Activation of Autophagy Ameliorates Age-Related Neurogenesis Decline and Neurodysfunction in Adult Mice

Na Yang1,2 · Xueqin Liu1 · Xiaojie Niu1 · Xiaoqiang Wang1 · Rong Jiang1 · Na Yuan3 · Jianrong Wang3 · Chengwu Zhang4 · Kah-Leong Lim5 · Li Lu1,2*

Accepted: 14 September 2021 / Published online: 21 September 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract
Adult neurogenesis is the ongoing generation of functional new neurons from neural progenitor cells (NPCs) in the mammalian brain. However, this process declines with aging, which is implicated in the recession of brain function and neurodegeneration. Understanding the mechanism of adult neurogenesis and stimulating neurogenesis will benefit the mitigation of neurodegenerative diseases. Autophagy, a highly conserved process of cellular degradation, is essential for maintaining cellular homeostasis and normal function. Whether and how autophagy affects adult neurogenesis remains poorly understood. In present study, we revealed a close connection between impaired autophagy and adult neurogenetic decline. Expression of autophagy-related genes and autophagic activity were significantly declined in the middle-adult subventricular/subgranular zone (SVZ/SGZ) homogenates and cultured NPCs, and inhibiting autophagy by siRNA interference resulted in impaired proliferation and differentiation of NPCs. Conversely, stimulating autophagy by rapamycin not only revitalized the viability of middle-adult NPCs, but also facilitated the neurogenesis in middle-adult SVZ/SGZ. More importantly, autophagic activation by rapamycin also ameliorated the olfactory sensitivity and cognition capacities in middle-adult mice. Taken together, our results reveal that compromised autophagy is involved in the decline of adult neurogenesis, which could be reversed by autophagy activation. It also shed light on the regulation of adult neurogenesis and paves the way for developing a therapeutic strategy for aging and neurodegenerative diseases.

Keywords Autophagy · Neural progenitor cell · Adult neurogenesis · Rapamycin · mTOR

Introduction
Neurogenesis is the process of formation of neurons de novo by neural progenitor cells (NPCs). In adult rodents, neurogenesis mainly occurs in two neurogenic niches: the subventricular zone (SVZ) located along the walls of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus [1]. Neurons born in the adult rodent SVZ migrate a long distance along the rostral migratory stream (RMS) to the olfactory neuroepithelium to initiate the neurogenesis of the olfactory bulb, while neurons born in the adult SGZ migrate a comparatively shorter distance to the granule cell layer of the hippocampus, which play a pivotal role in learning and memory [2, 3]. Adult neurogenesis in human brain has been supported by immunohistochemical analysis and non-invasive neuroimaging approaches [3–5]. However, adult neurogenesis undergoes a decline with aging. A sharp drop occurs even in as early as juvenile development of human (7–13 years old), which
is attributed to the loss of NPCs number and activity [5, 6]. In accordance with this, prior work by our group and others have revealed that adult neurogenesis exhibits a dramatic decline in mice from 6–9 months of age and becomes lower or undetectable with further aging [7–9]. Therefore, understanding the mechanism of adult neurogenesis and feasibly controlling it would help mitigate brain aging and neurodegenerative diseases.

Autophagy is an essential cellular homeostasis process involved in the degradation of damaged organelles and noxious proteins. Autophagic dysfunction has been implicated in age-related diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD), and Huntington’s disease (HD). Evidences have shown that inefficient autophagy leads to elevated α-synuclein (α-Syn) aggregation in the brain and significant dopaminergic neurons loss in the substantia nigra pars compacta in PD [10–12]. Autophagy is regulated by a series of autophagy-related genes (Atgs) acting sequentially. The deletion of Atgs (including Ambra1, Beclin1, Fip200 and Atg5) in mice results in blocked autophagic flux and attenuated LC3B-II, an autophagic marker, which is accompanied by a progressive loss of NPCs and a significant reduction in newborn neurons in the postnatal or early adult SVZ and hippocampus [13–15]. In addition, lacking of Atg7 expression leads to impaired autophagic activity, accompanied by the accumulation of cytoplasmic inclusion bodies and α-Syn protein in neurons or massive neuronal loss that contributes to PD onset [16, 17]. Interestingly, upregulated Atg7 and associated autophagic flux have been observed during the differentiation of mouse embryonic olfactory bulb stem cells (eOBSCs) [18]. Genetic suppression of Atg7 leads to deferral embryogenesis, premature death, massive neuronal loss and progressive neurodegeneration phenotypes [16, 19].

Currently, studies on the functional effects of autophagy on neurogenesis were mainly focused on the brain development stage and the early postnatal period, but little is known about its function in adult neurogenesis. Rapamycin is a well-established autophagy inducer, which can trigger autophagy initiation by selective inhibition of mTOR [20]. However, the effect of rapamycin on neurogenesis is controversial. Singh et al. have reported that rapamycin reverses the decline of neuronal makers’ levels in aged rats possibly via reducing the aging-induced oxidative stress, apoptotic cell death, and neurodegeneration makers [21]. Several studies have revealed that intraperitoneal injection of rapamycin leads to a decrease in progenitor stem cell pool and eliminates NPCs proliferation in early adult hippocampus [7, 22] or in the injured brain [23]. Hence, it is necessary to clarify how autophagy affects adult neurogenesis.

In present study, we explored whether autophagy impacts on the decline of adult neurogenesis, and whether pharmacological enhancement of autophagy by rapamycin affects adult neurogenesis and neurological function in both SVZ and SGZ of the hippocampus. Our study is aimed at providing clues for activating adult endogenous neurogenesis and delaying age-related brain dysfunction.

### Materials and Methods

#### Animals

All experimental procedures were executed in line with the institutional guidelines approved by the Animal Research Ethics Committee at the Shanxi Medical University. C57BL/6 mice were purchased from the Animal Center of Shanxi Medical University. GFP-LC3 mice (#BRC00806) were provided by J. Wang (Soochow University School of Medicine). All mice were kept under a 12-h light/dark cycle, with free access to food and water, and temperature at 22 °C. In this study, neonatal mice were defined as postnatal 24 h, young-adult mice as 2–3 months, and middle-adult mice as 7–8 months.

#### NPCs Culture and Differentiation

Primary NPCs were isolated by dissecting the SVZ of neonatal mice or middle-adult mice following the established protocols [8]. In brief, cells were cultured to form neurospheres in the proliferation medium consisting of DMEM-F12 medium supplemented with B27 (2%; Invitrogen), basic fibroblast growth factor (bFGF, 20 ng/mL; PeroTech, Rocky Hill, NJ), and epidermal growth factor (EGF, 20 ng/mL; PeroTech).

For differentiation, neurospheres were collected and digested to prepare a single cell suspension by Accutase. Then NPCs were seeded on the poly-L-ornithine (50 μg/mL, Sigma-Adrich, St Louis, MO) and laminin (20 μg/mL, Sigma-Aldrich) pre-coated coverslips. Following overnight incubation with proliferation medium, cells were replenished with the differentiation medium containing 0.5% fetal bovine serum and 1% B27 supplement for 5-day differentiation, followed by immunophenotypical identification of the neuronal marker TuJ1, as described below.

