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# Dynamic SAP102 expression in the hippocampal subregions of rats and APP/PS1 mice of various ages

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## Abstract

The hippocampus is a structurally and functionally complex brain area that plays important and diverse roles in higher brain functions, such as learning and memory, and mounting evidence indicates that different hippocampal subregions play distinctive roles. The hippocampus is also one of the first regions in the brain to suffer damage in Alzheimer's disease (AD). Synaptic dysfunction in the hippocampus, rather than neuronal loss *per se*, is paralleled by behavioural and functional deficits in AD. The membrane-associated guanylate kinase (MAGUK) family of proteins, including SAP102, PSD-95, PSD-93 and SAP97, have long been recognized as essential components of the postsynaptic density (PSD) at excitatory synapses. Hippocampal spines are the predominant synaptic transmission sites of excitatory glutamatergic synapses. During postnatal brain development, individual MAGUK members show distinct expression patterns. Although SAP102 has been confirmed as the dominant scaffold protein in neonatal synapses, its expression profiles in adult and ageing rodent hippocampi are discrepant. Furthermore, in AD brains, significantly reduced SAP102 protein levels have been found, suggesting that SAP102 may be related to AD progression; however, the precise mechanism underlying this result remains unclear. Herein, we observed distinct SAP102 expression profiles in the hippocampal CA1, CA3 and DG subregions of rats and APPswe/PS1dE9 (APP/PS1) mice at various ages using immunofluorescence. In Wistar rats, SAP102 was not only highly expressed in the hippocampal subregions of neonatal rats but also maintained relatively high expression levels in adult hippocampi and displayed no obvious decreases in the CA1 and DG subregions of aged rats. Surprisingly, we observed abnormally high SAP102 expression levels in the CA1 stratum moleculare and CA3 stratum polymorphum subregions of 2-month-old APP/PS1 mice, but low SAP102 levels in the DG and CA3 subregions of 7-month-old APP/PS1 mice, reflecting the subregion-specific reactivity and vulnerability of AD mouse models in different disease stages. Our findings provide fundamental data to support the functional differences of SAP102 in different hippocampal subregions during postnatal periods and may serve as the basis for additional functional studies on SAP102 in normal physiological conditions and different stages of AD.

**Key words:** Alzheimer's disease; APP/PS1; hippocampus; SAP102.

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## Introduction

The hippocampus is a structurally and functionally complex brain area that plays important and diverse roles in many higher brain functions, such as spatial and episodic memory, learning and emotion (Nakamura et al. 2011). Based on its

cell morphology, electrophysiological properties and synaptic connectivity, the hippocampus is histologically classified into three subregions, the Cornu Ammonis (CA)1, CA3 and dentate gyrus (DG; Johnston & Amaral, 2004). Mounting evidence indicates that different hippocampal subregions follow different developmental trajectories and play distinct roles, ranging from subregion-specific physiological functions in learning, memory and synaptic plasticity to malfunctions in pathological conditions and various diseases (Fujii et al. 2014).

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by gradual memory loss and cognitive impairments, and the hippocampus is one of the first regions in the brain to suffer damage in AD (Braak & Braak, 1991; Braak et al. 1993; Greene & Killiany, 2012). Increasingly, evidence suggests that synaptic dysfunction in the hippocampus, rather than neuronal loss *per se*, is paralleled by behavioural and functional deficits in AD (Terry et al. 1991; Selkoe, 2002).

The membrane-associated guanylate kinase (MAGUK) family of proteins, including SAP102, PSD-95, PSD-93 and SAP97, have long been recognized as essential components of the postsynaptic density (PSD) at excitatory synapses (Sheng & Hoogenraad, 2007). Hippocampal spines are predominant synaptic transmission sites of excitatory glutamatergic synapses (Sorra & Harris, 2000). As a bridge linking synaptic glutamate receptors to intracellular signalling molecules and cytoskeleton, MAGUK members can mediate synaptic plasticity and maintain synaptic transmission by compensating for one another (Funke et al. 2005; Schlüter et al. 2006; Murata & Constantine-Paton, 2013; Won et al. 2017).

During postnatal brain development, individual MAGUK members show distinct expression patterns. SAP102 is the dominant scaffold protein in neonatal synapses, whereas PSD-95 and PSD-93 are primarily expressed in mature synapses. In the adult rodent brain, unlike PSD95, SAP102 expression substantially decreases to levels insignificant for modulating synaptic expression (Sans et al. 2000; Elias et al. 2006; Murata & Constantine-Paton, 2013). However, some data have demonstrated that SAP102 is highly expressed in the ageing human brain (Proctor et al. 2010) and suggest that it plays more diverse roles, which need to be further elucidated. In addition, some researchers documented that SAP102 is required for hippocampal LTP induction and higher cognitive function, as SAP102 mutant mice showed cognitive impairments with a specific spatial learning deficit (Cuthbert et al. 2007). Furthermore, in the AD brain, significantly reduced protein levels of SAP102 were found (Proctor et al. 2010), suggesting that SAP102 may be related to the progression of AD, although the precise mechanism underlying this result is still unclear.

