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Gliogenesis but not neurogenesis occurs during the acute phase of vestibular compensation after unilateral vestibular neurectomy

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ABSTRACT

Following unilateral loss of vestibular input, recovery of motor symptoms is achieved within 2 weeks in rodents. Given that neurogenesis was only reported at 1 month post-lesion, whether there is neurogenesis in this early phase of vestibular compensation remains to be investigated. If not, what then is the major cell type that participates in this timeframe? We show abundant nestin-positive cells in the ipsilesional but not contralesional vestibular nucleus (VN) of rats after ablating cell bodies of vestibular nerve at the Scarpa's ganglion, as confirmed by both magnetic resonance imaging after surgery and histology. Bromodeoxyuridine (BrdU) uptake indicated that these cells actively proliferated. A high proportion of the cells were double-positive for nestin and GFAP as early as 4 days, and up to 2 weeks post-lesion, in contrast to none in control preparations. In contrast, the number of NeuN-positive neural lineage cells in the VN remained constant in both the control and lesioned rats. Furthermore, NeuN-positive cells were not positive for BrdU. However, a small number of proliferating cells stained positive for the immature neuron progenitor marker doublecortin. Taken together, we show that unilateral loss of vestibular input stimulates proliferation of neuroglial progenitors, and provide evidence that argues against occurrence of neurogenesis within the 2 week period in which recovery of postural and motor symptoms occurs.

1. Introduction

Imbalance in bilateral afferent inputs to the vestibular nucleus (VN) causes oculomotor and postural disequilibrium, as well as deranged egocentric cues for spatial navigation (Angelaki and Cullen, 2008; Basaldella et al., 2015; Bjerknes et al., 2015; Lai et al., 2016; Li et al., 2013). Vestibular suppressants offer temporary relieve of symptoms resultant from vestibular damage (Kolev and Sergeeva, 2016). However, resolution of loss in unilateral vestibular input in adults ultimately relies on adaptation by reinstating neural plasticity in VN circuits (Dutia, 2010) which are otherwise not plastic after the first postnatal week (Jiang et al., 2024; Lai et al., 2023). In rodents suffering from unilateral vestibular damage, partial compensation of static symptoms is achieved within 48 hours (Chen et al., 2019; El Mahmoudi et al., 2022) while recovery of dynamic symptoms requires one week (El Mahmoudi, et al., 2022).

Both changes in neuronal activity of VN neurons (Chan et al., 1999; Goto et al., 2000; Smith and Curthoys, 1988a, 1988b) and proliferation of neural precursors in the VN, occurring as early as 3 days post-injury (Dutheil et al., 2009; Dutheil et al., 2013), contribute to remodelling of central vestibular circuits for recovery of vestibular function (Chan et al., 1999; Dutia, 2010). While it was established that proliferation of VN cells within the first 3 weeks after unilateral vestibular neurectomy in the adult cat was required for motor recovery (Dutheil et al., 2009), the cell types involved were unclear. Notably, neurogenesis and astrogliogenesis were observed 1 month after unilateral vestibular

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neurectomy in cats (Tighilet et al., 2007) and rats (Rastoldo et al., 2021), but the specific cell types that proliferated prior to this remain unclear.

The expression of nestin protein, an embryonic intermediate filament, reflects the differentiation or proliferative state of neural precursors in the developing central nervous system of mammals (Kawaguchi et al., 2001; Mignone et al., 2004; Park et al., 2010). Adult stage expression of nestin is restricted to proliferative regions such as dentate gyrus of the hippocampus and subventricular zone of the lateral ventricle (Ming and Song, 2011). Re-expression of nestin in otherwise non-proliferating brain regions of the adult brain was reported to occur following cerebral lesions (Brook et al., 1999; Chen et al., 2002; Duggal et al., 1997; Sahin Kaya et al., 1999). However, it is unknown whether loss of vestibular inputs also stimulates upregulation of nestin and proliferation of endogenous neural precursors within the rat VN.

In the present study, we asked if unilateral loss of vestibular input causes proliferation of nestin-positive neural precursors in the rat VN within the acute phase at 2 weeks post-lesion. Since neurogenesis is observed in the later stage of vestibular compensation (Dutheil et al., 2016; Tighilet et al., 2007), we further used the mature neuron marker NeuN to assess if neurogenesis was already present within the acute phase. Given that recovery of static symptoms and motor coordination is known to occur within the first 2 post-leisonal weeks in rats (Chen et al. 2019; El Mahmoudi et al., 2022), absence of neurogenesis would suggest that gliogenesis is sufficient to provide the necessary support for neural plasticity (Allen, 2014; Araque and Navarrete, 2010; Buffo et al., 2010; Dutheil et al., 2013) required for remodelling of VN circuits after unilateral loss of vestibular input.

2. Methods and materials

2.1. Animal models and tissue preparation

Adult Sprague-Dawley rats (200–230 g) supplied by the Centre of Comparative Medicine Research (CCMR, The University of Hong Kong) were used in the present study. All procedures performed in studies involving animals were conducted in compliance with the NIH guidelines for Animal Welfare and were approved by The University of Hong Kong Committee on the Use of Live Animals in Teaching and Research. Efforts were made to minimize both the suffering and number of animals used.