#### RNA Interference

The siRNAs specifically targeting Atg7 (5’-GCAUCAUCU UUGAAUGUATT-3’) and Atg7 (5’-CAGCCUGGCAUU UGAUAATT-3’) were synthesized by Genepharma Corp (Shanghai, China). Both si-Atg7s were co-transfected into neonatal NPCs suspension via LONZA 4D-Nucleofector System (Lonz, Germany) according to the manufacturer’s instructions. Gene knockdown efficiency was confirmed by...
qPCR and Western blotting 48- and 72-h after incubation of siRNAs.

**Rapamycin Treatment**

Rapamycin (Sigma) was dissolved in DMSO at a concentration of 10 mg/ml and further diluted in 1× PBS with 5% Tween-80 and 5% PEG-400 for application. In vitro, rapamycin was added to the middle-adult NPCs suspension at concentrations of 10 nM, 20 nM and 50 nM for 7 days for proliferation and 3 days for differentiation. In vivo, mice were intraperitoneally injected with 4 mg/kg rapamycin every other day for 4 weeks. Upon completion of rapamycin treatments, cells and mice were prepared for the following tests.

**Neurosphere Assay**

To detect the neurosphere formation, the cultures with siRNAs transfection and rapamycin administration were observed and captured every day under a phase contrast microscope (Model CKX41, Olympus, Japan). Neurospheres were formed within 5 to 7 days, and the number and the diameter of neurospheres, with diameters larger than 30 μm, were measured using the Image J software (NIH, Bethesda, MD).

**Senescence-Associated β-Galactosidase Staining**

The senescent cells in NPCs subjected to siRNA and rapamycin were evaluated with SA-β-gal Staining Kit (Beyotime) as suggested in the instruction. The SA-β-gal positive cells were stained into blue and counted in a double-blind manner. In each sample, 10 different non-overlapping fields under 20-fold magnification were randomly selected, and the SA-β-gal positive ratio was calculated.

**RNA Extraction and Quantitative Real-Time PCR**

Total RNA was extracted from SVZ/SGZ homogenates and NPCs isolated from neonatal, young-adult and middle-adult mice using TRIzol reagent (Invitrogen), and was reverse-transcribed into cDNA utilizing the PrimeScript™ RT reagent kit (Takara, RR047A, Japan) following the instruction. Gene expression levels of Atg3, Atg5, Atg7 and Beclin1 were quantified using the TB Green® Premix Ex Taq™ GC (Takara, RR071A, Japan) on the StepOne Plus Real-Time PCR System (Applied Biosystems Inc., Foster City, CA). In a 20 μl reaction mixture, the amount of cDNA template added was 100 ng, and the final concentration of primer was 0.2 μM. The mRNA levels were quantitatively analyzed by 2^−ΔΔCt method and normalized with GAPDH. Sequences of primers utilized in the assays were listed in Table 1.

**Western Blot**

Total proteins were extracted from tissues and cells using RIPA lysis buffer supplemented with the protease inhibitor cocktail (Thermo Fischer, Pittsburgh, PA). Protein concentrations were determined by a BCA Protein Assay kit (Beyotime). Equal amount of proteins were separated by SDS-PAGE, and electro-transferred into PVDF membranes (Millipore, Billerica, MA, USA). After blocking with 5% skim milk in TBST (TBS containing 0.1% Tween-20) for 1 h at room temperature, the membranes were incubated with the primary antibodies overnight at 4 °C. On the next day, membranes were thoroughly rinsed in the TBST for three times, followed by incubation with goat anti-rabbit IgG or goat anti-mouse IgG secondary antibodies for 2 h at room temperature. After rinsing in the TBST, bands were then visualized through an enhanced ECL detection kit (GE Healthcare Life Science, Pittsburgh, PA), and documented on films. Intensities of bands were quantified using Image J software, and β-actin was used as a housekeeping control. Antibodies utilized here were listed in Table 2.

**BrdU Incorporation**

In vitro, NPCs with siRNAs transfection and rapamycin administration were seeded on the pre-coated coverslips and kept in the proliferation medium overnight. BrdU (10 μM) was added during the last 7 h of culture. Then, BrdU immunofluorescence detection was performed as detailed in the following methods.

In vivo, middle-adult mice were intraperitoneally injected with BrdU (100 mg/kg of body weight) daily for seven consecutive days, starting from the 8-th day during rapamycin administration. Upon finishing the 28-day rapamycin

---

### Table 1 Primers used for Real-time PCR

| Gene   | Forward primer sequence 5’ → 3’     | Reverse primer sequence 5’ → 3’     |
|--------|-----------------------------------|-----------------------------------|
| Atg3   | TGGTGATGGGGATGGTAGATAC            | TTCCTCACTCCTCCGTCTTTC             |
| Atg5   | GCGTTAGGATCCATTTATGTGTG          | TTCCATGAAGTTTCCGGCTGATG           |
| Atg7   | TCCAGTTGGATGATGAGTCTCC           | CACTGAGGTTACACATCT                |
| Beclin1| CCGGAGATGGTGCTCAGTGAAG           | GGGGTGATCCAGCTGGA                 |
| Gapdh  | TGTGTCCGTCGTGGATCTGA             | TTGCTGAGAAGTCGAGGAG              |

---

© Springer
injection, mice were sacrificed 24 h later. For the detection of new neurons generated from NPCs in the olfactory bulb, NeuN/BrdU double staining was executed as described below.

Immunofluorescence Staining

For immunocytochemistry analyses, cells were fixed with 4% paraformaldehyde (PFA), permeabilized with 0.3% Triton X-100 (Sigma-Aldrich, X100) in PBS and blocked with 10% goat serum for 10 min at room temperature. Thereafter, samples were incubated with primary antibodies overnight at 4 °C and with secondary antibodies for 2 h at 37 °C. For immunohistochemistry analyses, the brains were gained after perfusing with 4% PFA, post-fixed in 4% PFA for 12 h and dehydrated gradiently in sucrose (15% and 30%) until they sank to the bottom. Then, brains were frozen in OCT embedding medium at -20 °C for at least 2 h, and serial coronal sections with a thickness of 16 μm in SVZ (bregma 1.09 mm to 0.13 mm), 30 μm in SGZ (bregma -1.07 mm to -2.45 mm) and 16 μm in olfactory (bregma 4.57 mm to 4.07 mm) were sliced. Samples were permeabilized with 0.3% Triton X-100 in PBS, fixated and permeabilized further with acetone at -20 °C for 20 min, and blocked with 10% goat serum for 1 h at room temperature. Thereafter, samples were incubated with primary antibodies overnight at 4 °C and with secondary antibodies for 2 h at 37 °C. For BrdU staining, samples were denatured to expose antigen with 2 M HCL at 37 °C for 10 min in cells and 16 min in tissues before the permeabilization with Triton X-100.