Although the expression and function of PSD-95 in normal brain development and related neurological disorders have been widely studied (Roselli et al. 2005; Chang et al. 2009; Liu et al. 2010; Ling et al. 2012), the age-associated expression patterns and roles of other MAGUK family members, particularly SAP102, are far less defined (Elias & Nicoll, 2007; Zheng et al. 2010).

In the present study, using immunofluorescence labelling, we observed the distinct expression profiles of SAP102 in hippocampal CA1, CA3 and DG regions of Wistar rats and APPswe/PS1dE9 (APP/PS1) mice of different ages. In Wistar rats, SAP102 was not only highly expressed in the hippocampal subregions of neonatal rats but also maintained relatively higher expression levels in the adult hippocampus and was not significantly decreased in the CA1 and DG subregions of aged rats. Strikingly, in 2-month-old APP/PS1 mice, abnormally high expression levels of SAP102 were observed in the CA1 stratum (S.) moleculare and CA3 S. polymorphum regions, whereas lower SAP102 expression was observed in 7-month-old APP/PS1 mice, especially in the DG and CA3 regions, reflecting subregion-specific reactivity and vulnerability in different disease stages of AD mouse models.

Our findings provide fundamental data to support functional differences in SAP102 expression in distinct rodent hippocampal subregions at different postnatal ages and may serve as the basis for additional functional studies on SAP102 in normal physiological conditions and different stages of AD.

## Materials and methods

### Animals and tissue preparation

Wistar rats ($n = 6$ per age) of varying ages [postnatal (P) day 0 (day of birth), P4, P7, P10, P14, P21, P28, P56, P6 months (P6 m), P8 m, P20 m], APPswe/PS1dE9 (APP/PS1) mice carrying the K595N/M596L Swedish mutation and the exon 9-deleted variant of human PS1 on a C57BL/6J background and age-matched C57BL/6J wild-type (WT) mice (aged 2, 5 and 7 months; $n = 3$ per age) were used in this study. All rats and mice (males) were obtained from Beijing HFK Bioscience Co., Ltd (Beijing, China). All experimental procedures were approved by the Animal Ethics Committee of Capital Medical University. Animals were euthanized with 6% chloral hydrate, followed by rapid decapitation, and their brains were snap-frozen in prechilled isopentane and stored at −80 °C until cryosectioning (Mitev et al. 2003; Chang et al. 2012). Consecutive coronal sections (25 μm) were cut from each brain on a freezing microtome (CM1850, Leica, Mannheim, Germany). For the selected sections and delineation of the contours, rostral and/or caudal borders of the hippocampus, the rat brain atlas (Paxinos & Watson, 2005) and the mouse brain atlas (Paxinos & Franklin, 2001) were used as references.

### Immunofluorescence

Sections were fixed in ice-cold 4% paraformaldehyde (10 min), rinsed in phosphate-buffered saline (PBS), and permeabilized in PBS containing 0.3% Triton X-100 [30 min, room temperature (RT)]. Sections were blocked (3% horse serum in PBS, 30 min, RT) and incubated overnight (4 °C) with an SAP102 primary antibody (1 : 800, polyclonal rabbit antibody, cat. no.124213, Synaptic Systems, Göttingen, Germany). After thorough washing, the sections were

incubated with Alexa Fluor 594- or 488-conjugated goat anti-rabbit IgG (1 : 500; Molecular Probes, Eugene, OR, USA) for 2 h (RT). All antibodies were diluted in PBS containing 0.3% Triton X-100 and 0.3% horse serum. Control sections in which primary or secondary antibodies were omitted showed no labelled cells. Sections from all time points were simultaneously stained to ensure uniform conditions for subsequent quantitative analyses.

### Hoechst staining

Cellular nuclear chromatin was examined by bisbenzimide (Hoechst 33342, Sigma, St. Louis, MO, USA) staining. Following immunofluorescence staining, the sections were rinsed in PBS and incubated with Hoechst 33342 (1 : 1000 in PBS) for 15 min in the dark. After thorough washing, the sections were mounted in antifading medium.

### Quantitative analysis

To define the hippocampal boundaries in this study, we used atlases provided by Paxinos & Watson (2005) and Paxinos & Franklin (2001) as references. Digital images were captured with a Leica fluorescence microscope (DM5000 B; Leica) using Leica APPLICATION SUITE (version 2.20) software with excitation and emission wavelengths of 590 and 617 nm (Alexa Fluor 594), 470 nm and 525 nm (Alexa Fluor 488), and 340 and 425 nm (Hoechst 33342). The expression levels of SAP102 in the hippocampal subregions are represented as 'immunofluorescence intensity'. Immunofluorescence intensities were calculated using PHOTOSHOP CS3 software as mean optical density (OD) values, which were measured within the following hippocampal CA1, CA3 and DG sublayers: S. moleculare/molecular layer (Mol), granular cell layer (Gr) and S. polymorphum/polymorphic layer (Poly) of DG; S. lucidum (Luc), S. pyramidale/pyramidal cell layer (Py), and Poly of CA3; and Py, Poly and Mol of CA1 (Fig. 1).