Peripheral vestibular input to the central nervous system was perturbed by unilateral ablation of the Scarpa's ganglion (n = 47 rats). The animals were anesthetized with a ketamine-xylazine mix (80 mg/kg ketamine and 8 mg/kg xylazine, i.p., Alfasan, Netherlands). Part of the mastoid bone below the paraflocculus was removed without damage of the cerebellum to expose the Scarpa's ganglion for surgical ablation. Successful lesion was confirmed by obvious motor symptoms, e.g. severe ataxia, head tremor and repeated turning and by magnetic resonance imaging 2 days post-lesion (7 T PharmaScan, Bruker, Germany) in ketamine-xylazine anesthetized rats (Fig. 1c) and/or histology postmortem (Fig. 1b). After operation, animals were returned to individual cages and allowed to survive for 2 days (D2, n = 8 rats), 4 days (D4, n = 8 rats), 7 days (D7, n = 8 rats), and 14 days (D14, n = 8 rats). Sham-operated animals (n = 8 rats) were used as controls. Analgesic (buprenorphine, 0.05 mg/kg body weight, twice a day Schering-Plough, UK) was given twice daily for 3 days post-surgery. Anti-inflammatory drug (Meloxicam, 10 μg/ml in drinking water. Boehringer Ingelheim,

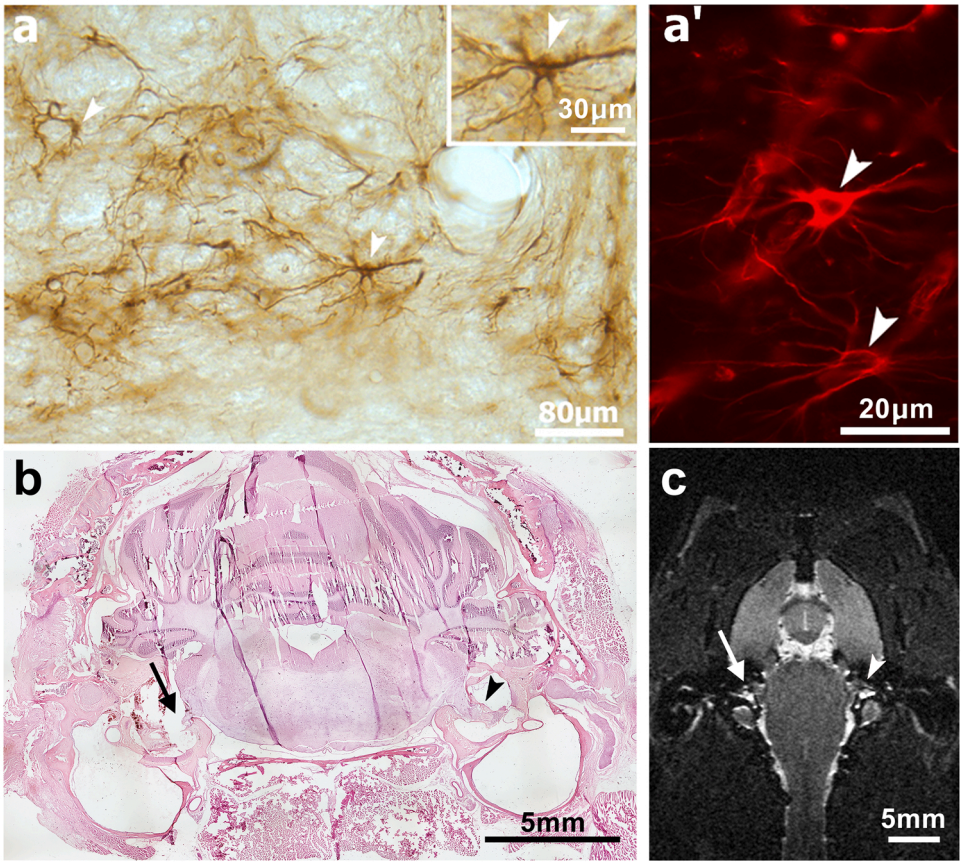

Fig. 1. Nestin-immunoreactive cells in the rat VN 14 days after lesion of the Scarpa's ganglia. a: Nestin-immunoreactive cells with astroglial morphology of multiple and radial processes were detectable using colorimetric reaction using DAB (a) or fluorophore conjugated secondary antibodies (a'). b and c: Unilateral ablation of Scarpa's ganglion (arrow) revealed by postmortem section stained with H&E (b) and magnetic resonance imaging in anesthetised rat at 2 days post-lesion (c). Intact vestibular nerve is denoted by arrowhead.

Germany) was given for 7 days post-surgery.

Administration of bromodeoxyuridine (BrdU) or 5-ethynyl-2′-deoxyuridine (EdU) was then performed to demonstrate nuclear DNA synthesis or cell proliferation state (Kee et al., 2002). BrdU (Sigma, USA) or EdU (Abcam, USA) was dissolved in saline (pH adjusted to 7.4 with NaOH) and injected into each animal (100 mg/kg, i.p. once) at 24 h before they were sacrificed. Immunohistochemistry using anti-BrdU antibody (Sigma, USA) or click reaction between EdU and TRITC-azide (Abcam, USA) was finally utilized to visualize nuclear incorporation of BrdU/EdU respectively.

At the required timepoints, animals were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p., Alfasan, Netherlands) and then transcardially perfused with 4 % paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brainstems were removed immediately and placed overnight in 0.1 M PB containing 30 % sucrose at 4 °C. After that, the brainstems containing VN were then serially cut into coronal sections (30 μm) with a frozen microtome and rinsed in 0.01 M phosphate buffered saline (PBS, pH 7.4).