Antibodies utilized in the above staining assays were listed in Table 2. Nuclei were counter-stained with DAPI (1 μg/ml, Cat# D9542, Sigma-Aldrich).

Imaging and Quantitative Analysis

Images were captured by a fluorescent microscope (Model BX51, Olympus, Tokyo, Japan) or a Leica SP8 confocal microscope (Leica Microsystems, Wetzlar, Germany) depending on the requirements. Staining was quantitatively analyzed in a double-blinded manner using MetaMorph software (Molecular Devices, Sunnyvale, CA) from at least three independent experiments. In vitro, for each coverslip, 6 different non-overlapping fields under 20-fold magnification were randomly selected, and the percentages of BrdU+ and Tuj1+ cells were calculated. In vivo, serial sections from comparable positions were used to count positive cells, as reported previously [24]. For each mouse, 8 sections were counted to calculate the average numbers of Ki67+ and DCX+ cells per section, which then multiplied the number of sections per SVZ and SGZ to get the total quantity for each mouse. For the olfactory bulb, 6 sections were counted.
to calculate the average numbers of BrdU+/NeuN+ cells per section.

**Odor Detection Test**

To assess the influence of rapamycin on the odor detection threshold of mice, we adopted an odor detection test [25, 26] with some minor modifications. This test includes two phases: habituation and testing. For habituation, mice were transferred to the test chamber (31×25×12.5 cm) to acclimatize to the environment for 30 min per time during the four consecutive times. During a 3-min testing period, each mouse in the test chamber was exposed to two odors on two separate filter papers, one of which was water and the other was different concentrations of novel odor (coconut powder, concentration: 1 mg/ml, 10 mg/ml and 100 mg/ml). Sniffing time was measured for three concentrations tested in separate 3 days, in ascending order. If animals can detect the novel odor was determined by the ratio of the time spent sniffing novel odor to the total sniffing time. 50% of sniffing duration was considered that mice were able to detect the olfactory stimulus.

**Olfactory Avoidance Test**

The olfactory avoidance test was performed using a component of fox feces, nTMT (nondehydrogenated 2, 4, 5-trimethylthiazole), to investigate the influence of rapamycin on the odor detection ability of mice [27, 28]. Prior to the test, mice were habituated to the experimental environment, in which mice were placed in a test cage (31×25×12.5 cm) for 30 min per time during the four consecutive times, under the weak-light condition (<5 lx). In the testing phase, the test cage was divided into two equal areas and three different volumes (0, 0.1, and 1 μl) of undiluted nTMT was dropped on the filter paper (2 cm×2 cm) in every side of test cage. Then, mouse was placed on the opposite side of the test cage and the behavior was recorded with a video camera during the 10-min test duration. There was an interval of 24 h among different volumes of nTMT. Avoidance time was defined as when mouse nasal tip was less than 2 cm from the object. Avoidance behavior was determined by an avoidance index as follows: avoidance index = (P—50)/50, where P is the percentage of avoidance time during the 10 min test period.

**Y-Maze Test**

To assess the hippocampus-dependent spatial recognition and memory performance of the mice with rapamycin intraperitoneal injection, Y-Maze was performed as previously described [24]. Briefly, mice were transferred to the behavior room for at least 30 min for environment habituation. In testing stage, each mouse was placed at the end of one arm and allowed to explore freely through the three arms for 5 min. The number of arm entries and alternations were recorded using the Smart 3.0 Video Tracking System (Harvard Apparatus, Cambridge, Massachusetts). An arm entry is defined as when all four paws are completely within the arm. Spontaneous alternation is referred to that mouse entries all three arms in the overlapping triplet sets. The alternation percentage was calculated as follows: [spontaneous alternation / (total number of arm entries-2)]×100.

**Novel Object Recognition Test**

The novel object recognition (NOR) test was performed, as previously reported [24], to evaluate the hippocampus-dependent non-spatial memory retention for the mice with rapamycin injection. It was performed in an open field area (a 40×40×35 cm test box) with two different kinds of objects. Both objects are generally consistent in height and volume, but are different in shape and appearance. This test includes three phases: habituation, training, and testing. First, the mouse was habituated to the test box for 30 min daily for 3 consecutive days. In the training phase, the mouse was allowed to explore two identical objects placed at a symmetric position of the box for 10 min. 1.5 h later, one object was replaced by a novel object, and the animal was allowed to explore freely for 5 min. The time spent exploring each object in the training and testing phases was recorded using the Smart 3.0 Video Tracking System (Harvard Apparatus, Cambridge, Massachusetts). An exploration was considered as when mouse nasal tip was less than 2 cm from the object. The object preference index was defined as the percentage of time spent exploring any one of the two objects (training session) or the novel one (retention session) over the total time exploring both objects.

**Statistical Analysis**

All data in this study were analyzed using SPSS Software and presented as mean ± standard deviations (SD). The unpaired Student’s t-test was used for comparisons between two groups, and One-Way ANOVA with Tukey’s post-hoc test was used for multi-group comparisons. The statistically significant level was determined by $P < 0.05$.

**Results**

**Compromised Autophagy Activity of NPCs with Aging**

LC3 family includes three members: LC3A, LC3B and LC3C, of which LC3B is widely used as a marker for
autophagic assessment [29]. The conversion of LC3B-I (cytosolic form) to LC3B-II (membrane-bound lipidated form) is a hallmark of autophagosome formation [30]. To clarify the activity of autophagy in NPCs in different aging stage, LC3B-II in homogenates from the SVZ and the SGZ of hippocampal dentate gyrus was detected with Western Blotting. As shown in Fig. 1A, the level of LC3B-II was decreased in the SVZ and SGZ homogenates from middle-adult mice compared to that from neonatal ones. Similarly, a 40% reduction of LC3B-II was observed in cultured NPCs from middle-adult mice (Fig. 1B). To further monitor autophagy activity, NPCs from GFP-LC3 mice were treated with 10 nM lysosomal degradation blocker, bafilomycin A1, for 4 h to capture GFP-LC3 puncta [31, 32]. As shown in Fig. 1B, bright puncta were formed in the cytoplasm of NPCs from neonatal mice, but rare in that