### Statistical analysis

Nonparametric Kruskal–Wallis *H*-tests or one-way analysis of variance (ANOVA) were used to evaluate age-dependent differences in all groups, followed by the Mann–Whitney *U*- test to determine significant differences between two specific groups. Paired *t*-tests were applied to evaluate the differences between WT and APP/PS1 mice. $P < 0.05$ was considered statistically significant. All data are expressed as the mean ± SEM and were analysed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

## Results

### SAP102 is highly expressed in neonates, adult rat hippocampi and CA1 and DG subregions of aged rat hippocampi

SAP102 is well known to be the main scaffold protein in neonatal synapses and is critical during synaptogenesis and development (Sans et al. 2000; Van et al. 2004; Elias et al. 2006; Murata & Constantine-Paton, 2013). Partially consistent with previous reports, our results showed that SAP102 was unevenly distributed throughout the hippocampus. Under observation with a high-powered microscope, SAP102 was intensely expressed in the cytoplasms throughout the process areas but not in the nuclei (Figs 1–4). The fluorescence intensities of SAP102 in the hippocampal subfields CA1, CA3 and DG increased gradually with age from P0 to P56; in adulthood, SAP102 maintained relatively higher levels from P56 to P8 m. In aged rats (P20 m), SAP102 unexpectedly displayed no significant decrease in the CA1 and DG regions ($P > 0.05$) but did decrease in the CA3 subregion ($P < 0.05$, P20 m vs. P8 m), which contrasted with the results of previous studies (Sans et al. 2000; Matsunaga et al. 2016).

### SAP102 maintained higher levels in the rat hippocampal DG and CA1 molecular layers

The pyramidal cell layer, the main cellular layer in the CA region, is tightly packed in CA1 and more loosely packed in CA3. The pyramidal cell basal dendrites extend into the Poly and apical dendrites primarily are located in the Mol. In addition, the CA3 molecular layer contains a narrow acellular zone with distinct physiological properties, called the Luc, where the axons from granule cell (mossy fibres) synapse with the dendrites of CA3 pyramidal cells. In three CA1 sublayers (Fig. 2), weak SAP102 fluorescence intensity was detectable from P0, and a sharp increase was observed from P4 to P7. From P10, the SAP102 fluorescence intensity continually increased, peaking at approximately P8 m and then decreasing slightly to P20 m ($P > 0.05$). Generally, SAP102 expression in the Mol of CA1 was significantly higher than that in the Poly and Py layers ($P < 0.05$, Mol vs. Poly or Py in P10, P28, P56 and P6 m). In the three CA3 layers (Fig. 3), partially similar to the trend in CA1, the SAP102 fluorescence intensity increased slightly before P8 m but declined significantly from P8 m to P20 m ($P < 0.05$, in Py, Poly and Luc), which was unlike the changes observed in the CA1 and DG regions. There was no significant difference in SAP102 expression between Luc, Poly or Py in the CA3 region ($P > 0.05$).

Granule cells are the principal cell type making up the DG, and their apical dendrites primarily occupy the molecular layer, wherein axons from the entorhinal cortex and mossy cells terminate mainly on the dendritic spines of granule cells. In the DG, the SAP102 expression trend varied with age very similarly to that in the CA1 region (Fig. 4). Remarkably, an obviously increased immunofluorescence intensity was observed in the Mol of the DG from P4 to P7 ($P < 0.05$). Moreover, the fluorescence intensity of SAP102 in Mol continually increased and always maintained higher levels than those in Gr and Poly ($P < 0.05$, Mol vs. Gr or Poly in P7, P10, P14, P28, P6 m and P20 m; Fig. 4). Simultaneously, the fluorescence intensity of SAP102 in Gr was higher than that in Poly from P28 to P20 m, which was not observed in the CA1 and CA3 pyramidal layers. This alteration is potentially due to the persistent neurogenesis ability of granule cells throughout adulthood.

Dendritic spines are widely recognized as the predominant sites of excitatory synapses in the hippocampus. As an