2.2. Immunohistochemistry and double immunofluorescence

The brainstem sections were processed for nestin with an avidin-biotin peroxidase complex (ABC) method. Briefly, the sections were incubated with monoclonal mouse anti-nestin (1:200 dilution, Rat-401, Developmental Studies Hybridoma Bank of The University of Iowa, USA) in 0.01 M PBS (pH 7.4) containing 1 % bovine serum albumin, 3 % normal donkey serum and 0.1 % Triton X-100 for 48 h at 4°C. This mouse anti-nestin serum was generated against nestin protein and characterized by Western blotting. Specificity of nestin antibody had been confirmed in our previous studies (Chen et al., 2002, 2004, 2006; Shi et al., 2002). Subsequently, the sections were rinsed in 0.01 M PBS and then incubated for 4 h at room temperature with goat anti-mouse IgG-biotinylated (Vector; 1:200 dilution) solution, followed by incubation with ABC complex (Vector; 1:200 dilution). Finally, the sections were reacted with DAB/H_2O_2 (0.05 %/0.005 %) solution in Tris-HCI buffer (pH, 7.6) for about 20 min at room temperature. After being washed, the sections were mounted on gelatin-coated glass slides, air-dried, dehydrated, cleared, and coverslipped with DPX, and then examined under an Axiophot 2 microscope (Zeiss, Germany).

Double immunofluorescence was performed on serial sections to visualize the co-localization of either nestin with GFAP, with BrdU or EdU, or with NeuN. The sections were incubated for 48 h at 4°C with a mixture of mouse anti-nestin (1:100 dilution) and rabbit anti-GFAP (DAKO, 1:500 dilution) IgG in 0.01 M PBS containing 1 % bovine serum albumin, 3 % normal donkey serum and 0.1 % Triton X-100. Subsequently, the sections were rinsed in 0.01 MPBS, and then incubated for 24 h at 4°C with a mixture of dichorotriazinylamino-fluorescein (DTAF)-conjugated donkey anti-mouse IgG and tetramethyl rhodamine isothiocyanate (TRITC)-conjugated donkey anti-rabbit IgG (Chemicon, USA; 1:100 dilution). For sequential visualization of nestin and BrdU, the sections were first incubated with mouse anti-nestin (1:100) and DTAF-conjugated donkey anti-mouse IgG. After being washed, the sections were then incubated with mouse anti-BrdU biotin conjugated monoclonal antibody (Sigma, USA, 1:100) and avidin-TRITC. Similar procedures were performed for double immunofluorescence of nestin and NeuN, with sequential incubation of mouse anti-nestin (1:100) and rabbit anti-NeuN (Chemicon, USA, 1:1000). After being washed, the sections were then incubated with mouse anti-BrdU biotin conjugated monoclonal antibody (Sigma, USA, 1:100), followed by avidin-FITC and goat anti-rabbit IgG-Alexa 594 (Invitrogen, USA). The sections were mounted on gelatin-coated glass slides, and coverslipped in 0.01 M PBS containing 50 % glycerine and 2.5 % triethylenediamine, prior to imaging (Axiophot 2, Zeiss, Germany).

For control experiments, the primary antibody was substituted with normal mouse serum (for nestin, BrdU and NeuN immunocytochemistry) or normal rabbit serum (for GFAP immunocytochemistry). The sections were then processed with the same procedures as described above. Negative controls for non-specific binding of secondary antibody were produced by incubating sections in normal mouse or rabbit serum in place of the primary antibodies. In these preparations, nestin-immunoreactive (nestin-ir) and GFAP-ir cells were not detected. In addition, the fluorescence staining intensity in the control was used as a reference to establish the threshold for identifying immunoreactive cells.

For semi-quantification, nestin single-, GFAP single-, BrdU single-, nestin/GFAP double-, and nestin/BrdU double-labelled cells were counted on 10 representative fields (20 ×) within the VN (mean ± S.E.M., n = 8 rats). VN subnuclei in 4 levels of the medulla were assessed: medial and superior VN at 10.2 mm caudal to Bregma; lateral, medial, spinal and superior VN at 10.6 mm caudal to Bregma; medial and spinal VN at 11.6 and 12.0 mm caudal to Bregma. The diameters of immunoreactive glial cell somas were measured by their short and long diameters with a morphmetric micrometer and the average value was presented as mean ± S.E.M. The nomenclature and demarcation of brain structures were adapted from the stereotaxic rat brain atlas (Paxinos and Watson, 2013). Intensity of immunoreactivity was identified using published methods (Chen et al., 2003) and ranked (on a scale of –, +, ++, and +++, for negative, weak, average, and intense immunoreactivity, respectively) independently by 3 researchers who were blinded to the experimental grouping. The total number of nestin-positive cell bodies in each VN sub-nucleus was recorded and the average number was reported in Table 2.

2.3. Statistical analysis

T-test was used to compare means between 2 groups. One-way ANOVA followed by post-hoc Tukey's test was used to compare the number of immunoreactive cells between control, 4 days post-lesion, and 14 days post-lesion groups. Statistical analysis was conducted with GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA). $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Distribution pattern of nestin-immunoreactivity in the VN

Unilateral ablation of the Scarpa's ganglion was used since this, but not unilateral labyrinthectomy, induced neurogenesis at 1 month post-lesion (Rastoldo et al., 2021). Ablation of Scarpa's ganglion was confirmed both in anesthetized rats by magnetic resonance imaging (Fig. 1c) and/or by histology at the respective time points (Fig. 1b). Such ablation led to abundant nestin-immunoreactivity in various VN subnuclei ipsilateral to the side of the lesioned Scarpa's ganglia as early as 2 days post-lesion (Fig. 1a, Fig. 2b-f), but was hardly detected in the contralateral VN (Fig. 2a). Nestin-immunoreactivity was predominately localized in somas and processes of astroglial-like cells (Fig. 1a').