Fig. 1 Compromised autophagy activity in NPCs of different aging stages. (A) Expression of LC3B-II in SVZ and SGZ of neonatal and middle-adult mice (n = 3). (B) GFP-LC3 puncta and the expression of LC3B-II in NPCs from neonatal and middle-adult mice. Scale bar = 10 μm. (C) The mRNA levels of autophagy-related genes (Atg7, Atg3, Atg5 and Beclin1) in SVZ, SGZ, and cultured NPCs (n = 3) from neonatal, young-adult and middle-adult mice. Data were from three independent experiments, and presented as means ± SD. *P < 0.05, **P < 0.01 and ***P < 0.001 vs. neonatal group.
Fig. 2 *Atg7* gene knockdown attenuated the proliferation and differentiation capacity of neonatal NPCs. (A) siRNA inhibited the *Atg7* protein expression in neonatal NPCs. (B) *Atg7* knockdown induced the alteration of LC3B-II and p62 in neonatal NPCs. (C) *Atg7* knockdown retarded the neurospheres’ formation of neonatal NPCs, Scale bar = 100 μm. (D) *Atg7* knockdown hindered the proliferation of neonatal NPCs as shown by BrdU staining, Scale bar = 100 μm. (E) *Atg7* knockdown induced the senescence of neonatal NPCs, Scale bar = 100 μm. (F) *Atg7* knockdown attenuated neuronal differentiation of neonatal NPCs as evaluated by relative Tuj1/DAPI ratio, Scale bar = 100 μm. Data were from six independent experiments, and presented as means ± SD. *P < 0.05, **P < 0.01 and ***P < 0.001 vs. Ctrl.
from middle-adult mice. To further explore the relationship between autophagy and self-renewal of NPCs with aging, we assessed the expression of Atgs in SVZ and SGZ homogenates, as well as in cultured NPCs from neonatal, young-adult...
and middle-adult mice by qRT-PCR. Both in vivo and in vitro data indicated the mRNA levels of Beclin 1, Atg7, Atg3 and Atg5 were dramatically decreased in homogenates and cultured NPCs from young-adult and middle-adult mice (Fig. 1C). The protein level of Beclin 1, Atg7, Atg3 and Atg5 also showed a decline trend as proved by Western Blotting (Supplementary Fig. 1). It is reasonable to postulate that the impaired autophagy may be involved in the decline of the viability of adult NPCs, which was previously reported by our group and others [8, 33, 34].

**Regulation of Self-Renewal and Differentiation of NPCs by Autophagy**

To validate the role of autophagy in maintaining NPCs proliferation and differentiation, neonatal NPCs were transfected with siRNA that targeted the Atg7 gene, a key component in the formation of autophagosome and serving as an E1-like activating enzyme required for the conversion of LC3B-I to LC3B-II [35–37]. The Atg7 protein and Atg7 mRNA level were efficiently down-regulated after transfection with the Atg7 specific siRNA (si-Atg7) (Fig. 2A). Besides, LC3B-II level also reduced in NPCs following si-Atg7 transfecting, accompanied by elevated p62 (also known as SQSTM1/sequestosome 1) (Fig. 2B), which could directly bind to LC3 and be selectively degraded by autolysosome in the downstream steps of autophagosome formation [35, 38, 39]. These data indicated that autophagy was down-regulated by si-Atg7 in neonatal NPCs. Meanwhile, knocking down Atg7 suppressed neonatal NPCs proliferation, characterized by forming fewer and smaller neurospheres (Fig. 2C) and significantly down-regulated BrdU incorporation (Fig. 2D). As shown in Fig. 2E, neonatal NPCs with Atg7 knockdown also displayed the senescence-like phenotype, with the increased β-galactosidase signal. To further investigate whether si-Atg7-induced autophagic dysfunction affected the neuronal differentiation of neonatal NPCs, cells transfected with either scrambled siRNA or si-Atg7 were cultured in the differentiation medium for 5 days and quantified the percentage of cells expressing immature neuronal marker Tuj1. As illustrated in Fig. 2F, compromised neuronal differentiation was found in neonatal NPCs with Atg7 knockdown. Taken together, these data demonstrate the correlation between the autophagy dysfunction and impaired self-renewal and differentiation of NPCs, suggesting that activation of autophagy may be a feasible strategy to counteract the down-regulation of self-renewal in adult NPCs.

Given that compromised autophagy recapitulates the phenotypes of aging NPCs, we wished to know if enhanced autophagy could stimulate the potency of NPCs. We applied rapamycin, a well-known autophagy inducer, to the middle-adult NPCs and observed the autophagic level as well as NPCs potency [40]. As shown in Fig. 3A, rapamycin (50 nM) application increased GFP-LC3 puncta. The western blotting results showed that rapamycin-stimulated NPCs had an increased level of LC3B-II, along with attenuated p62 (Fig. 3B). It was reported that rapamycin triggered autophagy via the mTOR-ULK pathway [41]. To confirm this, we detected the levels of phosphorylated mTOR and ULK1. As displayed in Fig. 3B, the phosphorylation levels of mTOR at Ser 2448 and ULK1 at Ser757 were significantly declined in the rapamycin-treated NPCs compared to control. In line with these findings, rapamycin-treated middle-adult NPCs formed more neurospheres with larger size (Fig. 3C) and incorporated more BrdU than control (Fig. 3D). Moreover, rapamycin treatment increased Tuj1+ cells (Fig. 3F) and decreased SA-β-gal positive cells (Fig. 3E) in a concentration-dependent manner in middle-adult NPCs. Those results suggest that pharmacological activation of autophagy renews the proliferation and differentiation of middle-adult NPCs.

**Rapamycin Induced In Vivo Neurogenesis Via Activating Autophagy**

Encouraged by the in vitro results, we sought to determine whether in vivo administration of rapamycin could ameliorate the decline of autophagy in the brain and facilitate adult neurogenesis. To confirm this, 7–8 month-old mice were intraperitoneally injected with rapamycin every other day for 4 weeks (Figs. 4A and 5A) according to previous reports [42]. As displayed in Fig. 4B, rapamycin elevated endogenous autophagy activity was observed within the SVZ, a main neurogenic niche, represented by increased LC3B-II accumulation, down-regulated p62 and reduced phosphorylated mTOR and ULK1 levels in the rapamycin group. In vivo NPCs proliferation and neural differentiation were determined by endogenous proliferation marker Ki67 and immature neuron-specific marker doublecortin (DCX). Notably, the numbers of Ki67+ and DCX+ cells were significantly increased in rapamycin-treated mice (Fig. 4C and D). Since newly formed neurons in the SVZ can migrate and integrate into the olfactory bulb circuits [2], we next examined the olfactory bulb neurogenesis with BrdU

