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RESEARCH ARTICLE

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Modelling the dopamine and noradrenergic cell loss that occurs in Parkinson's disease and the impact on hippocampal neurogenesis

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Abstract

Key pathological features of Parkinson's Disease (PD) include the progressive degeneration of midbrain dopaminergic (DA) neurons and hindbrain noradrenergic (NA) neurons. The loss of DA neurons has been extensively studied and is the main cause of motor dysfunction. Importantly, however, there are a range of 'non-movement' related features of PD including cognitive dysfunction, sleep disturbances and mood disorders. The origins for these non-motor symptoms are less clear, but a possible substrate for cognitive decline may be reduced adult-hippocampal neurogenesis, which is reported to be impaired in PD. The mechanisms underlying reduced neurogenesis in PD are not well established. Here we tested the hypothesis that NA and DA depletion, as occurs in PD, impairs hippocampal neurogenesis. We used 6-hydroxydopamine or the immunotoxin dopamine-β-hydroxylase-saporin to selectively lesion DA or NA neurons, respectively, in adult Sprague Dawley rats and assessed hippocampal neurogenesis through phenotyping of cells birthdated using 5-bromo-2′-deoxyuridine. The results showed no difference in proliferation or differentiation of newborn cells in the subgranular zone of the dentate gyrus after NA or DA lesions. This suggests that impairment of hippocampal neurogenesis in PD likely results from mechanisms independent of, or in addition to degeneration of DA and NA neurons.

KEYWORDS
catecholamines, dentate gyrus, endogenous stem-cells, neurodegeneration

1 | INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease that mostly affects individuals over 60 years old. The pathology of PD is generally described as a degeneration of dopamine (DA) neurons of the substantia nigra pars compacta (SNpc), which leads to motor impairments that include rigidity, tremor, and bradykinesia (Samii, Nutt, & Ransom, 2004). However, PD is a multisystem disorder, and the progressive pathology also impacts various other nuclei including the locus cœruleus (LC; Halliday, 2012; Zweig, Cardillo, Cohen, Giere, & Hedreen, 1993), basal forebrain (Hall et al., 2014; Nakano & Hirano, 1984), olfactory structures (Pearce, Hawkes, & Daniel 1995) and the raphe nuclei (Halliday, Blumbergs, Cotton, Blessing, & Geffen, 1990).

While often described as a motor disease, PD patients also exhibit a wide range of non-motor symptoms, including olfactory impairment, depression, and mild cognitive impairments progressing to dementia in late-stage disease (Barbosa, 2013). Thus, exploring the relationship between nonmotor symptoms and patterns of pathology in specific brain regions is becoming an increasingly important area in PD research.

Hippocampal neurogenesis has been linked to cognitive function and is reportedly reduced in parallel with the onset of age-related cognitive decline (Small, Schobel, Buxton, Witter, & Barnes, 2012). Furthermore, hippocampal neurogenesis has been suggested to be deficient in PD patients, with MRI studies showing that PD patients with dementia have smaller hippocampi than patients without dementia (Camicioli et al., 2003; Laakso et al., 1996). Höglinger et al. (2004) also investigated hippocampal neurogenesis levels in PD patients, reporting fewer numbers of neuronal precursors.

Since the reports correlating impaired hippocampal neurogenesis to nonmotor symptoms in PD patients, a number of studies have focused on identifying factors responsible for regulating hippocampal neurogenesis in order to identify therapeutic targets (Geraerts, Krylyshkina, Debyser, & Baekelandt, 2007; Kotani, Yamauchi, Teramoto, & Ogura, 2008; Malberg, Eisch, Nestler, & Duman, 2000; Meneghini et al., 2014; Vaidya, Vadodaria, & Jha, 2007). DA degeneration has been suggested to be a key factor underlying a decrease in neurogenesis in the hippocampus (Höglinger et al., 2004; Park & Enikolopov, 2010; Suzuki et al., 2010). Indeed, Suzuki et al. (2010) used 6-hydroxydopamine (6-OHDA) to remove midbrain DA neurons and observed a reduction of proliferating cells in the subgranular zone (SGZ) of the dentate gyrus (DG), a finding also reported in a study using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Höglinger et al., 2004). However, the specificity of both 6-OHDA and MPTP are not limited to DA. 6-OHDA targets all catecholamines (Breese & Traylor, 1970) and MPTP also affects noradrenaline (NA) and serotonin (5HT) neurons (Gupta, Felten, & Gash, 1984; Namura et al., 1987). Consequently, it is possible that NA depletion was underestimated in these previous studies, and may play an important role in the reduced hippocampal neurogenesis reported.

Supporting this hypothesis, we recently demonstrated that the DG, which receives a dense innervation from NA neurons originating in the LC, completely lacks innervation from midbrain DA neurons (Ermine, Wright, Parish, Stanic, & Thompson, 2016). Furthermore, reports that NA neuronal degeneration may precede DA neuronal degeneration in PD (Braak & Del Tredici, 2008), combined with nonmotor symptoms that arise prior to motor dysfunction (Tolosa, Compta, & Gaig 2007), provides correlative support for the hypothesis that NA plays a role in regulating cell proliferation and neurogenesis in the SGZ. To test this, we compared the effect of DA depletion and NA depletion alone, or together, on cell proliferation and neurogenesis in the SGZ of adult rats.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult female Sprague Dawley (SD) rats were used in this study. All procedures were conducted in accordance with the Australian National Health and Medical Research Council's published Code of Practice for the Use of Animals in Research, and experiments were approved by the Florey Institute of Neuroscience and Mental Health Animal Ethics Committee. All animals were group housed in individually ventilated cages under a 12 hr light/dark cycle with ad libitum access to food and water.