In Scarpa's ganglia lesioned animals, nestin-ir cells were substantially distributed in the VN complex (including lateral, medial, spinal, and superior nuclei) and its subgroups (groups x and y) (Fig. 2). Nestin-ir soma in the VN were mostly oval or multipolar in shape and their mean diameter was 8 ± 6 μm (n = 30 cells). The majority of nestin-ir cells exhibited multiple and radial processes (Fig. 1a').

The temporal pattern of nestin re-expression was examined from 2 days post-lesion during recovery of static symptoms and at 14 days post-lesion when dynamic symptoms were also recovered (Chen et al., 2019; Curthoys, 2000). From semi-quantitative data shown in Table 1, peak expression of nestin occurred 4 –7 days post-lesion. By 14 days post-lesion, nestin-immunoreactivity was decreased, but was still higher than that of the control (Table 1). Quantitatively, the number of nestin-positive cells was significantly increased by 20-fold 4 days post-lesion (control 18 ± 15, 4 days post-lesion 357 ± 48, n = 8 rats per group, $p < 0.05$). This was decreased 12-fold by 2 weeks post-lesion, but

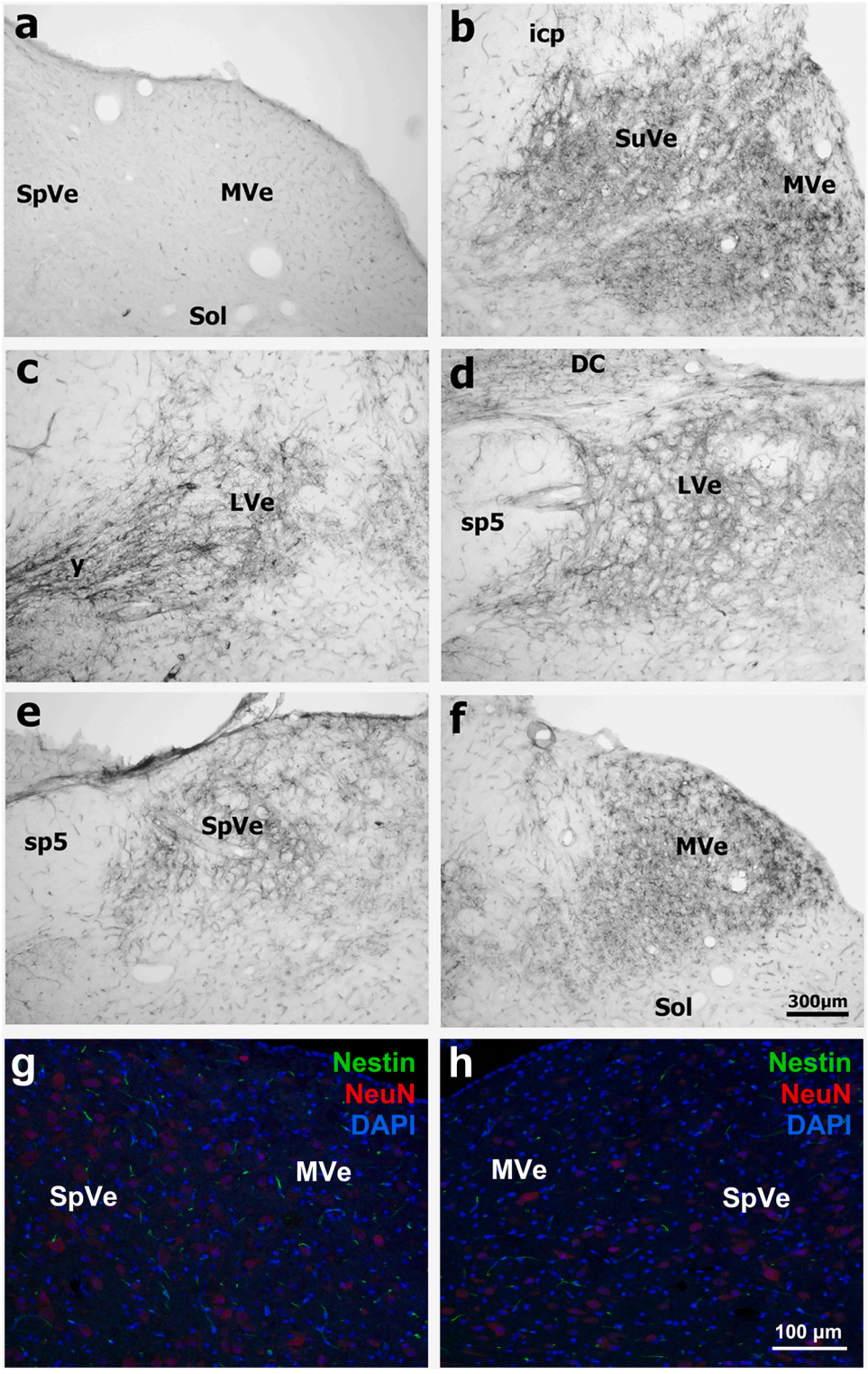

Fig. 2. Distribution of nestin-immunoreactivity in distinct regions of deafferentated VN. a: Nestin-immunoreactivity is hardly detected in the contralateral VN. b–f: Abundant nestin-immunoreactivity is found in VN complex and subgroups on the lesioned side of a representative animal at 14 days after surgical lesion of the Scarpa's ganglion. g-h: Double immunofluorescence staining of Nestin and NeuN in ipsilateral (g) and contralateral (h) VN 14 days after lesion. *Abbreviations:* DC, dorsal cochlear nucleus; icp, inferior cerebellar pedunule; LVe, lateral vestibular nucleus; MVe, medial vestibular nucleus; Sol, nucleus of solitary tract; sp5, spinal trigeminal tract; SpVe, spinal vestibular nucleus; SuVe, superior vestibular nucleus; y, group y subnucleus.