---

**Fig. 3** Rapamycin rejuvenated autophagy activity and the proliferation and differentiation of NPCs derived from middle-adult mice. (A) Rapamycin increased the number of GFP-LC3 puncta in middle-adult NPCs, Scale bar = 10 μm. (B) Rapamycin affected the expression of LC3B-II, p62, p-mTOR/mTOR and p-ULK1/ULK1 in middle-adult NPCs. (C) Rapamycin stimulated the neurosphere’s formation of middle-adult NPCs, Scale bar = 100 μm. (D) Rapamycin enhanced the proliferation of middle-adult NPCs as shown by BrdU staining. (E) Rapamycin attenuated the senescence of middle-adult NPCs, Scale bar = 100 μm. (F) Rapamycin increased the number of Tuj1+ cells in middle-adult NPCs, in a concentration-dependent manner, Scale bar = 100 μm. Data were from there independent experiments, and presented as means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 vs. DMSO group.
through six-day consecutive injections. The number of NeuN+/BrdU+ colabeled cells in the granule cell layer (GCL) of the OB in the rapamycin group was significantly higher than that of control (Fig. 4E). The olfactory behavior was examined using odor detection test and olfactory avoidance test. Rapamycin-treated mice were able to detect lower odor concentration (10 mg/ml), whereas DMSO control mice only detected an odor at 100 mg/ml, indicating improved detection sensitivity after rapamycin application (Fig. 4F). Furthermore, we performed an olfactory avoidance test using nTMT which was reported to evoke fear responses [27, 28]. Interestingly, when a lower amount (0.1 μl) of nTMT was used, rapamycin-treated mice showed significant avoidance responses compared with DMSO control mice, but no difference to a high amount of nTMT (1 μl) (Fig. 4G), indicating that rapamycin-treated mice have increased sensitivity to detect odorants. These observations imply that activation of autophagy by rapamycin can enhance neurogenesis in the SVZ/OB and improve olfactory sensitivity in middle-adult mice. MetaMorph Microscopy Automation and Image Analysis Software was applied in automatic cell counting in present study, although a stereological method may be more unbiased and preferred in future investigation.

The other major site of neurogenic niche in the brain is the hippocampus where NPCs predominantly accumulated within the SGZ of dentate gyrus [5]. We wondered whether intraperitoneal injection of rapamycin also stimulates adult neurogenesis in middle-adult SGZ. As expected, rapamycin administration significantly increased the expression of LC3B-II in DG, while reduced the levels of p62, phosphorylated mTOR and ULK1 (Fig. 5B). These data showed that rapamycin raised the autophagic level of middle-adult hippocampus. Concurrently, there were more proliferative NPCs and new neurons in SGZ in rapamycin group than those in control group, as evidenced by Ki67+, DCX+ staining (Fig. 5C and D). Further, rapamycin-treated mice showed improved spatial working memory as documented by the Y-Maze test. Compared to DMSO controls, rapamycin-treated mice obtained approximately 10% higher spontaneous alternation (Fig. 5E left panel), while there was no significant difference for exploratory activities between the two groups, as indicated by the total numbers of arm entries (Fig. 5E, right panel). To evaluate learning and memory, we next performed the NOR test, which was based on the natural tendency of rodents to investigate new objects. During the training phase, the time percentages spent exploring the two objects were the same between rapamycin-treated and DMSO mice (Fig. 5F, left), indicating approximately similar levels of motivation and curiosity to the novel object. However, in the testing phase, rapamycin-treated mice spent more time with novel objects than familiar ones, whereas DMSO control mice showed no such difference (Fig. 5F, right). These data suggest that activation of autophagy by rapamycin promotes the new neurons generation and functionally improves cognitive ability in middle-adult mice.

**Discussion**

Adult neurogenesis is of great importance in maintaining brain function [43, 44]. Understanding how adult neurogenesis is regulated attracts the attention of neuroscientists. In the present study, we have demonstrated that autophagy plays a pivotal role in adult neurogenesis. With aging, the expression and activity of autophagy-related proteins in middle-adult SVZ/SGZ and cultured NPCs declined significantly. Inhibiting Atg7 by siRNA impaired the proliferation and differentiation of NPCs. Conversely, activating autophagy by rapamycin revitalized NPCs’ viability and differentiation potency. In vivo, injection of rapamycin stimulated neurogenesis in middle-adult SVZ/SGZ as well as functional recovery such as improved olfactory sensitivity and cognitional capacities. Consequently, drugs explorations or other interventions targeting the activation of endogenous neurogenesis will be of great utility, potentially serving as effective strategies for the prevention and treatment of age-related brain dysfunction.

Many factors, including diet, exercise, hormones, neurotransmitters, have been reported to affect adult neurogenesis [45–47]. Nevertheless, the mechanism intrinsically underlying adult neurogenesis remains to be illustrated. Protein homeostasis is the precondition to maintain normal cellular function, such as neurogenesis. Our previous work has shown that proteasome dysfunction leads to declined NPCs proliferation and differentiation [8]. Autophagy, as the major alternative protein degradation pathway, plays a vital role in brain development and function. During the developmental stage, constitutive autophagy is essential for embryonic neurogenesis [48]. Impaired autophagy influences the neuron differentiation and proliferation of NPCs, which leads to severe neural tube defects [49–51]. At early postnatal stages, basal autophagy is required for maintaining the viability and differentiation potency of NPCs [13–15]. Compromised autophagy is also implicated in the pathogenesis
Fig. 5 Rapamycin improved hippocampus neurogenesis and cognitive capability of middle-adult mice. (A) Schematic experimental design of rapamycin treatment in vivo and efficiency evaluation. (B) Rapamycin affected the expression of LC3B-II, p62, p-mTOR/mTOR and p-ULK1/ULK1 in hippocampus of middle-adult mice (n=3). (C, D) Rapamycin increased the Ki67, DCX positive cell number in the SGZ of the middle-adult mice, Scale bar=50 μm (n=6). (E) Rapamycin improved the alternation rate in Y-Maze test of middle-adult mice (n=10). (F) Rapamycin enhanced the tendency of exploring novel objects by middle-adult mice in NOR test (n=10). Data were presented as means±SD. *P<0.05, **P<0.01, ***P<0.001 vs. DMSO group.
of neurodegenerative diseases [52, 53], among which neurogenesis is likely affected.

Atgs are required for the formation of the isolation membrane and the autophagosome, which involves two major steps: nucleation and elongation of the isolation membrane. Among these genes, Beclin1 is essential for the nucleation of isolation membrane, and Atg7, Atg3 and Atg5 are required for the elongation of isolation membrane [31]. In present study, we found that the expression of Atg3, Atg5, Atg7, Beclin1 and LC3B-II in SVZ and hippocampus of middle-adult mice decreased, compared with that in neonatal mice, which is accompanied by declined adult neurogenesis. These observations were further supported by our in vitro data from cultured NPCs. Consistently, other researchers have reported deletions of Fip200, a gene essential for autophagy induction, and Atg5 result in progressively loss of adult NPCs and deteriorated neuronal differentiation [13, 15]. Among these Atgs declined in middle-adult group, the decrease of Atg7 was particularly significant. Atg7 level in middle-adult NPCs dropped approximately 72% of that in neonatal NPCs. We further demonstrated that inhibition of Atg7 by siRNA in neonatal NPCs suppressed autophagy activity as well as cell proliferation and differentiation, a phenomenon that is confirmed by the smaller neurospheres observed, fewer BrdU+ and TuJ1+ cells, along with increased β-galactosidase. In line with our findings, Atg7 deficiency has also been implicated in neuronal loss, axonal degeneration and memory impairment [16, 54, 55]. Besides that, a higher Atg7 level was essential for the neuronal differentiation of the embryonic olfactory bulb [18]. Noteworthy, in some pathological conditions, increased expression of Atg7 is also involved in inflammation and microglia activation, which are associated with cell death and childhood neurological disorders [35, 56, 57]. Thus, enhanced autophagy might not always be beneficial, and further investigations utilizing different animal models are needed to elucidate the precise role of Atgs-mediated autophagy in neurogenesis.