2.2 | Surgical procedures

The rats were anesthetised with isoflurane (5% at 1 L/min) and maintained under anaesthesia for the duration of the surgery (2%, 1 L/min). The animals were placed in a stereotaxic frame (Kopf, Germany) and a small burr-hole was drilled in the skull to administer the following toxins at a rate of 1 μL/min: (1) 14 μg of 6-OHDA [Tocris, in 0.02% ascorbic acid (Sigma) and saline (0.9% NaCl)] into the medial forebrain bundle (MFB) (4.2 mm anterior and 1.2 mm lateral to bregma, 7.8 mm below dura) with i.p. injection of desipramine (20 mg/kg) 30 min prior to surgery to protect NA neurons; (2) 1 μg of dopamine-β-hydroxylase saporin (DBH-saporin) in the intra cerebral ventricle (i.c.v.; 0.6 mm anterior and 1.5 mm lateral to bregma, 3 mm below dura); (3) both 6-OHDA (14 μg in MFB) and DBH-saporin (1 μg in i.c.v.); (4) saline in MFB and/or i.c.v.

Animals were sacrificed at either four or nine weeks postlesion to confirm selective neuronal (DA and/or NA) ablation and assess the impact of the treatments on SGZ cell proliferation. The four-week time-point was chosen for its relevance to study designs in the existing literature (Baker, Baker, & Hagg, 2004; Höglinger et al., 2004; Suzuki et al., 2010). The nine-week time-point allowed a sufficient time for the newborn cells to differentiate into their neuronal phenotype and investigates a longer-term effect of the lesions on neurogenesis.

2.3 | Lesion validation by assessing DA and NA levels using HPLC

To validate the DA and NA lesions, we assessed DA and NA levels in the brain four weeks after lesioning using high performance liquid chromatography (HPLC). DA and NA levels were assessed at the level of terminal fields in the striatum or hippocampus, as described previously in detail (Parish et al., 2001). In brief, animals were decapitated and the striatum and hippocampi were rapidly dissected, weighed, and snap-frozen for storage at −80 °C until analysis, at which point samples were homogenised in an extraction buffer containing 0.4M perchloric acid, 7.9 mM sodium metabisulphite, 1.34 mM disodium ethylenediaminetetra-acetic acid (EDTA) in distilled water. To rupture vesicular membranes, samples were sonicated for 15 s and centrifuged at 10,000g (3 × 5 min). The resultant supernatant was transferred to HPLC vials and placed in an autosampler for injection onto the HPLC. The HPLC consisted of a LC-20AT pump (Shimadzu), SIL-20A Autosampler (Shimadzu), and C18 reverse phase column (Bio-Rad, Hercules, USA). Detection was via a 3 mm VT-03 flow cell with glassy carbon working electrode (Antec Leyden) and Decade II Electrochemical Detector (Antec Leyden). The mobile phase consisted of 17% v/v methanol in purified deionized water containing 70 mM KH_2PO_4 (Merck), 0.5 mM EDTA (Merck), and 8.0 mM sulfonic acid (Merck), pH 3.0 and was run at a flow rate of 0.5 mL/min.

2.4 | 5-Bromo-2′-deoxyuridine (BrdU) treatment

Four weeks postsurgery, 56 rats across the different treatment groups were injected with BrdU (50 mg/kg, i.p.) every 12 hr for seven days to label dividing cells over a one week period.

2.5 | Tissue collection and immunohistochemistry

Animals were sacrificed by a terminal dose of pentobarbitone (100 mg/kg; Virbac, Peakhurst, Australia) and transcardially perfused with paraformaldehyde media (PFA, 4% in 0.4M phosphate buffer with 0.2% picric acid). Brains were collected and further postfixed for 2 hr in PFA, followed by cryo-protection in 20% sucrose PBS solution for 1–2 days. After freezing the brains on dry ice, 40 μm-thick coronal sections were collected in 1:12-series using a freezing-microtome (Leica, Wetzlar, Germany).

Free-floating immunohistochemistry for tyrosine hydroxylase (TH), DBH, BrdU, Ki67, and Prox1 were performed on a 1:12 or 1:6 series as previously described (Thompson, 2005). Tissues used for BrdU labelling were pretreated by incubation in Omnipur deionized formamide (Merck Millipore) at 65 °C for 2 hr, in 2M HCL at 37 °C for 30 min and in Borate buffer at room temperature for 20 min. Primary antibodies and dilutions used were: sheep anti-BrdU (Exalpha, A205P, 1:1000); mouse anti-DBH (Millipore, MAB308, 1:5000); rabbit anti-TH (Pel-freeze, P40101-0, 1:1000); rabbit anti-Ki67 (Thermo Fisher, LBVRM-9106-S1, 1:1000); rabbit anti-prox1 (Millipore, ABN278, 1:2000). Secondary antibodies and dilution factors were: antirabbit and antisheep conjugated to Dylight Fluorophores 488 and 549 (Jackson ImmunoResearch, 1:200) for fluorescent staining and antimouse, antirabbit, antigoat, and antisheep conjugated to biotin (Jackson ImmunoResearch,1:400) for chromogenic staining.

2.6 | Analysis

Fluorescent microscopic analysis and images were performed using a Zeiss Meta confocal microscope (LSM 780) and chromogenic images were captured using a Leica DM6000 microscope.

The number of BrdU+ and Ki67+ cells in the SGZ were quantified across six sections located at ~2.6, 2.84, 3.08, 3.32, 3.56, and 3.8 mm caudal to bregma. The number of BrdU/Prox1 double positive cells was also quantified, by random selection of 80 BrdU+ cells located in the SGZ across three to six sections (ranging from 2.6 to 3.8 mm caudal to bregma) and analysis of double-labelling through orthogonal reconstruction using the confocal microscope, to identify them as Prox1 positive or negative.