remained significantly increased compared to controls (control 18 ± 15, 14 days post-lesion 220 ± 26, n = 8 rats per group, $p < 0.05$).

3.2. *Nestin-positive VN cells express GFAP but not NeuN*

Cells exhibiting both nestin- and GFAP-immunoreactivities were observed in the VN on the lesion side. The majority of nestin/GFAP

Table 1
Comparison of nestin-immunoreactivity in ipsilesional VN subnuclei of adult rats after unilateral vestibular neurectomy.

	SuVe	LVe	SpVe	MVe	Group x	Group y
Control	–	–	–	–	–	–
Day 2	+	+	+	++	+	+
Day 4	+++	++	++	+++	++	++
Day 7	+++	++	++	+++	+	+
Day 14	++	+	+	++	+	+

Semi-quantitative evaluation of immunoreactivity in the VN of adult rats (n = 8). Density of nestin-immunoreactivity is ranked as high (+++), moderate (++), low (+) and negative (–). Control, sham operated animals; Day 2 –14, days after unilateral vestibular neurectomy. *Abbreviations:* LVe, lateral VN; MVe, medial BN; SpVe, spinal VN; SuVe, superior VN.

double-labelled cells exhibited multiple and extensive radial processes. Their somas were also oval or fusiform in shape, and their mean diameter was 9 ± 5 μm (n = 30 cells). Representative examples of double-labelled cells in the spinal VN are shown in Fig. 3.

GFAP-ir cells increased significantly by 88 % of the control at 4 days post-lesion to 132 % by 2 weeks post-lesion, indicating a steady increase in GFAP-positive cells during this period (Table 2). At 4 days post-lesion, 59 % of nestin-ir cells showed GFAP-immunoreactivity, and these nestin/GFAP double-labelled cells constituted 46 % of GFAP-ir cells in the ipsilesional VN of lesioned animals (Table 2). By14 days post-lesion, a significantly higher proportion of nestin-ir cells (85.3 %) were double-labelled with GFAP compared to 4 days post-lesion, but these double-labelled cells only constituted 32.7 % of GFAP-ir cells (Table 2).

We further stained for the mature neuron marker NeuN to discern if neurogenesis, which had been reported at 1 month post-lesion (Dutheil et al., 2016; Tighilet et al., 2007), was initiated in the first 2 weeks of vestibular compensation. It was found that the number of NeuN-positive neurons was unchanged during the first 2 weeks post-lesion (Table 2). Furthermore, nestin/NeuN double-labelled cells were not detected in the deafferented VN (Fig. 2g-h, Table 2). In other words, no genesis of mature neurons was present in the acute phase of vestibular compensation after unilateral vestibular neurectomy. However, sparse immunoreactivity of the migrating neuron progenitor marker doublecortin (DCX) could be observed by 14 days post-lesion (Fig. 4, right column), implying that lesion-induced neurogenesis was underway at this time point but neuronal progenitors had not yet matured into functional neurons.

3.3. *Nestin-expressing cells show nuclear incorporation of BrdU/EdU*

BrdU or EdU incorporation was used to investigate whether increase in GFAP-ir was due to upregulation of GFAP expression or proliferation of GFAP-positive cells. BrdU/EdU-ir was observed in both nestin-ir cells (Fig. 4, left column and Table 2) and GFAP-ir cells (Fig. 4, middle column and Table 2) in the deafferented VN. Such cells double-positive for proliferation marker EdU/BrdU and glial cell markers were absent both in the labyrinth-intact side and in controls. Cells expressing BrdU increased significantly at 4 days after lesion. A significant increase in the number of BrdU labelled cells by 13-fold was observed in the VN at 4 days post-lesion compared to controls (Table 2). Although these cells progressively declined in number, the number of BrdU labelled cells remained 5-fold higher than the control at 2 weeks post-lesion (Table 2). At day 4 after lesion, 27 % of nestin-ir cells showed BrdU-immunoreactivity, and these nestin/BrdU double-labelled cells constituted 94 % of BrdU-ir cells in the VN of the lesioned side (Table 2). At day 14 after lesion, only 12.8 % of nestin-ir cells showed BrdU-immunoreactivity, and these double-labelled cells decreased to 63.6 % of BrdU-ir cells (Table 2). Occurrence of nestin-positive neural progenitor cells with and without BrdU labelling suggested co-existence of proliferative neural progenitors and non-proliferative nestin-expressing cells within the VN. This, together with the occurrence of GFAP and nestin double-labelled cells, implied that both proliferative and endemic neural progenitors in the VN contributed to gliogenesis after unilateral vestibular neurectomy.

