repeatedly treated with methamphetamine: involvement of allosteric potentiation of nicotinic acetylcholine receptors and dopaminergic-ERK1/2 systems. Int J Neuropsychopharmacol 13:1343–1354.

Nordberg A, Lundqvist H, Hartvig P, Lilja A, Långström B (1995) Kinetic analysis of regional (S)(-)11C-nicotine binding in normal and Alzheimer brains – *in vivo* assessment using positron emission tomography. Alzheimer Dis Assoc Disord 9:21–27.

Ouchi Y, Banno Y, Shimizu Y, Ando S, Hasegawa H, Adachi K, Iwamoto T (2013) Reduced adult hippocampal neurogenesis and working memory deficits in the Dgcr8-deficient mouse model of 22q11.2 deletion-associated schizophrenia can be rescued by IGF2. J Neurosci 33:9408–9419.

Perry E, Martin-Ruiz C, Lee M, Griffiths M, Johnson M, Piggott M, Haroutunian V, Buxbaum JD, Nãsland J, Davis K, Gotti C, Clementi F, Tzartos S, Cohen O, Soreq H, Jaros E, Perry R, Ballard C, McKeith I, Court J (2000) Nicotinic receptor subtypes in human brain ageing. Alzheimer and Lewy body diseases. Eur J Pharmacol 393:215–222.

Resende RR, Alves AS, Britto LR, Ulrich H (2008) Role of acetylcholine receptors in proliferation and differentiation of P19 embryonal carcinoma cells. Exp Cell Res 314:1429–1443.

Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, Fabbri ME, Tessarollo L, Maffei L, Berardi N, Caleo M (2006) Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. Eur J Neurosci 24:1850–1856.

Samochocki M, Höffle A, Fehrenbacher A, Jostock R, Ludwig J, Christner C, Radina M, Zerlin M, Ullmer C, Pereira EF, Lübbert H, Albuquerque EX, Maelicke A (2003) Galantamine is an allosterically potentiating ligand of neuronal nicotinic but not of muscarinic acetylcholine receptors. J Pharmacol Exp Ther 305:1024–1036.

Schänzer A, Wachs FP, Wilhelm D, Acker T, Cooper-Kuhn C, Beck H, Winkler J, Aigner L, Plate KH, Kuhn HG (2004) Direct stimulation of adult neural stem cells *in vitro* and neurogenesis *in vivo* by vascular endothelial growth factor. Brain Pathol 14:237–248.

Schilström B, Ivanov VB, Wiker C, Svensson TH (2007) Galantamine enhances dopaminergic neurotransmission *in vivo* via allosteric potentiation of nicotinic acetylcholine receptors. Neuropsychopharmacology 32:43–53.

Shruster A, Offen D (2014) Targeting neurogenesis ameliorates danger assessment in a mouse model of Alzheimer's disease. Behav Brain Res 261:193–201.

Song G, Ouyang G, Bao S (2005) The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med 9:59–71.

Tanaka K, Yagi T, Shimakoshi R, Azuma K, Nanba T, Ogo H, Tamura A, Asanuma M (2009) Effects of galantamine on L-NAME-induced behavioral impairment in Y-maze task in mice. Neurosci Lett 462:235–238.

Tashiro A, Makino H, Gage FH (2007) Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. J Neurosci 27:3252–3259.

Veyrac A, Gros A, Bruel-Jungerman E, Rochefort C, Kleine Borgmann FB, Jessberger S, Laroche S (2013) Zif268/egr1 gene controls the selection, maturation and functional integration of adult hippocampal newborn neurons by learning. Proc Natl Acad Sci USA 110:7062–7067.

Wadenberg ML, Fjällström AK, Federley M, Persson P, Stenqvist P (2011) Effects of adjunct galantamine to risperidone, or haloperidol, in animal models of antipsychotic activity and extrapyramidal side-effect liability: involvement of the cholinergic muscarinic receptor. Int J Neuropsychopharmacol 14:644–654.

Wang D, Noda Y, Zhou Y, Mouri A, Mizoguchi H, Nitta A, Chen W, Nabeshima T (2007) The allosteric potentiation of nicotinic acetylcholine receptors by galantamine ameliorates the cognitive dysfunction in beta amyloid 25–35 i.c.v.-injected mice: involvement of dopaminergic systems. Neuropsychopharmacology 32:1261–1271.

Wishka DG, Walker DP, Yates KM, Reitz SC, Jia S, Myers JK, Olson KL, Jacobsen EJ, Wolfe ML, Groppi VE, Hanchar AJ, Thornburgh BA, Cortes-Burgos LA, Wong EH, Staton BA, Raub TJ, Higdon NR, Wall TM, Hurst RS, Walters RR et al. (2006) Discovery of N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]furo [2,3- c]pyridine-5-carboxamide, an agonist of the $\alpha 7$ nicotinic acetylcholine receptor, for the potential treatment of cognitive deficits in schizophrenia: synthesis and structure–activity relationship. J Med Chem 49:4425–4436.

Yano K, Koda K, Ago Y, Kobayashi H, Takuma K, Matsuda T (2009) Galantamine improves apomorphine-induced deficits in prepulse inhibition via muscarinic ACh receptors in mice. Br J Pharmacol 156:173–180.

Zhao C, Teng EM, Summers RG, Ming GL, Gage FH (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. J Neurosci 26:3–11.

Zhao C, Deng W, Gage FH (2008) Mechanisms and functional implications of adult neurogenesis. Cell 132:645–660.