Statistical analysis was performed using a Kruskal-Wallis with Dunn's multiple comparison test to assess the difference of DA and NA levels at their terminal fields between the lesioned and saline control groups. The difference among the groups in BrdU+ cells, Ki67+ cells, and BrdU+/Prox1+ double positive cells in the DG was assessed via one-way ANOVA, with a Dunnett's multiple comparison test. The difference between the groups in the number of BrdU+ cells and Ki67+ cells in the SVZ was assessed via an unpaired *t*-test.

3 | RESULTS

3.1 | Unilateral ablation of midbrain DA neurons or hindbrain NA neurons results in a robust reduction of each neurotransmitter in the respective striatal or hippocampal terminal fields

To test whether the DA system and/or the NA system are involved in regulating adult hippocampal neurogenesis, we performed three different lesions: (1) selective ablation of NA neurons in the LC using the toxin DBH-saporin; (2) selective ablation of DA neurons from the SNpc/ventral tegmental area (VTA) using 6-OHDA and desipramine; and (3) double lesions of DA and NA neurons from the SNpc and LC, respectively, using 6-OHDA and DBH-saporin. Four weeks post-lesion, BrdU was administered i.p. twice daily for seven days. Animals were perfused at either four weeks after lesioning, to verify success of the lesions, or nine weeks after lesioning, to assess the impact on cell-proliferation and neurogenesis (Figure 1a).

HPLC was performed four weeks after toxin injection to allow for initial validation of both lesions and to give a quantitative measure of the impact of the lesions on NA and DA transmitter levels in the terminal fields (Figure 1b,c). Administration of DBH-saporin resulted in a 13-fold reduction of NA levels in the hippocampus compared with the control group (NA lesion: mean ± SEM = 0.2290 ± 0.036 nM/mg of tissue, $n = 8$; control: mean ± SEM = 2.998 ± 0.2475 nM/mg of tissue, $n = 15$; $p = .0006$; DA lesion: mean ± SEM = 3.268 ± 0.15 nM/mg of tissue, $n = 8$, Figure 1b), but did not affect the level of striatal DA (Figure 1c). The 6-OHDA + desipramine lesion resulted in an 8.8-fold reduction in striatal DA compared with controls (DA lesion: mean ± SEM = 53.76 ± 21.88 nM/mg of tissue, $n = 8$; control: mean ± SEM = 218.8 ± 6.621 nM/mg of tissue, $n = 16$; $p = .0008$; NA lesion: mean ± SEM = 249 ± 21.78 nM/mg of tissue, $n = 8$, Figure 1c) but did not affect the level of hippocampal NA (Figure 1b).

Immunohistochemical identification of NA and DA neurons and fibres nine weeks after toxin injection, allowed for final validation of both the NA and DA lesions. In animals injected with DBH-saporin the number of NA cell bodies in the LC and corresponding fibres in the hippocampus were markedly reduced compared with saline injected controls (Figure 1d–g). Similarly, animals that received 6-OHDA + desipramine treatment showed a significant loss of TH immunoreactivity in the SNpc and striatum, (Figure 1h–k). Thus, robust DA and NA lesions were validated both at neuroanatomical and biochemical levels.

3.2 | Unilateral removal of either the DA or NA projection systems does not affect hippocampal cell proliferation or neurogenesis

To determine the number of adult-born cells in the SGZ and their phenotypic fate, we pulsed adult rats with BrdU for a period of seven days beginning four weeks after lesioning, and performed histological analysis after a further four week period - nine weeks post-lesion. We observed no difference in the number of BrdU+ cells in the SGZ between all treatment groups (saline control group: mean ± SEM = 182 ± 16.96, $n = 15$; NA lesion: mean ± SEM = 178.5 ± 18.44, $n = 8$; DA lesion: mean ± SEM = 177.4 ± 18.0, $n = 8$; double lesion: mean ± SEM = 181.3 ± 24.96, $n = 7$; $p = .9979$; Figure 2a–e).

Because the number of BrdU+ cells at nine weeks postlesion reflects the survival of cells generated during the seven-day pulse period, we also quantified the number of actively dividing Ki67+ cells in the SGZ as a more specific measure of proliferation. Lesioning of the DA or NA systems alone, or in combination, did not significantly affect the number of Ki67+ cells in the SGZ quantified across six sections