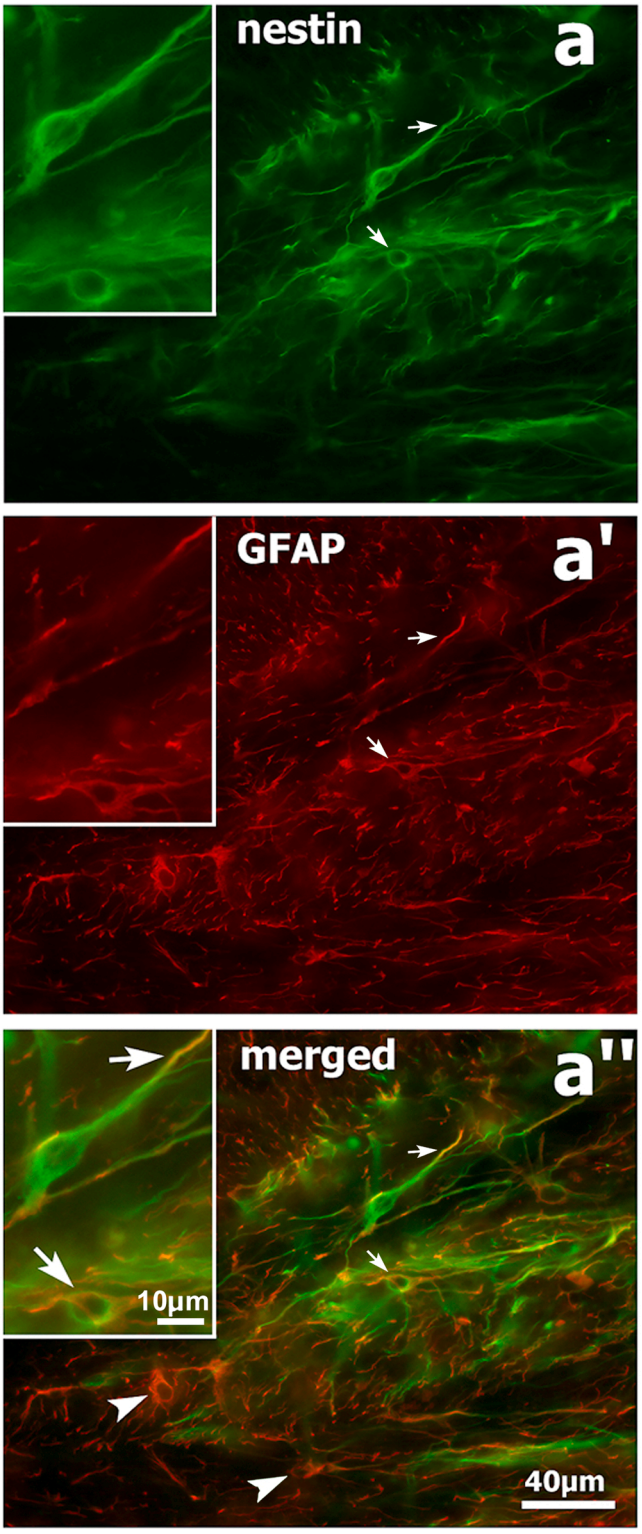

Fig. 3. Co-expression of nestin and GFAP in astroglial cells within the deafferentated VN. Both nestin-(a) and GFAP-(a') immunoreactive cells are present in the SpVe 14 days after lesion. Arrows point to nestin/GFAP double-immunoreactive cells. GFAP-only cells are indicated by arrowheads.

Table 2
Double-labelling of nestin with GFAP, NeuN or BrdU within the VN of rats after unilateral vestibular neurectomy.

	Control			4 days post-lesion					14 days post-lesion			
Cell type marker	Marker-positive cells	Nestin-positive cells	Double-labeled cells	Marker-positive cells	Nestin-positive cells	Double-labeled cells	% double-labeled cells among nestin-positive cells	% double-labeled cells among marker-positive cells	Marker-positive cells	Nestin-positive cells	Double-labeled cells	% double-labeled cells among nestin-positive cells	% double-labeled cells among marker-positive cells
GFAP	245 ± 36	21 ± 9	0	461 ± 21*	359 ± 26*	212 ± 14*	59.1 ± 3.9	46.0 ± 3.0	569 ± 38*#	218 ± 13*#	186 ± 15*	85.3 ± 6.9#	32.7 ± 2.6#
NeuN	578 ± 29	18 ± 10	0	586 ± 36	337 ± 25*	0	0	0	553 ± 28	226 ± 17*#	0	0	0
BrdU	8 ± 6	16 ± 6	0	108 ± 14*	376 ± 31*	102 ± 6*	27.1 ± 1.6	94.4 ± 5.6	44 ± 8*#	215 ± 14*#	28 ± 5*#	12.8 ± 2.3#	63.6 ± 11.4#

The number of immunoreactive cells (mean ± S.E.M., n = 8 rats) in 10 representative fields within the VN of rats in control group, as well as at 4 and 14 days post-lesion. Double-immunostaining of nestin and cell type markers GFAP, NeuN, or the proliferation marker BrdU was performed for each group. * $P < 0.05$ compared to the control group. # $P < 0.05$ compared to the 4 days post-lesion group.