Autophagy could also be modulated by certain compounds. Rapamycin, a well-established autophagy activator, promotes autophagic activation through inhibiting the phosphorylation of mTOR at Ser2448 [58]. mTOR inhibition can further suppress the phosphorylation of ULK1 (an Atg1 homolog) at Ser757, which is required for autophagic initiation and recruitment of other autophagy related proteins [41, 42]. Here, we demonstrated that in vivo administration of rapamycin could ameliorate the decline of autophagy in the middle-adult brain and facilitate adult neurogenesis through autophagy activation via the suppression of mTOR. Specifically, we showed that autophagic activation by rapamycin not only facilitated the endogenous neurogenesis in the middle-adult SVZ and hippocampus, but also improved the olfactory sensitivity and cognitive capability in middle-adult mice. We further confirmed in vitro that rapamycin-triggered autophagy activation via mTOR suppression rejuvenated the viability and differentiation potency of middle-adult NPCs. Reports from other groups also support our observations. Singh et al. have found that activating autophagy through oral administration of rapamycin plays a neuroprotective role by alleviating the oxidative damage, inflammatory response and cell death in the aged brain [21]. Spilman et al. have reported that rapamycin administration protects hippocampus neurons against injury and improves the learning and memory ability through mTOR inhibition in AD mice [59]. Rapamycin might stimulate adult neurogenesis via other pathways. Majumder et al. reported that rapamycin reduced interleukin-1β and enhanced NMDA signaling which could promote neurogenesis [60, 61]. In certain circumstances, autophagy activation might not alter neurogenesis. Kodali et al. reported that activation of autophagy by metformin ameliorated cognitive function of aged mice, but neurogenesis was not affected [62]. One possible reason is that in aged mice the neurogenesis capacity is weak and autophagy activation could not render a triggering effect. Studies report that less dysfunctional proteins and organelles occur in early life, so intervention increases the autophagy, such as mTOR inhibition, can be detrimental [63, 64]. In present study, we demonstrated that activating autophagy in middle-adult mice ameliorated the declination of neurogenesis. This illustrates autophagy levels should be precisely regulated, and too high or too low level is unfavorable for the organism. DMSO, the solvent of rapamycin, especially in higher dose might affect the cognition of the mice. So naive control mice or DMSO administration mice should be included so as to exclude the potential effect of mice handling or solvent. It is worth noting that rapamycin administration could also cause side effects, such as suppressing immunity and increasing the risk of cancer [65]. Thus, further studies are required to determine the clinical applications of rapamycin in different scenarios [66].

In summary, our results revealed that autophagy was closely correlated with adult neurogenesis. Genetic suppression of Atg7 compromised neurogenesis; conversely, pharmacological activation of autophagy enhanced middle-adult neurogenesis and restored middle-adult brain function. This shed light on the regulation of adult neurogenesis. Genetic or pharmacological activation of autophagy could serve as an attractive therapeutic strategy for age-related brain dysfunction and neurodegenerative diseases where neurogenesis is compromised.

Supplementary Information The online version contains supplemental material available at https://doi.org/10.1007/s12015-021-10265-0.

Authors’ Contributions N.Y., XQ.L., XJ.N., XQ.W. and R.J. performed and analyzed the experiments. N.Y. and R.J. helped in some animal experiments. KL. L. and L.L. conceived and designed the research. The manuscript was written by XQ. L., L.L. and CW. Z.

All authors read and approved the final manuscript.
Funding  This work was supported by National Natural Science Foundation of China (81200254, 81571381), Research Project Supported by Shanxi Scholarship Council of China (2020–085) and Teaching Innovation Programs of Higher Education Institutions in Shanxi (J2020094).

Data Availability  Not applicable.

Code Availability  Not applicable.

Declarations

Ethics Approval  All animal studies were approved by the Committee for Animal Care and Ethical Review at Shanxi Medical University.

Consent to Participate  Not applicable.

Consent for Publication  Not applicable.

Competing Interests  The authors declare that they have no competing interests.