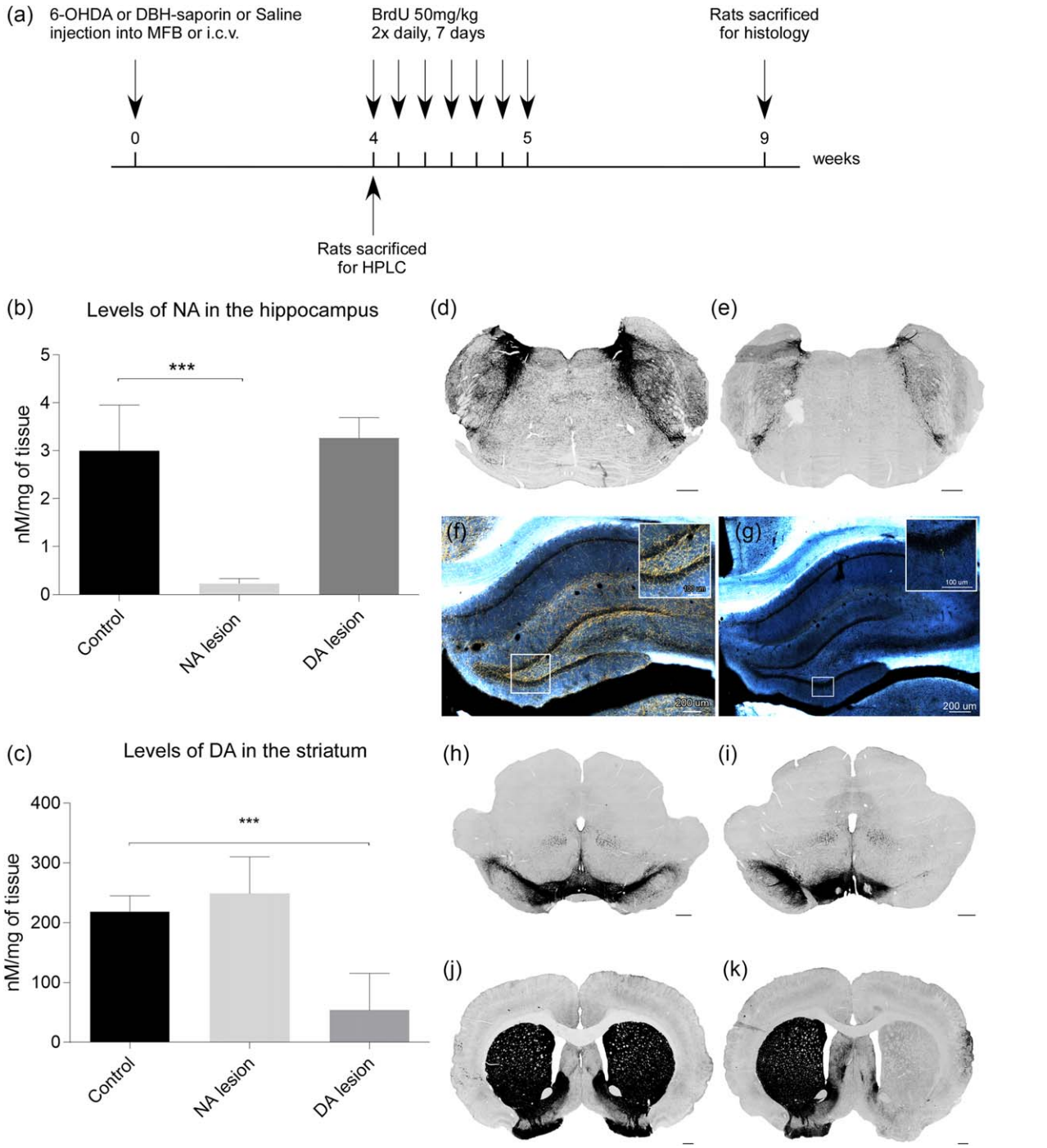

FIGURE 1 DBH-saporin and 6-OHDA successfully lesion the NA and DA systems, respectively, in adult SD rats. Lesioned animals were killed at 4 weeks for confirmation of selective lesioning, or killed at 9 weeks (after 7 days of bi-daily BrdU injections at 4 weeks), to assess hippocampal neurogenesis (a). As confirmed by HPLC 4 weeks after toxin administration, NA lesions induced a 13-fold reduction in NA levels in the hippocampus (b) and DA lesions induced a 9-fold reduction of DA levels in the striatum (c), compared with their respective control groups. Immunohistochemical detection of DBH using a brightfield microscope in controls (d) and lesioned animals (e), reveals a reduction in the number of NA cell bodies in the LC 9 weeks after DBH-saporin administration. Darkfield imaging of chromogenically labelled DBH in the hippocampus shows the intact NA system in control animals (f), compared with an almost complete ablation of NA fibres in lesioned animals (g). The analysis of TH immunoreactivity 9 weeks after 6-OHDA administration showed an almost complete ablation of DA cell bodies in the right SNpc (h vs. i) and axon terminals in the striatum (j vs. k). Statistical analysis: Kruskal-Wallis with Dunn's multiple comparison test, *** $p \leq .0006$ (b) and *** $p = .0008$ (c), error bars = Standard deviation. For control groups $n = 16$, for lesion groups $n = 8$. Scale bars: d, e,h,i,j,k = 500 μm and f,g = 200 μm. Abbreviations: DBH, dopamine-β-hydroxylase; TH, tyrosine hydroxylase; NA, noradrenaline; DA, dopamine; LC, locus cœruleus; HPLC, high performance liquid chromatography; SD, Sprague Dawley; SNpc, substantia nigra pars compacta [Color figure can be viewed at wileyonlinelibrary.com]