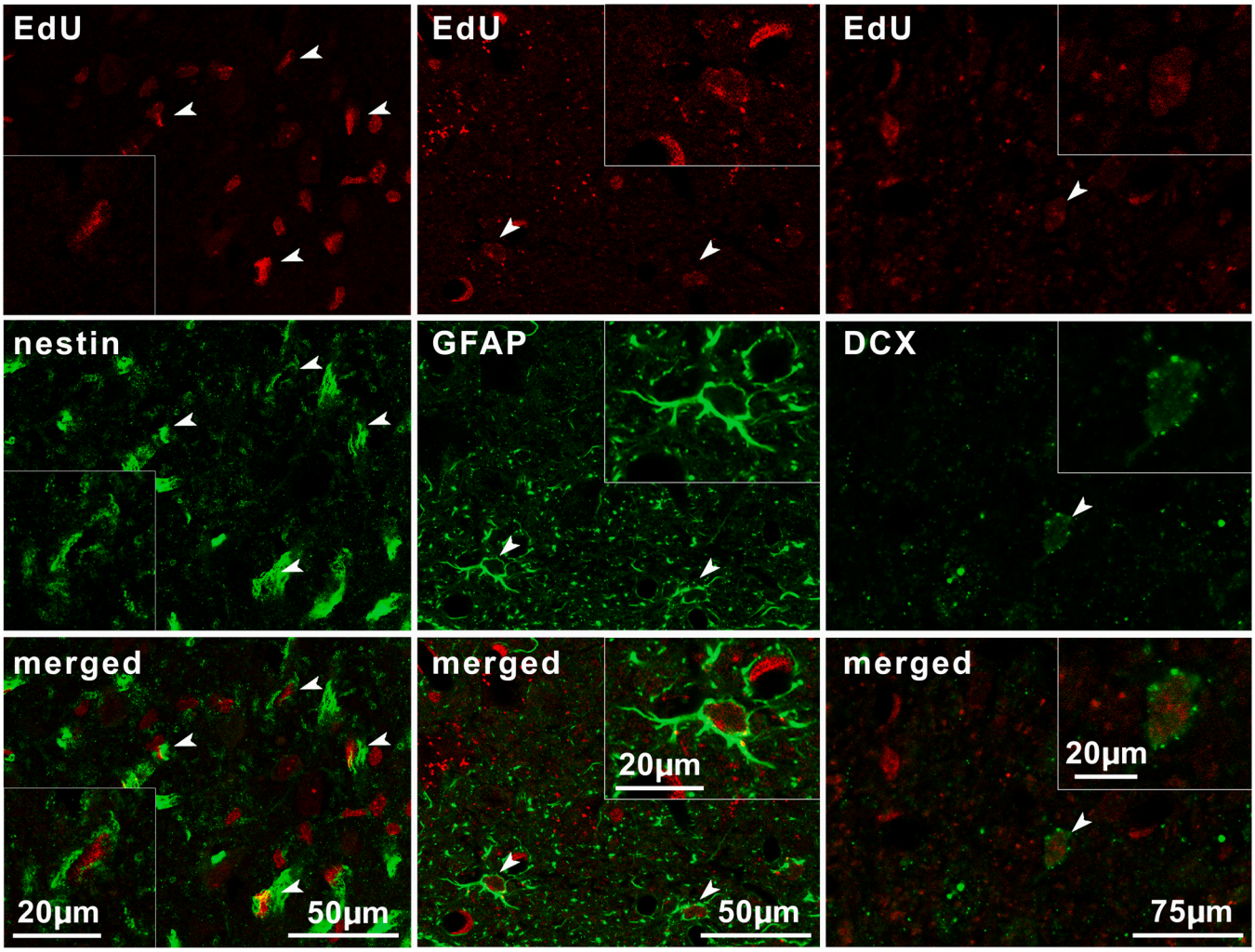

Fig. 4. Proliferation of astroglial cells and immature neurons in the VN of lesioned animals. Left column: EdU and nestin double-labelled cells (arrowheads) are abundant in the de-afferented VN at 14 days after lesion. Middle column: EdU and GFAP double-labelled cells (arrowheads) are also found in these rats. Right column: EdU and DCX double-labelled cells (arrowhead) are rarely observed at this time point.

4. Discussion

While it is well documented that postural symptoms resultant from unilateral loss of vestibular input can be recovered within 2 weeks (Péricat et al., 2017) and that cell proliferation in the VN is required for such postural recovery (Dutheil et al., 2009), the neural elements that support this process of recovery have remained unclear. In this study, we demonstrated that unilateral vestibular neurectomy was sufficient to cause a significant increase in the number of nestin- and GFAP-expressing cells in VN within 2 weeks post-lesion, but the number

of NeuN-expressing cells remained unchanged compared to unlesioned animals. A large portion of these nestin- or GFAP-expressing cells were resultant from lesion-induced proliferation of endogenous progenitors, since they co-stained with the proliferation marker BrdU or EdU.

GFAP can be expressed by both neuronal and glia progenitors at an early stage (Liu et al., 2010). While both neuro and gliogenesis have been observed 1 month post-lesion (Marouane et al., 2021; Rastoldo et al., 2021; Dutheil et al., 2016; Tighilet et al., 2007), it is not known if neurogenesis participates during the critical first 2 week post-lesional period where postural symptoms are recovered. We did not observe post-lesion increase in number of NeuN-expressing cells nor co-staining of nestin and NeuN in the post-lesion VN within the first 2 weeks post lesion. Our data therefore suggests that the newborn nestin- and GFAP-expressing progenitors observed post-lesion were likely to be destined for glia cell fates. Glial cells can stimulate rewiring of existing circuits. For example, astrocytes can increase plasticity of neurons (Allen, 2014; Araque and Navarrete, 2010; Lawal et al., 2022; Mederos et al., 2018), thereby promoting functional recovery through rebalancing of the bilateral vestibular outputs.