References

1. Gage, F. H. (2000). Mammalian neural stem cells. Science, 287(5457), 1433–1438.
2. Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. Cell, 132(4), 645–660.
3. Seki, T., Hori, T., Miyata, H., Maehara, M., & Namba, T. (2019). Analysis of proliferating neuronal progenitors and immature neurons in the human hippocampus surgically removed from control and epileptic patients. Science and Reports, 9(1), 18194.
4. Ho, N. F., Hooker, J. M., Sahay, A., Holt, D. J., & Roffman, J. L. (2013). In vivo imaging of adult human hippocampal neurogenesis: Progress, pitfalls and promise. Molecular Psychiatry, 18(4), 404–416.
5. Kumar, A., Pareek, V., Faiq, M. A., Ghosh, S. K., & Kumari, C. (2019). Adult neurogenesis in humans: A review of basic concepts, history, current research, and clinical implications. Innov Clin Neurosci, 16(5–6), 30–37.
6. Sorrells, S. F., Paredes, M. F., Cebrian-Silla, A., Sandoval, K., Qi, D., Kelley, K. W., & Alvarez-Buylla, A. (2018). Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature, 555(7696), 377–381.
7. Romine, J., Gao, X., Xu, X. M., So, K. F., & Chen, J. (2015). The proliferation of amplifying neural progenitor cells is impaired in the aging brain and restored by the mtor pathway activation. Neurobiology of Aging, 36(4), 1716–1726.
8. Zhao, Y., Liu, X., He, Z., Niu, X., Shi, W., Ding, J. M., & Lu, L. (2016). Essential role of proteasomes in maintaining self-renewal in neural progenitor cells. Science and Reports, 6, 19752.
9. Kalamakis, G., Brune, D., Ravichandran, S., Bolz, J., Fan, W., Ziebell, F., & Martin-Villalba, A. (2019). Quiescence modulates stem cell maintenance and regenerative capacity in the aging brain. Cell, 176(6), 1407–1419 e1414.
10. Friedman, L. G., Lachenmayer, M. L., Wang, J., He, L., Pouloue, S. M., Komatsu, M., & Yue, Z. (2012). Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of alpha-synuclein and lrrk2 in the brain. Journal of Neuroscience, 32(22), 7585–7593.
11. Fernandes, H. J., Hartfield, E. M., Christian, H. C., Emmanoulidou, E., Zheng, Y., Booth, H., & Wade-Martins, R. (2016). Er stress and autophagic perturbations lead to elevated extracellular alpha-synuclein in gba-n370s parkinson’s ipsc-derived dopamine neurons. Stem Cell Reports, 6(3), 342–356.
12. Hunn, B. H. M., Vingill, S., Threlfell, S., Alegre-Abarrategui, J., Magdelyns, M., Deltheil, T., & Wade-Martins, R. (2019). Impairment of macroautophagy in dopamine neurons has opposing effects on parkinsonian pathology and behavior. Cell Rep, 29(4), 920–931 e927.
13. Wang, C., Liang, C. C., Bian, Z. C., Zhu, Y., & Guan, J. L. (2013). Fip200 is required for maintenance and differentiation of postnatal neural stem cells. Nature Neuroscience, 16(5), 532–542.
14. Yazdankhah, M., Farioli-Vecchioli, S., Tonchev, A. B., Stoykova, A., & Cecconi, F. (2014). The autophagy regulators ambral1 and beclin 1 are required for adult neurogenesis in the brain subventricular zone. Cell Death Dis, 5, e1403.
15. Xi, Y., Dhalwal, J. S., Ceizar, M., Vaculik, M., Kumar, K. L., & Lagace, D. C. (2016). Knockout of atg5 delays the maturation and reduces the survival of adult-generated neurons in the hippocampus. Cell Death Dis, 7, e2127.
16. Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., & Tanaka, K. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature, 441(7095), 880–884.
17. Chen, D., Pang, S., Feng, X., Huang, W., Hawley, R. G., & Yan, B. (2013). Genetic analysis of the atg7 gene promoter in sporadic parkinson’s disease. Neuroscience Letters, 534, 193–198.
18. Vazquez, P., Arroba, A. I., Cecconi, F., de la Rosa, E. J., Boya, P., & de Pablo, F. (2012). Atg5 and ambral1 differentially modulate neurogenesis in neural stem cells. Autophagy, 8(2), 187–199.
19. Revuelta, M., & Matheu, A. (2017). Autophagy in stem cell aging. Aging Cell, 16(5), 912–915.
20. Ravikumar, B., Vacher, C., Berger, Z., Davies, J. E., Luo, S., Oroz, L. G., & Rubinstein, D. C. (2004). Inhibition of mtor induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of huntington disease. Nature Genetics, 36(6), 585–595.
21. Singh, A. K., Singh, S., Tripathi, V. K., Bissoy, A., Garg, G., & Rizvi, S. I. (2019). Rapamycin confers neuroprotection against aging-induced oxidative stress, mitochondrial dysfunction, and neurodegeneration in old rats through activation of autophagy. Rejuvenation Research, 22(1), 60–70.
22. Raman, L., Kong, X., & Kernie, S. G. (2013). Pharmacological inhibition of the mtor pathway impairs hippocampal development in mice. Neuroscience Letters, 541, 9–14.
23. Wang, X., Seekaew, P., Gao, X., & Chen, J. (2016). Traumatic brain injury stimulates neural stem cell proliferation via mammalian target of rapamycin signaling pathway activation. eNeuro, 3(5), 1–14.
24. Niu, X., Zhao, Y., Yang, N., Zhao, X., Zhang, W., Bai, X., & Lu, L. (2020). Proteasome activation by insulin-like growth factor-1/nuclear factor erythroid 2-related factor 2 signaling promotes exercise-induced neurogenesis. Stem Cells, 38(2), 246–260.
25. Breton-Provencher, V., Lemasson, M., Peralta, M. R., & Saghatelian, A. (2009). Interneurons produced in adulthood are required for the normal functioning of the olfactory bulb network and for the execution of selected olfactory behaviors. Journal of Neuroscience, 29(48), 15245–15257.
26. Takahashi, H., Ogawa, Y., Yoshiihara, S., Ashina, R., Kinoshita, M., Kitano, T., & Tsuboi, A. (2016). A subtype of olfactory bulb interneurons is required for odor detection and discrimination behaviors. Journal of Neuroscience, 36(31), 8210–8227.
27. Fendt, M., & Endres, T. (2008). 2,3,5-trimethyl-3-thiazoline (tmt), a component of fox odor - just repugnant or really fear-inducing? *Neuroscience and Biobehavioral Reviews, 32*(7), 1259–1266.

28. Takahashi, L. K., Nakashima, B. R., Hong, H., & Watanabe, K. (2005). The smell of danger: A behavioral and neural analysis of predator odor-induced fear. *Neuroscience and Biobehavioral Reviews, 29*(7), 1157–1167.

29. Klionsky, D. J., Abdelmohsen, K., Abe, A., Abedin, M. J., Abeliovich, H., Acevedo Arzenoza, A., & Zaughaier, S. M. (2016). Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy, 12*(1), 1–222.

30. Mizushima, N., Yoshimori, T., & Levine, B. (2010). Methods in mammalian autophagy research. *Cell, 140*(3), 313–326.

31. Pugsley, H. R. (2017). Quantifying autophagy: Measuring LC3 puncta and autolysosome formation in cells using multispectral imaging flow cytometry. *Methods, 112*, 147–156.

32. Mauthé, M., Orthon, I., Rocchi, C., Zhou, X., Luhr, M., Hijlkema, K. J., & Reggiori, F. (2018). Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. *Autophagy, 14*(8), 1435–1455.

33. Smith, L. K., White, C. W., 3rd., & Villeda, S. A. (2018). The systemic environment: At the interface of aging and adult neurogenesis. *Cell and Tissue Research, 371*(1), 105–113.

34. Cheng, Z., Li, Y. Q., & Wong, C. S. (2016). Effects of aging on hippocampal neurogenesis after irradiation. *International Journal of Radiation Oncology Biology Physics, 94*(5), 1181–1189.

35. Xie, C., Ginet, V., Sun, Y., Koike, M., Zhou, K., Li, T., & Zhu, C. (2016). Neuroprotection by selective neuronal deletion of atg7 in neonatal brain injury. *Autophagy, 12*(2), 410–423.

36. Su, L. Y., Luo, R., Liu, Q., Su, J. R., Yang, L. X., Ding, Y. Q., & Yao, Y. G. (2017). Atg5- and atg7-dependent autophagy in dopaminergic neurons regulates cellular and behavioral responses to morphine. *Autophagy, 13*(9), 1496–1511.

37. Donde, A., Sun, M., Jeong, Y. H., Wen, X., Ling, J., Lin, S., & Wong, P. C. (2020). Upregulation of atg7 attenuates motor neuron dysfunction associated with depletion of tardbp/tdp-43. *Autophagy, 16*(4), 672–682.

38. Gao, W., Chen, Z., Wang, W., & Stang, M. T. (2013). E1-like activating enzyme atg7 is preferentially sequestered into p62 aggregates via its interaction with lc3-i. *PLoS One, 8*(9), e73229.

39. Bjorkoy, G., Lamark, T., Brech, A., Outzen, H., Perander, M., Overvatn, A., & Johansen, T. (2005). P62/sqstm1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-in cell death disease. *Journal of Cell Biology, 171*(4), 603–614.