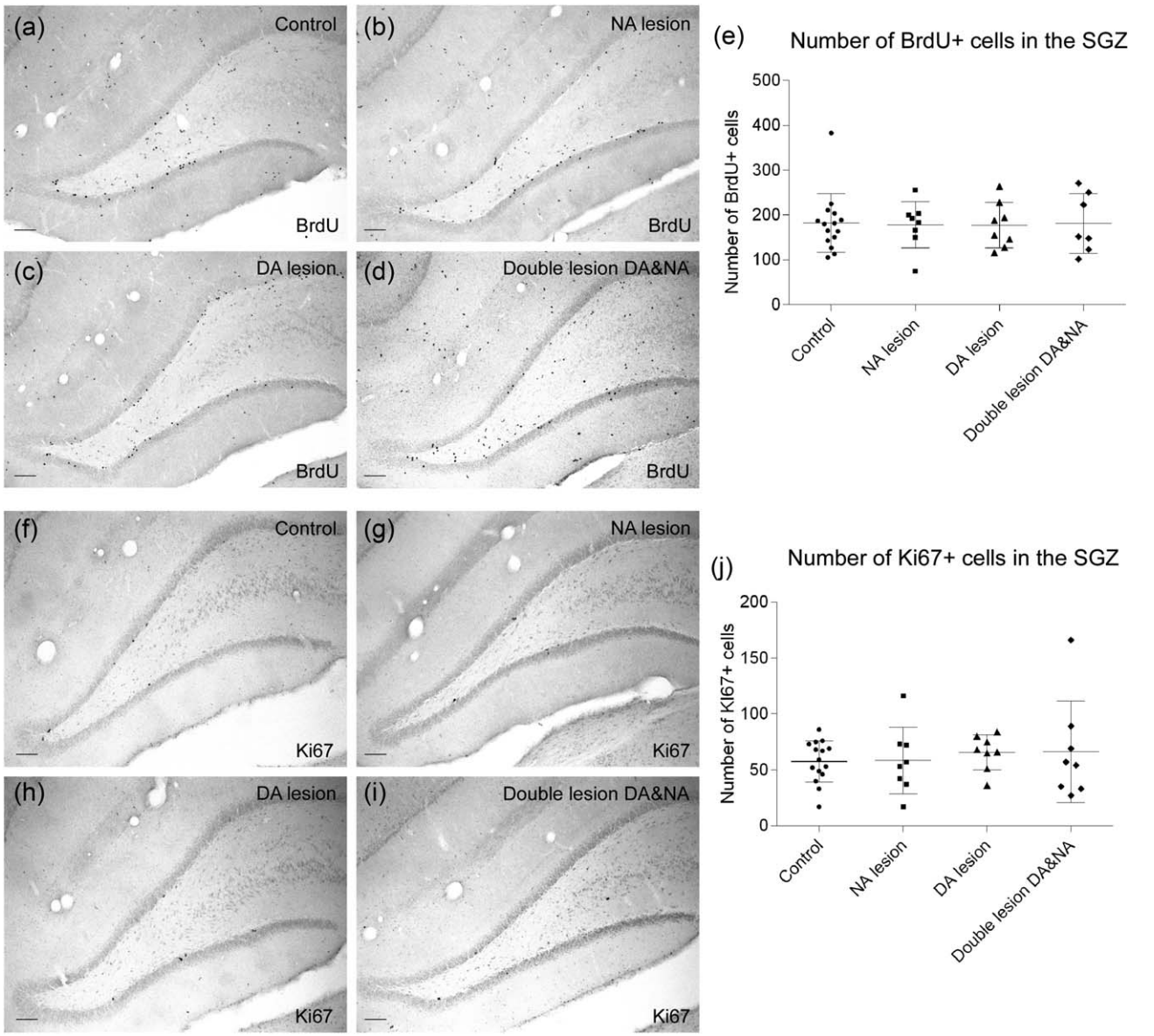

FIGURE 2 The number of surviving new-born and proliferative cells in the SGZ of adult rats is unchanged 9 weeks following DA or NA lesions, or combined double lesions. Immunohistochemistry for BrdU in representative sections from the control (a), NA lesion (b), DA lesion (c), and the double lesion DA & NA group (d) 4 weeks after the last BrdU injection. Quantification in the SGZ across 6 sections did not reveal any difference in BrdU+ numbers between the control and experimental groups (e). Immunohistochemistry for Ki67 in representative sections from the control (f), NA lesion (g), DA lesion (h), and double lesion groups (i) 9 weeks after the initial lesioning. Quantification in the SGZ across 6 sections did not reveal any difference in Ki67+ numbers between the control and experimental groups (j). Statistical analysis: (e,j) One-way ANOVA with Dunnett's test for multiple comparison, $F_{3,34} = 0.013$ and $p = .9979$ (e), $F_{3,35} = 0.2696$ and $p = .8468$ (j). Error bars = Standard deviation. For control groups $n = 16$, for lesion groups $n = 8$. Scale bars: a–d and f–i 100 μm. Abbreviations: BrdU, 5-bromo-2′-deoxyuridine; NA, noradrenaline; DA, dopamine; SGZ, subgranular zone

spanning the temporal hippocampus (control: mean ± SEM = 57.53 ± 4.80, $n = 15$; NA lesion: mean ± SEM = 58.38 ± 10.512, $n = 8$; DA lesion: mean ± SEM = 65.63 ± 5.558, $n = 8$; double lesion: mean ± SEM = 66.25 ± 16.0, $n = 8$; $p = .8468$; Figure 2f–j).

To assess the effect of NA or DA loss on the differentiation of new-born cells in each group, the proportion of BrdU+ cells that adopted a Prox1+ phenotype (broadly expressed in granular neurons of the SGZ) was quantified. Four weeks after BrdU administration, the percentage of BrdU+ cells to adopt a Prox1+ identity in the SGZ was not significantly different from control numbers in any of the treatment groups (control: mean ± SEM = 76.34% ± 1.92, $n = 15$; NA lesion: mean ± SEM = 79.53% ± 1.89, $n = 8$; DA lesion: mean ± SEM = 75.54% ± 2.82, $n = 7$; double lesion: mean ± SEM = 78.57% ± 2.43, $n = 7$; $p = .6016$; Figure 3a–e).