Further experiments, are required to unveil the final cell fate of these nestin- and GFAP-expressing cells, as well as the mechanism by which gliogenesis brings about the changes in VN neuron activity to support vestibular compensation. Interestingly, recent studies suggest that the cell fate of such progenitors is not fixed but instead depends on rehabilitation paradigms (Marouane et al., 2021). This adds a layer of complexity toward understanding the process of vestibular compensation.

Taken together with previous reports of neurogenesis VN of cats (Dutheil et al., 2016; Tighilet et al., 2007) and rats (Rastoldo et al., 2021) at 1 month after vestibular neurectomy, our results suggest that lesion-induced neurogenesis occurs within the 3rd and 4th weeks after unilateral vestibular neurectomy. This is consistent with of a small number of cells double-positive for the immature neuron progenitor marker DCX and the proliferation marker EdU at 14 days post-lesion. Our results therefore suggest that neuron progenitors proliferated within the first 2 weeks remained immature and could only mature into new neurons at a later stage of vestibular compensation. While neurogenesis was only elicited by vestibular neurectomy but not labyrinthectomy (Chen et al., 2019; Rastoldo et al., 2021), lack of NeuN-EdU double-positive cells at 14 days after unilateral vestibular neurectomy in our study showed that recovery of static and dynamic symptoms, known to occur within this period (Chen et al., 2019; Rastoldo et al., 2021), did not depend on neurogenesis. Notably, rapid recovery from vestibular neurectomy by sensory-motor rehabilitation was accompanied by significant reduction in neurogenesis with increase in microgliogenesis (Marouane et al., 2021). This further supported the notion that neurogenesis is not required for recovery from unilateral vestibular loss.

The source of these new GFAP-positive cells after unilateral vestibular neurectomy has not been definitively determined. Presence of endemic precursors, migration of precursors from other brain regions, as well as proliferation of mature cells after re-expression of neural progenitor markers have all been suggested (Tighilet et al., 2007). Existence of neurogenic and gliogenic precursors in the ependymal layer of the fourth ventricle (Gomez-Gonzalez et al., 2022; Luo et al., 2015), directly above the VN, imply possible influx of progenitors post-lesion. The rapid increase in the number of nestin-positive cells throughout the lesioned VN within a short time frame (2 days post-lesion), however, suggest existence of neural progenitors within the VN. Furthermore, the majority of nestin-positive cells were not positive for the proliferation marker BrdU in the post-lesional VN. This suggests that endemic progenitors within the VN upregulate the expression of nestin and GFAP in response to unilateral loss of vestibular input. A recent study provided evidence for Sox2-positive progenitors in the VN as the source for GFAP-positive glial cells post-lesion (Rastoldo et al., 2021). This is in line with the occurrence of GFAP and nestin double-positive cells observed in our current study, given that nestin and Sox2 are often co-expressed by the same population of precursors in the developing brain (Albright et al., 2016). Furthermore, decrease in the percentage of co-labelling of GFAP cells with nestin from 4 days to 14 days post-lesion is in line with downregulation of nestin expression as progenitors mature (Li et al., 2022; Liu et al., 2022).

In the embryonic and early postnatal cerebral cortex, nestin-positive progenitor cells can give rise to both neurons and glial cells (Luo et al., 2015; Parnavelas and Nadarajah, 2001; Siddiqi et al., 2014; Sun et al., 2005), depending on the local environment in each specific brain region (Malatesta et al., 2003). For example, neural precursors in the adult telencephalon retain neurogenic potential (Liu et al., 2010; Seri et al., 2001) but those in the developing diencephalon were gliogenic (Guo et al., 2018). Increase in GFAP and nestin double-labelled cells within the first 2 weeks post-lesion as observed in the current study, combined with neurogenesis at 1 month post-lesion reported by other groups (Dutheil et al., 2013), suggested that the VN acts as a gliogenic niche during the acute phase of vestibular compensation, but turns into a more neurogenic microenvironment towards the later stage. The recent observation of low level constitutive neurogenesis in the VN (Rastoldo et al., 2022), raises the question of whether "post-lesional neurogenesis" in earlier studies simply observed the basal neurogenesis within the VN.

In all, our results revealed that gliogenesis, but not neurogenesis, occurred within the first 2 weeks after unilateral vestibular neurectomy, coinciding with the reported time frame for recovery of static and dynamic deficits (El Mahmoudi et al., 2022). This suggests glial cell proliferation precedes neuronal proliferation over the course of vestibular compensation. Newly generated GFAP-positive glial cells originating from local progenitors are well poised to facilitate re-instatement of neural plasticity required for vestibular compensation via non-neurogenic mechanisms.

CRediT authorship contribution statement

Wu Kenneth Lap Kei: Formal analysis, Investigation, Project administration, Supervision, Visualization, Writing – review & editing. **Chen Liang-Wei:** Formal analysis, Investigation, Writing – original draft, Methodology, Visualization. **Shum Daisy Kwok-Yan:** Project administration, Supervision, Writing – review & editing. **Chan Ying-Shing:** Formal analysis, Project administration, Resources, Supervision, Writing – review & editing. **Ma Chun-Wai:** Formal analysis, Investigation, Visualization, Writing – review & editing. **Tam Kin-Wai:** Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Lai Chun-Hong:** Investigation, Methodology, Supervision, Writing – original draft. **Tsui Yat-Ping:** Formal analysis, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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