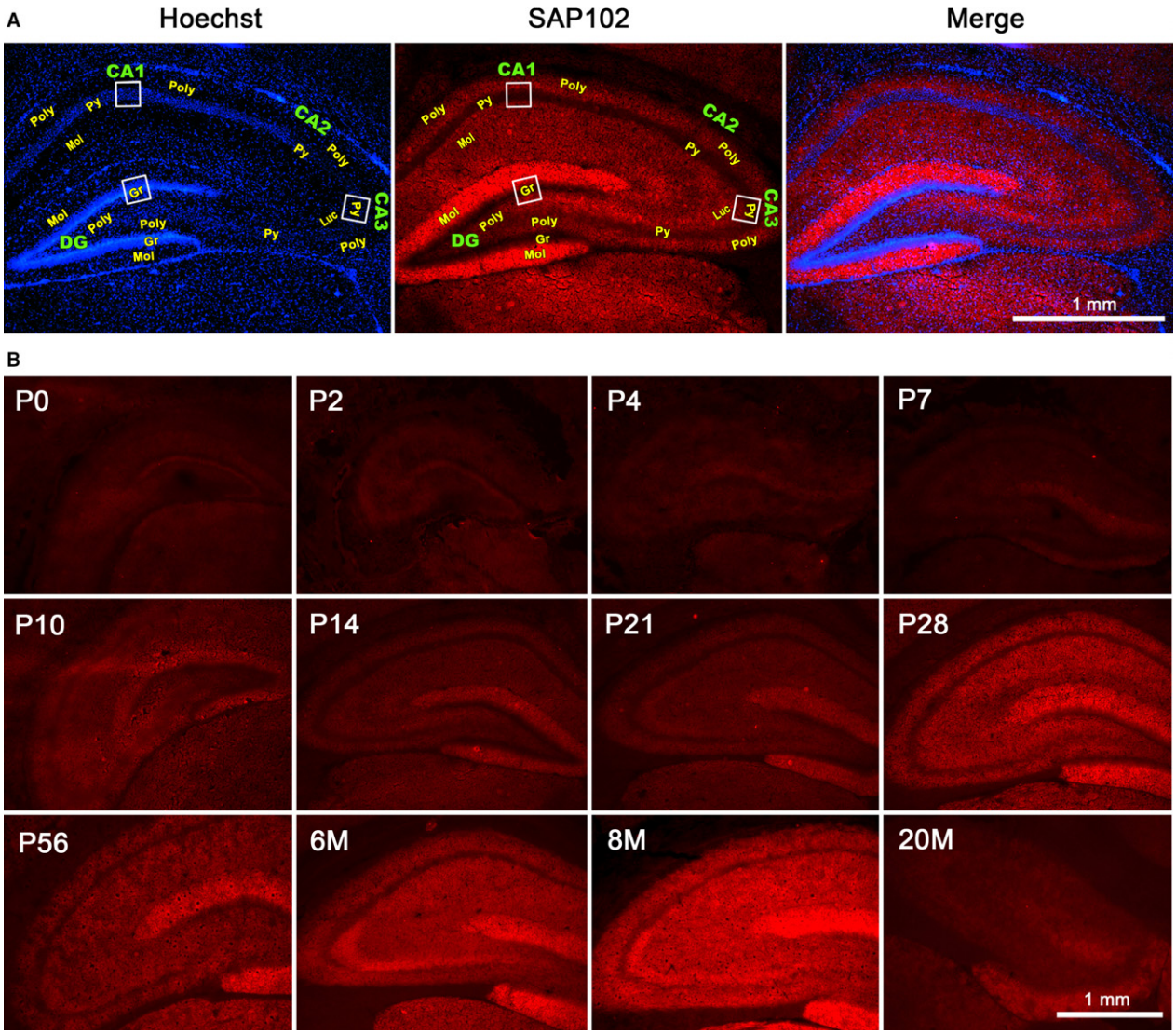


**Fig. 1** Micrographs of rat hippocampal subregions and SAP102 expression in the hippocampi of rats at various ages obtained by immunofluorescence staining. (A) Micrographs showing rat hippocampal subregions stained with immunofluorescence (SAP102 is shown in red and Hoechst 33342 in blue). The rodent hippocampus includes the Cornu Ammonis (CA) 1, CA2 and CA3 regions as well as the dentate gyrus (DG). The CA1 and CA3 regions consist of the stratum (S.) polymorphum (Poly), S. pyramidale (Py) and S. moleculare (Mol). The S. lucidum (Luc) is part of the S. moleculare in CA3. The DG consists of the S. polymorphum (Poly), granular cell layer (Gr) and S. moleculare (Mol). The white rectangles indicate the regions outlined in Figs 2–5 shown at higher magnifications. (B) Photomicrographs showing SAP102 expression in rat hippocampi at various ages as detected by immunofluorescence staining.

essential component of the PSD at excitatory synapses, SAP102 was highly expressed in Mol of the DG and CA1 regions, further supporting spines as the main loci wherein SAP102 functions.

## Abnormal SAP102 expression in the hippocampal subregions of APP/PS1 mice

In the past decade, because of their early onset of pathological alterations and stable genetic background, APP/PS1 mice have become a very popular AD mouse model (Radde et al. 2006; Bilkei-Gorzo, 2014). The APPswe/PS1dE9 double-transgenic mice used in this study develop amyloid plaques at 4 months, and the number of plaques increases with age (Garcia-Alloza et al. 2006; Ruan et al. 2009; Malm et al. 2011; Edwards et al. 2014; Jin et al. 2015; Kelly et al. 2017). Therefore, to investigate and compare dynamic changes in SAP102 expression in the hippocampal subregions, we chose APP/PS1 mice at the time points of 2 months (no plaques; Savonenko et al. 2005; Ruan et al. 2009; Kamphuis et al. 2012; Zhang et al. 2012; Végh et al. 2014), 5 months (sparse plaques; Garcia-Alloza et al. 2006;