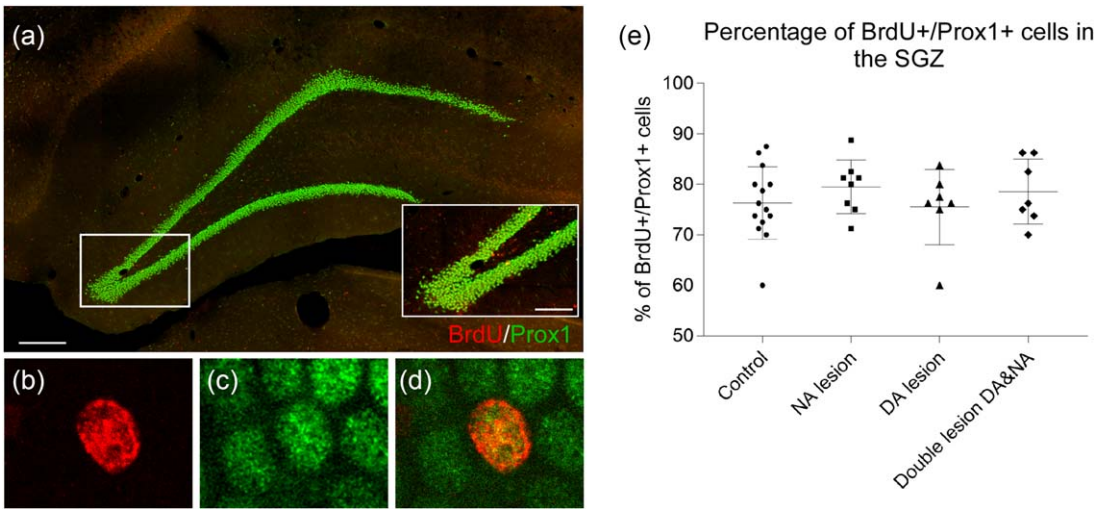

FIGURE 3 Neurogenesis in the SGZ of adult rats is unchanged at 9 weeks following DA or NA lesions, or combined double lesions. A representative image from a control animal showing immunohistochemistry for BrdU and Prox1 at 4 weeks after the 7-day BrdU pulse period that was used to quantify the number of neurons generated in that time (a–d). (b–d) represents a high magnification example of a BrdU+/Prox1+ cell. A total of 80 BrdU+ cells in the dentate SGZ were assessed for Prox1 immunoreactivity by orthogonal reconstruction of confocal images in order to determine the fractional contribution to BrdU+ cells (e). Statistical analysis: (e) One-way ANOVA with Dunnett's test for multiple comparison, $F_{3,32} = 0.6291$ and $p = .6016$. Error bars = Standard deviation. For control groups $n = 16$, for lesion groups $n = 8$. Scale bars: A 200 μm. Abbreviations: BrdU, 5-bromo-2′-deoxyuridine; NA, noradrenaline; DA, dopamine; SGZ, subgranular zone [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Depletion of striatal DA with 6-OHDA does not affect the number of BrdU+ cells or proliferating Ki67+ cells in the SVZ

While a recent study found fewer proliferating cells in the SGZ after midbrain DA neuron lesions in adult rats (Suzuki et al., 2010), reduced proliferation in the SVZ has been more widely reported following DA deficiency (Baker et al., 2004; Höglinger et al., 2004; Winner et al., 2006). We aimed to confirm these reports of reduced SVZ proliferation in our experimental setting, to obtain an 'internal control' from which to contextualize the negative findings from our analysis of the hippocampus. We therefore quantified the number of BrdU+ and Ki67+ cells in the SVZ across three coronal sections from animals nine weeks after lesioning (four weeks after the one-week BrdU pulse period) and compared the DA lesion and control groups. Given the almost complete absence of NA innervation of the striatum and SVZ, the NA lesion group was not assessed.

In contrast to previous reports, we found that depletion of striatal DA by lesioning SNpc neurons with 6-OHDA, did not affect SVZ cell turnover based on the number of BrdU+ cells labelled over seven days, four to five weeks after 6-OHDA lesioning (Figure 4a–c) (control: mean ± SEM = 808 ± 30.98, $n = 8$; DA lesion: mean ± SEM = 808.3 ± 49.88, $n = 8$; $p = .9968$) or Ki67+ cells nine weeks after lesioning (Figure 4d–f) (control: mean ± SEM = 1430 ± 120.8, $n = 8$; DA lesion: mean ± SEM = 1513 ± 91.54, $n = 8$; $p = .5926$).

3.4 | Cell proliferation in the SGZ and the SVZ is not transiently affected acutely after DA and NA lesions

In the context of the existing literature, we were surprised at the lack of impact that DA lesions had on the number of adult-born surviving BrdU+ cells and actively proliferating Ki67+ cells in the SGZ, and particularly the SVZ. While we were originally focused on evidence for stable and potentially long-term changes, we considered we may have missed a more transient reduction in cell turnover after lesioning. To test this, NA or DA ablation was performed in a new cohort of animals, and sacrificed four weeks post-lesion (Figure 5a) to assess cell-proliferation based on immunohistochemistry for Ki67. The extent of lesioning at four weeks was similar to the DA or NA neuronal cell loss seen at the nine-week post-lesion time-point, including a robust reduction of DBH+ cell bodies in the LC and associated fibres in the hippocampus in DBH-saporin-treated animals (Figure 5b–e), and similarly robust loss of midbrain DA neurons and associated striatal projections following 6-OHDA treatment (Figure 5f–i).

The effect of lesioning on SGZ and SVZ cell proliferation was assessed by comparing the number of Ki67+ cells in the NA-lesion, DA-lesion and control groups (Figure 5j,k). In the SGZ, no difference in the number of Ki67+ cells was found when comparing NA and DA lesions with control (Control: mean ± SEM = 100 ± 10.38, $n = 8$; NA lesion: mean ± SEM = 112.4 ± 15.22, $n = 7$; DA lesion: mean ± SEM = 114.9 ± 20.15, $n = 8$; $p = .7726$). Similarly, there was no significant impact of DA lesions on the number of Ki67+ cells in the SVZ (Control: mean ± SEM = 1598 ± 121.2, $n = 7$; DA lesion: mean ± SEM = 1673 ± 82.25, $n = 7$; $p = .6199$).

4 | DISCUSSION

These results of DA and/or NA denervation having no effect on cell proliferation and neurogenesis suggest that hippocampal neurogenesis is not directly regulated by the DA or NA systems in the adult rat brain,