40. Liu, Q., Zhou, X., Li, C., Zhang, X., & Li, C. L. (2018). Rapamycin promotes the anticancer action of dihydroartemisinin in breast cancer mda-mb-231 cells by regulating expression of atg7 and dapk. *Oncology Letters, 15*(4), 5781–5786.

41. Kim, Y. C., & Guan, K. L. (2015). Mtor: A pharmacologic target for autophagy regulation. *The Journal of Clinical Investigation, 125*(1), 25–32.

42. Li, G., Miskimen, K. L., Wang, Z., Xie, X. Y., Tse, W., Gouilleux, F., & Bunting, K. D. (2010). Effective targeting of stat5-mediated survival in myeloproliferative neoplasms using abt-737 combined with rapamycin. *Leukemia, 24*(8), 1397–1405.

43. Ming, G. L., & Song, H. (2011). Adult neurogenesis in the mammalian brain: Significant answers and significant questions. *Neuron, 70*(4), 687–702.

44. Deng, W., Aimone, J. B., & Gage, F. H. (2010). New neurons and new memories: How does adult hippocampal neurogenesis affect learning and memory? *Current Reviews in Neuroscience, 11*(5), 339–350.

45. Cutler, R. R., & Kokovay, E. (2020). Rejuvenating subventricular zone neurogenesis in the aging brain. *Current Opinion in Pharmacology, 50*, 1–8.

46. Katsimpardi, L., & Lledo, P. M. (2018). Regulation of neurogenesis in the adult and aging brain. *Current Opinion in Neurobiology, 53*, 131–138.

47. Livingston-Thomas, J., Nelson, P., Karthikeyan, S., Antonescu, S., Jeffers, M. S., Marzolini, S., & Corbett, D. (2016). Exercise and environmental enrichment as enablers of task-specific neuroplasticity and stroke recovery. *Neurotherapeutics, 13*(2), 395–402.

48. Fimia, G. M., Stoykova, A., Romagnoli, A., Giunta, L., Di Bartolomeo, S., Nardacci, R., & Ceconi, F. (2007). Ambral1 regulates autophagy and development of the nervous system. *Nature, 447*(7148), 1121–1125.

49. Mizushima, N., & Levine, B. (2010). Autophagy in mammalian development and differentiation. *Nature Cell Biology, 12*(9), 823–830.

50. Casares-Crespo, L., Calatayud-Basela, I., Garcia-Corzo, L., & Mira, H. (2018). On the role of basal autophagy in adult neural stem cells and neurogenesis. *Frontiers in Cellular Neuroscience, 12*, 339.

51. Wu, X., Fleming, A., Ricketts, T., Pavel, M., Virgin, H., Menzies, F. M., & Rubinsztein, D. C. (2016). Autophagy regulates notch degradation and modulates stem cell development and neurogenesis. *Nature Communications, 7*, 10533.

52. Nikoletopoulou, V., Papandreou, M. E., & Tavernarakis, N. (2015). Autophagy in the physiology and pathology of the central nervous system. *Cell Death and Differentiation, 22*(3), 398–407.

53. Menzies, F. M., Fleming, A., Caricasole, A., Bento, C. F., Andrews, S. P., Ashkenazi, A., & Rubinsztein, D. C. (2017). Autophagy and neurodegeneration: Pathogenic mechanisms and therapeutic opportunities. *Neuron, 93*(5), 1015–1034.

54. Komatsu, M., Wang, Q. J., Holstein, G. R., Friedrich, V. L., Jr., Ivata, J., Kominami, E., & Yue, Z. (2007). Essential role for autophagy protein atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. *Proc Natl Acad Sci U S A, 104*(36), 14489–14494.

55. Gupta, V. K., Scheunemann, L., Eisenberg, T., Mertel, S., Bhukel, A., Koemans, T. S., & Sigrist, S. J. S. (2013). Restoring polyamines protects from age-induced memory impairment in an autophagy-dependent manner. *Nature Neuroscience, 16*(10), 1453–1460.

56. Wang, Y., Zhou, K., Li, T., Xu, Y., Xie, C., Sun, Y., & Zhu, C. (2017). Inhibition of autophagy prevents irradiation-induced neural stem and progenitor cell death in the juvenile mouse brain. *Cell Death Dis, 8*(3), e2694.

57. Wang, Y., Zhou, K., Li, T., Xu, Y., Xie, C., Sun, Y., & Zhu, C. (2019). Selective neuronal deletion of the atg7 gene reduces irradiation-induced cerebellar white matter injury in the juvenile mouse brain by ameliorating oligodendrocyte progenitor cell loss. *Frontiers in Cellular Neuroscience, 13*, 241.

58. Selvakumar, G. P., Iyer, S. S., Kempruj, D., Ahmed, M. E., Thangavel, R., Dubova, I., & Zaheer, A. (2019). Molecular association of glia maturation factor with the autophagic machinery in rat dopaminergic neurons: A role for endoplasmic reticulum stress and mapk activation. *Molecular Neurobiology, 56*(6), 3865–3881.

59. Spilman, P., Podlutskaya, N., Hart, M. J., Debnath, J., Gorostiza, O., Bredesen, D., & Galvan, V. (2010). Inhibition of mtor by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer’s disease. *PLoS One, 5*(4), e9979.

60. Majumder, S., Caccamo, A., Medina, D. X., Benavides, A. D., Javors, M. A., Kraig, E., & Oddo, S. (2012). Lifelong rapamycin administration ameliorates age-dependent cognitive deficits by reducing il-1beta and enhancing nmda signaling. *Aging Cell, 11*(2), 326–335.

61. Yamada, J., & Jinno, S. (2019). Potential link between antidepressant-like effects of ketamine and promotion of adult neurogenesis in the ventral hippocampus of mice. *Neuropharmacology, 158*, 107710.
62. Kodali, M., Attaluri, S., Madhu, L. N., Shuai, B., Upadhye, R., Gonzalez, J. J., & Shetty, A. K. (2021). Metformin treatment in late middle age improves cognitive function with alleviation of microglial activation and enhancement of autophagy in the hippocampus. *Aging Cell, 20*(2), e13277.

63. Schmeisser, K., & Parker, J. A. (2019). Pleiotropic effects of mtor and autophagy during development and aging. *Front Cell Dev Biol, 7*, 192.

64. Paliouras, G. N., Hamilton, L. K., Aumont, A., Joppe, S. E., Barnabe-Heider, F., & Fernandes, K. J. (2012). Mammalian target of rapamycin signaling is a key regulator of the transit-amplifying progenitor pool in the adult and aging forebrain. *Journal of Neuroscience, 32*(43), 15012–15026.

65. Blagosklonny, M. V. (2019). Rapamycin for longevity: Opinion article. *Aging (Albany NY), 11*(19), 8048–8067.

66. Kaeberlein, M., & Galvan, V. (2019). Rapamycin and alzheimer's disease: Time for a clinical trial? *Sci Transl Med, 11*(476), eaar4289.

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.