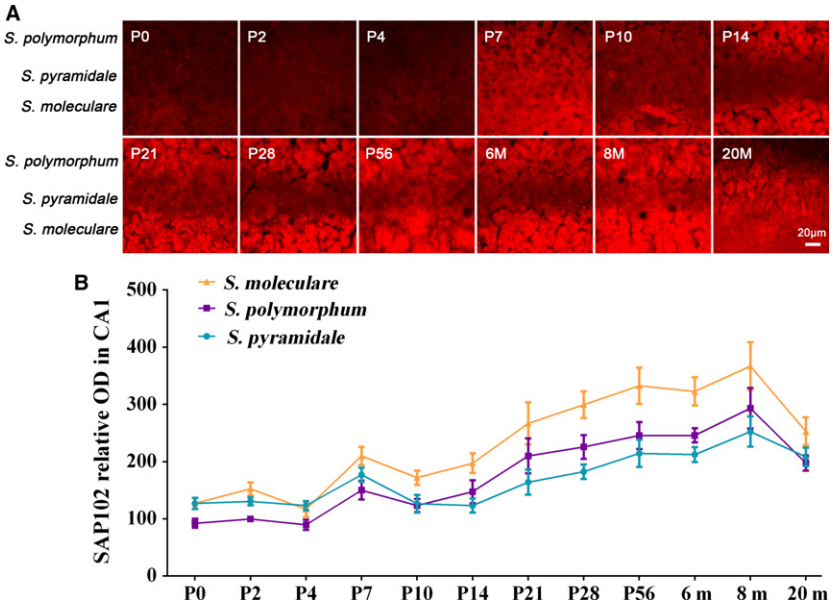


**Fig. 2** SAP102 expression in the CA1 sublayers in rats of different ages. (A) Higher magnification images of SAP102 expression in the CA1 sublayers during postnatal development. (B) Line diagram illustrating SAP102 expression (relative OD) in the hippocampal CA1 sublayers during postnatal development (relative OD: % of OD in the granular layer of the DG at P0).Values are expressed as mean ± SEM ($n$ = 6 animals per age).

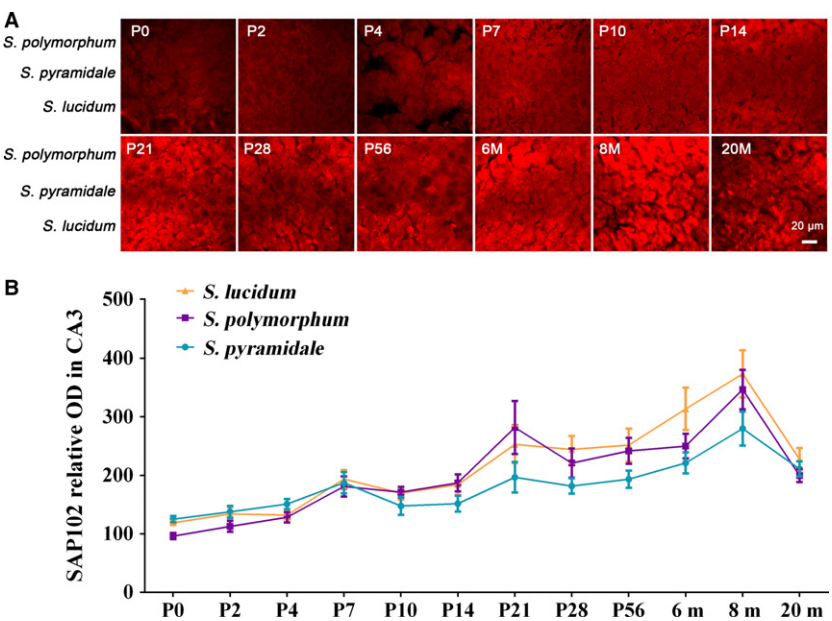


**Fig. 3** SAP102 expression in the CA3 sublayers in rats of different ages. (A) Higher magnification images of SAP102 expression in the CA3 sublayers during postnatal development. (B) Line diagram illustrating SAP102 expression in the hippocampal CA3 sublayers during postnatal development (relative OD: % of OD in the granular layer of the DG at P0). Values are expressed as mean ± SEM ($n$ = 6 animals per age).

Chen et al. 2009; Kelly et al. 2017) and 7 months (marked number of plaques; Savonenko et al. 2005; Garcia-Alloza et al. 2006; Jin et al. 2015), respectively mimicking the preclinical, early and early to middle stages of AD (Jiang et al. 2017).