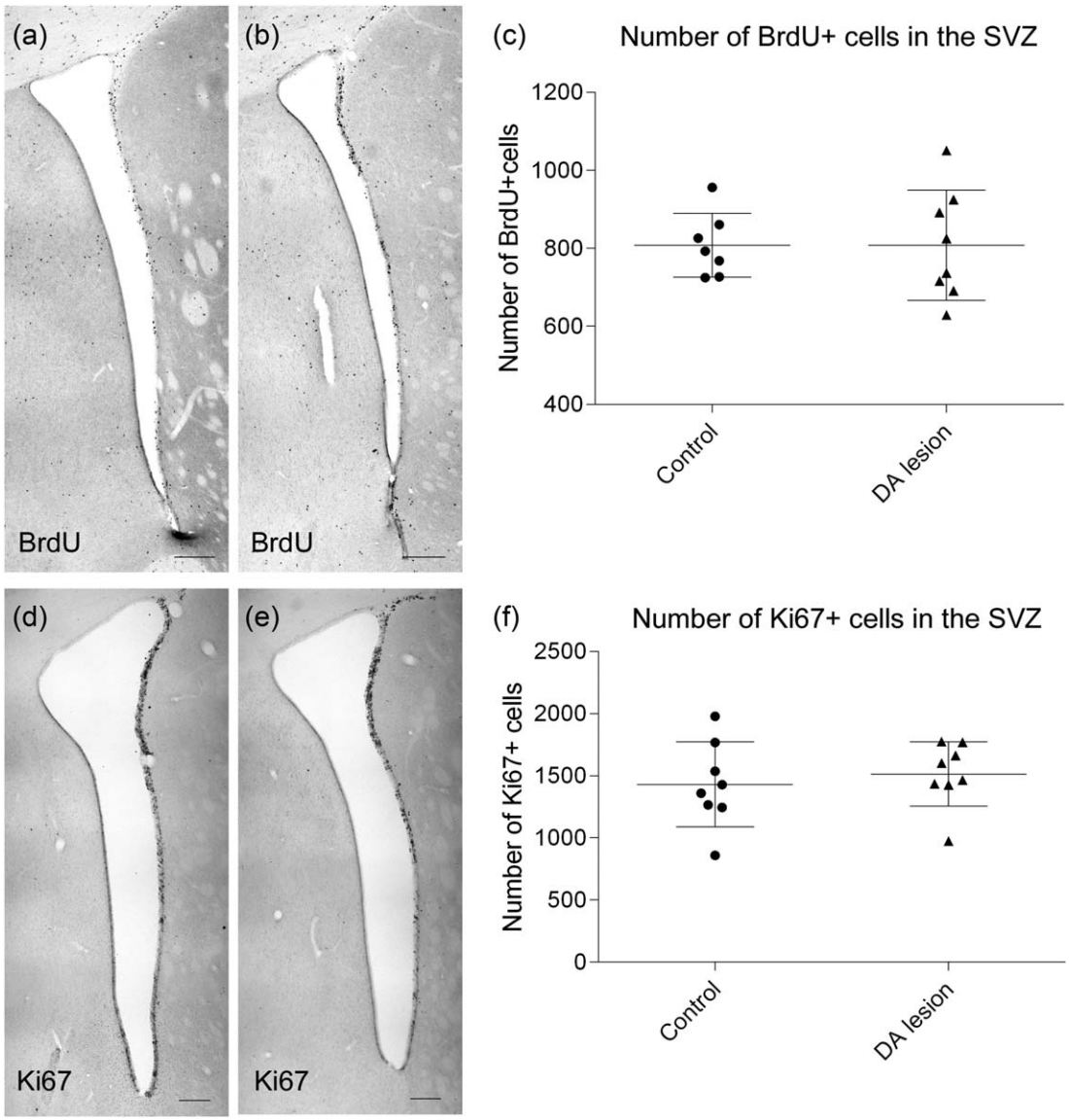

FIGURE 4 Adult-born and proliferating cells in the SVZ remain unchanged after 6-OHDA-induced striatal DA lesions in adult rats. Representative examples of BrdU immunoreactivity in the SVZ of control (a) and DA lesion groups (b). Cell counts showed no difference in the number of BrdU+ cells generated 4–5 weeks after lesioning (c). Representative examples of Ki67 immunoreactivity in the SVZ of control (d) and DA lesion groups (e). Cell counts showed no difference in the number of Ki67+ cells detected 9 weeks after lesioning (f). Statistical analysis: (c,f) unpaired t-test, $p = .9968$ (c) $p = .5926$ (f). Error bars = Standard deviation. For all groups $n = 8$. Scale bars: a–e, 200 μm. Abbreviations: BrdU, 5-bromo-2'-deoxyuridine; SVZ, subventricular zone

and thus is unlikely to directly underlie a link between loss of these neurons and cognitive decline in PD. This is consistent at a neuroanatomical level with our recent report on the lack of direct innervation of the hippocampus from midbrain DA neurons (Ermine et al., 2016). At a functional level however, the results contrast to previous studies, where DA has been described as a regulator of adult hippocampal neurogenesis (Broussard, 2012; Höglinger et al., 2004; Mu, Zhao, & Gage, 2011; Suzuki et al., 2010; Wisman, Sahin, Maingay, Leanza, & Kirik, 2008).

The existing literature is notably mixed with respect to conclusions that DA either positively or negatively regulates hippocampal neurogenesis. For example, in MPTP-induced rodent models of PD, low doses of MPTP reportedly decrease proliferation (Höglinger et al., 2004) while higher doses increase proliferation (Park & Enikolopov, 2010). Further confusion comes from pharmacological replacement studies, where the D2 antagonist haloperidol has been reported as having no effect (Malberg et al., 2000), decreasing (Wakade, Mahadik, Waller, & Chiu, 2002) or increasing (Keilhoff, Grecksch, & Becker, 2010) SGZ proliferation levels. Haloperidol has also been reported to have no effect on (Halim, Weickert, McClintock, Weinberger, & Lipska, 2004), and to increase (Keilhoff, Grecksch, Bernstein, Roskoden, & Becker, 2010) survival of adult-born cells in the DG.