We first observed SAP102 expression in WT mice, and the trends were generally the same as those in age-matched rats; the SAP102 fluorescence intensities in the hippocampal subfields of WT mice increased slightly from 2 to 7 months of age (Fig. 5). However, in the APP/PS1 mouse model, the observed expression trend of SAP102 was quite different. While the SAP102 fluorescence intensities in most sublayers of the hippocampi of 2-month-old APP/PS1 mice were not significantly different from those of WT mice (Fig. 5), they were obviously higher than those in the CA1 Mol and CA3 Poly regions of WT mice ($P < 0.05$). Interestingly, this difference disappeared at 5 months of age ($P > 0.05$). Strikingly, in 7-month-old APP/PS1 mice, the SAP102 fluorescence intensity became significantly lower than that in WT mice in all three layers of the DG and CA3 ($P < 0.05$) and in the pyramidal layer of CA1 ($P < 0.05$; Fig. 5). These results suggested that CA1 apical dendrites and CA3 basal dendrites are early responsive areas during the prodromal stage in APP/PS1 mice, and the DG and CA3 subfields may be more vulnerable and prone to both impairment and disease progression.

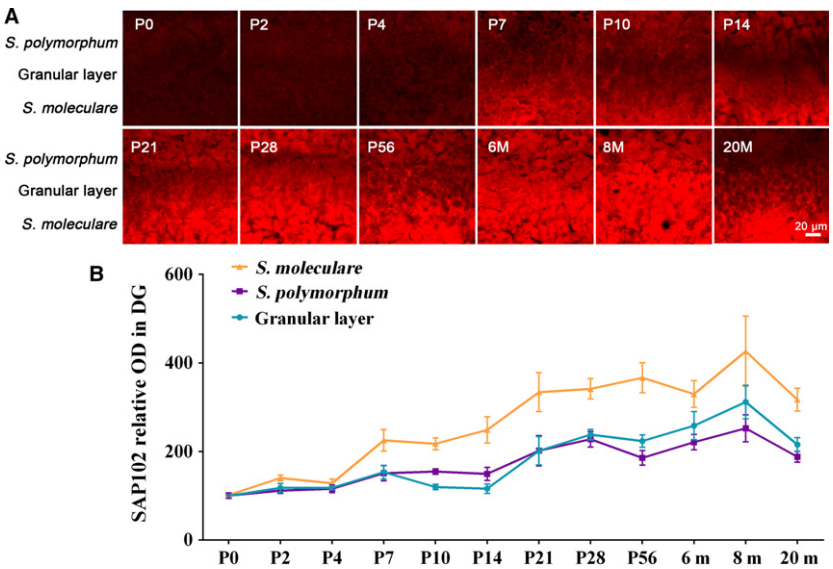


**Fig. 4** SAP102 expression in the DG sublayers in rats of different ages. (A) Higher magnification images of SAP102 expression in the DG sublayers during postnatal development. (B) Line diagram illustrating SAP102 expression in the hippocampal DG sublayers during postnatal development (relative OD: % of OD in the granular layer of the DG at P0). Values are expressed as mean ± SEM ($n$ = 6 animals per age).

## Discussion

SAP102 is a significant multidomain protein in the PSD (Zheng et al. 2011). Similar to its family protein PSD-95, SAP102 plays important roles in synaptogenesis, trafficking and anchoring certain receptors (Elias et al. 2008), and organizing various signalling transduction cascades (Chen et al. 2011; Murata & Constantine-Paton, 2013). SAP102 is expressed in the cytoplasm, dendrites, axonal growth cones and postsynaptic sites (Zheng et al. 2010). As it is involved in synaptic plasticity and contributes to cognition development, changes in SAP102 expression are related to variable neurological diseases, such as mental retardation (Crocker-Buque et al. 2016) and AD (Proctor et al. 2010).

During postnatal brain development, individual MAGUK proteins show distinct expression profiles, and SAP102 is widely recognized as being abundantly expressed during the early stages of postnatal life. However, there are many discrepancies in its expression patterns during adulthood and ageing stages. Our data herein showed that SAP102 was highly expressed in the rat hippocampus from P56 to P8 m and displayed no significant decreases in the CA1 and DG regions at P20 m. Numerous reports have demonstrated that SAP102 is expressed in the hippocampus at young ages and is then mostly replaced by PSD95 in later stages of life (Sans et al. 2000; Elias et al. 2006; Murata & Constantine-Paton, 2013). Matsunaga reported that the SAP102 level in the Wistar rat hippocampus increased from P35 to P5 m (Matsunaga et al. 2016), which is unlike the reduced level of SAP102 expression from P35 to P6 m found by Sans et al. (2000). In humans, researchers examined SAP102 expression in the brain tissues of humans aged 57–87 years, finding that SAP102 maintained relatively higher expression levels in the inferior temporal lobe, occipital lobe and hippocampus (Proctor et al. 2010). Possible explanations for these discrepancies in the literature include sampling variability, use of different morphometric methods, and regional and species differences. Our results herein suggested that SAP102 plays diverse roles in rat adulthood and ageing stages that need to be further investigated.

In addition, we surprisingly found that the SAP102 levels in P20 m rats significantly declined in the CA3 region but not in the CA1 and DG regions. This finding can be explained by some evidence showing that, unlike CA1, the hippocampal CA3 area shows obvious age-related dysfunction during ageing (Stephens et al. 2011). Place cells in CA3, but not in CA1, fail to remap their place fields and display 'cognitive rigidity' in aged rats (Wilson et al. 2003, 2005). Similar findings were observed in healthy aged human subjects, who showed impairments in pattern separation and increased MRI BOLD activity in the CA3 region (Yassa et al. 2011). Previous studies have long-focused on the CA1 subregion, whereas the CA3 region has received relatively less attention despite its selective contribution to age-related memory impairment (Simkin et al. 2015); our data further support the vulnerability of CA3 to ageing.

Because SAP102 plays so many important roles in physiological conditions throughout life, changes in its levels and function are related to many neurological disorders, such as AD. According to Proctor's study, SAP102 levels were markedly decreased in the inferior temporal cortex of human AD autopsy brain tissues, especially in patients who carried a copy of the APOEε4 allele (Proctor et al. 2010), but no significant difference was found in the hippocampus, suggesting that SAP102 plays specific roles in different brain subregions during AD progression. In this study, we analysed APP/PS1 mice aged 2, 5 and 7 months, imitating the early stages of AD, to observe dynamic changes in SAP102 expression. We found abnormally increased SAP102 expression in the CA1 S. moleculare and CA3 S. polymorphum

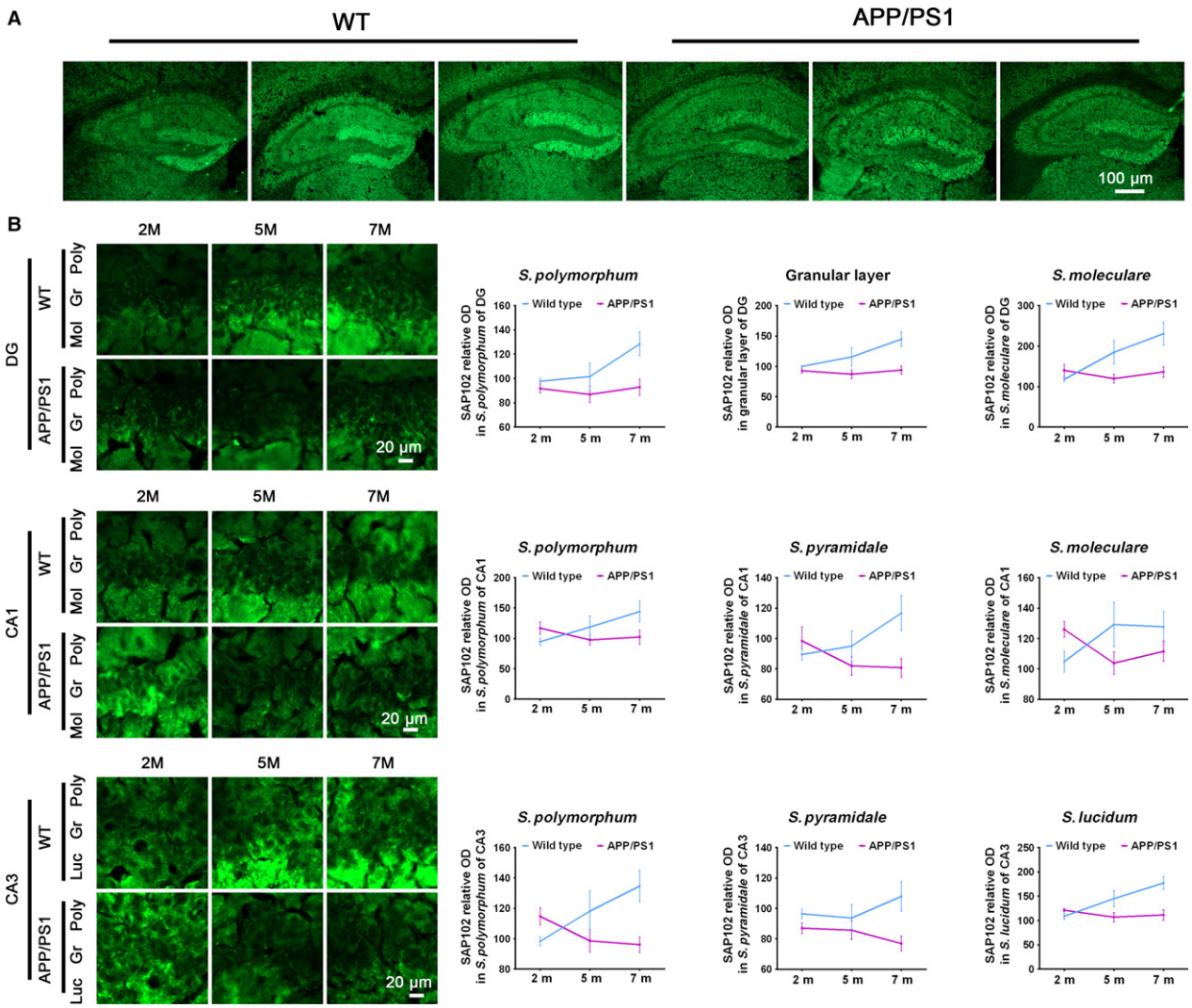


**Fig. 5** SAP102 expression in WT and APP/PS1 mice aged 2, 5 and 7 months. (A) Photomicrographs showing the different expression levels of SAP102 in the hippocampi of wild-type (WT) and APP/PS1 mice aged 2, 5 and 7 months as detected by immunofluorescence staining. (B) Higher magnification images and corresponding line diagrams illustrating SAP102 expression in WT and APP/PS1 mice hippocampal DG, CA1 and CA3 sublayers at 2, 5 and 7 months (SAP102 relative OD: % of OD in the granular layer of the DG in 2-month-old WT mice). Values are expressed as mean ± SEM ($n$ = 3 animals per age).

subregions of 2-month-old APP/PS1 mice. However, this difference disappeared at 5 months of age, and SAP102 expression in 7-month-old APP/PS1 mice was lower than that in WT mice, especially in the DG and CA3 subregions. This alteration trend was consistent with a report on the human hippocampal proteome at different AD pathological stages, which exhibited an early-up, late-down SAP102 expression pattern (Hondius et al. 2016). We speculated that the increased SAP102 expression in the CA1 S. moleculare and CA3 S. polymorphum subregions of 2-month-old APP/PS1 mice implied that some early synaptic impairments are caused by increasing amounts of toxic soluble Aβ oligomers (Végh et al. 2014). In fact, the increased SAP102 expression more likely reflects a reduction in afferent innervation from the entorhinal cortex (Ahmed et al. 2015; Lv et al. 2015; Mufson et al. 2015). Simultaneously, this increase could be a compensatory response in the prodromal stage of the disease, which could help maintain normal synaptic function. We believe that 5 months of age is a balance stage for SAP102 expression. PSD-93 is a primary member of the MAGUK family and is abundantly expressed in the PSD. Yu et al. (2017) recently found that PSD-93 expression was significantly decreased in the hippocampi of 6-month-old APP/PS1 mice, but no alterations in PSD-95, SAP97 or SAP102 expression were observed, indicating that PSD-93 might serve as an important biomarker to assess early synaptic injuries induced by Aβ. Given that the APP/PS1 mouse strain used herein has shown sparse plaque

deposition and some cognitive deficits at 5 months of age (Garcia-Alloza et al. 2006; Pistell et al. 2008; Kelly et al. 2017), concomitant abnormalities in some other synaptic proteins, such as PSD-93, are possible and need to be elucidated in future investigation. At 7 months of age, we showed that SAP102 expression levels were lower than those in WT mice, which possibly indicated a state of decompensation. In this stage, robust plaques have been observed in the hippocampi of APP/PS1 mice and spatial learning deficits are apparent (Savonenko et al. 2005; Garcia-Alloza et al. 2006; Reiserer et al. 2007), and more severe synaptic dysfunctions are possible. As a well-studied member of the MAGUK family, PSD-95 is the major scaffold protein of dendritic spines, which is involved in ageing, AD and numerous psychiatric disorders (Savioz et al. 2014). Several reports have demonstrated that hippocampal PSD-95 levels are significantly decreased in 9-month-old APP/PS1 mice (Inestrosa et al. 2013; He et al. 2016; Liu et al. 2017). Another study showed that PSD-95 expression was significantly increased in the frontal cortex of aged cognitively impaired rats and unaltered in the hippocampus (Preissmann et al. 2012). In contrast, Nyffeler et al. (2007) indicated that PSD-95 levels were selectively increased in the hippocampi of aged learning-impaired rats. Proctor et al. (2010) found PSD-95 and SAP-102 protein levels to be downregulated in autopsied AD inferior temporal cortex tissue but not in hippocampal tissue, whereas another study on AD patients observed significantly increased PSD-95 expression in the hippocampus and entorhinal cortex (Leuba et al. 2008). These conflicting results imply that alterations in MAGUK proteins vary with species, brain subregion, progressive disease stage and age, and the precise changes and related mechanisms need to be further elucidated.

Our findings herein indicated that in APP/PS1 mice, the CA1 S. moleculare and CA3 S. polymorphum regions are more responsive during the preclinical stage of disease, and the DG and CA3 regions become more vulnerable as the disease progresses.

In summary, we herein described the dynamic expression profiles of SAP102 in the hippocampi of differently aged rats and APP/PS1 mice. SAP102 was not only highly expressed in early postnatal stages but also maintained relatively higher expression levels in the adult hippocampus and displayed no significant decreases in the CA1 and DG subregions of aged rats, suggesting that it has extensive functions in different life stages. In APP/PS1 mice, alterations in SAP102 expression in the CA1 S. moleculare and CA3 S. polymorphum regions served as early responsive markers in the prodromal disease stage, while those in the DG and CA3 were more vulnerable as the disease progressed. The diverse functions of SAP102 and its related mechanisms are worthy of further exploration.

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## Author contributions

Dongning Su carried out the immunofluorescence study and contributed to the measurements, data analysis and the writing of the manuscript. Hui Liu, Tianrong Liu, Zhang Xin, Wei Yang and Yizhi Song contributed to the measurements and data analysis. Lirong Chang participated in the design of the study and the writing of the manuscript. Jinping Liu and Yan Wu revised the paper.

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