The hippocampus receives a dense innervation from the locus coeruleus NA system (Loughlin, Foote, & Bloom, 1986; Loy, Koziell,

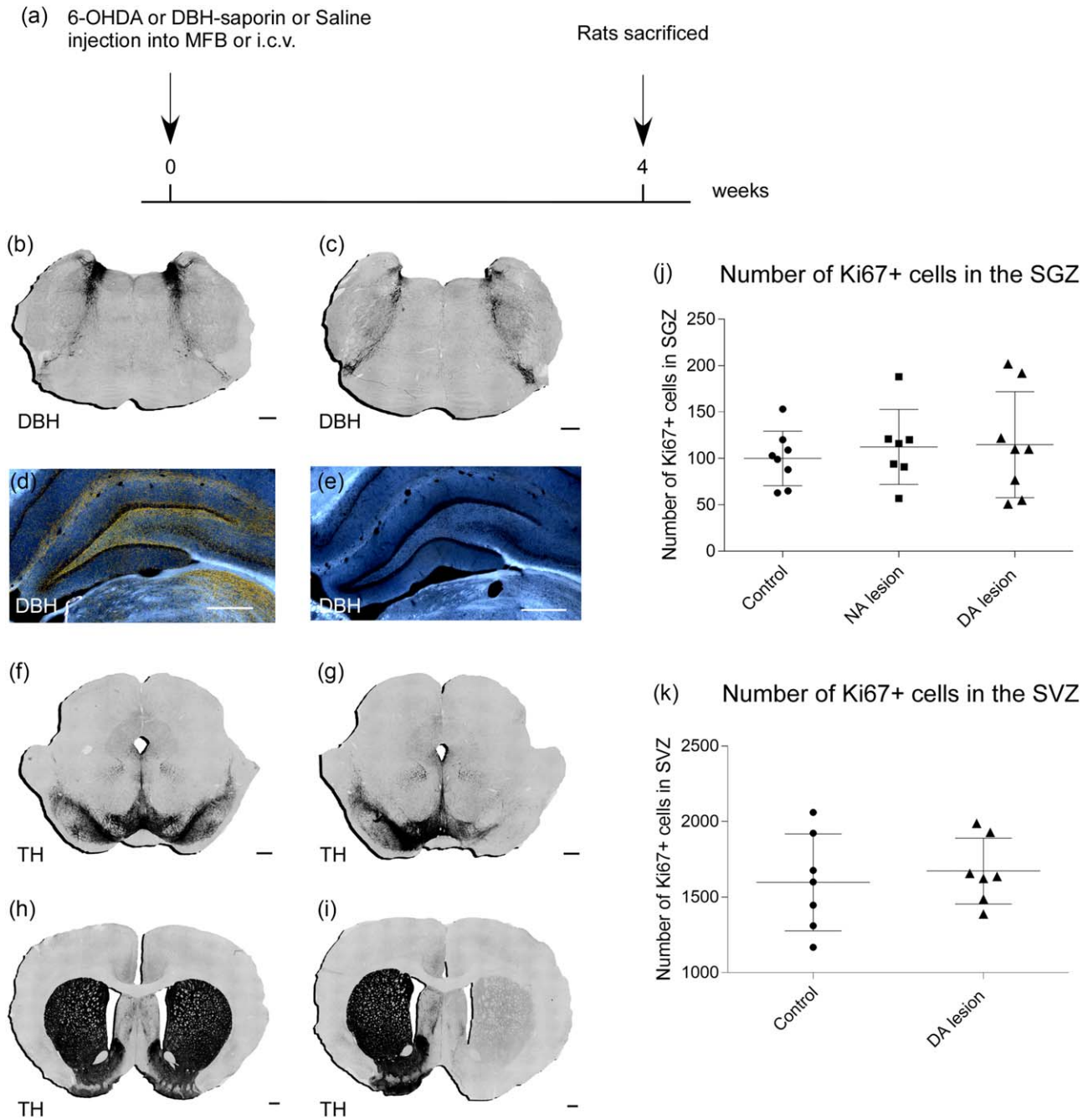

FIGURE 5 Lesions of DA or NA projection systems does not affect cell proliferation in the SVZ or SGZ of adult rats 4 weeks postlesion. Immunohistochemistry in representative coronal sections of the LC and hippocampus showed that compared with sham lesion (c, e), the i.c.v. injection of DBH-saporin toxin robustly ablated NA neurons (c, e). TH immunoreactivity in controls (f, h) compared with the DA lesion group, in which a near complete ablation of DA neurons in the SNpc and DA fibres occurred in the striatum after 6-OHDA administration (g, i). Analysis of the number of Ki67+ cells revealed no difference in proliferating cells in the SGZ between the NA lesion, DA lesion and control groups (j) or in the SVZ between the DA lesion and controls (k). Statistical analysis: (j) one-way ANOVA, $F_{2,\ 20} = 0.2614$, $p = .7726$; (k) unpaired t-test, $p = .6199$. Error bars = Standard deviation. Scale bars: b–i, 500 μm. Abbreviations: DBH, dopamine-β-hydroxylase; LC, locus cœruleus; TH, tyrosine hydroxylase; SGZ, subgranular zone; SNpc, substantia nigra pars compacta; SVZ, subventricular zone [Color figure can be viewed at wileyonlinelibrary.com]

Lindsey, & Moore, 1980); and particularly within the SGZ (Ermine et al., 2016) where the entirety of TH+ fibres coexpress the NA-specific marker DBH but not the DA-specific transporter (DAT). In light of this, we hypothesised that in a recent study reporting reduced hippocampal neurogenesis in response to 6-OHDA lesion of midbrain DA neurons, "off-target" depletion of the nearby locus coeruleus NA neurons may be the relevant underlying mechanism. Surprisingly, however, we report here that hippocampal neurogenesis was insensitive to near complete depletion of the DA or NA projection systems, alone or in combination, under similar experimental conditions.

TABLE 1 Summary of studies that investigated the role of DA on SVZ proliferation and their conclusion [Color table can be viewed at wileyonlinelibrary.com]

Study	Experimental design	Concluded role of DA on proliferation
Baker et al., 2004	D3 agonist, rats	↑
Van Kampen et al., 2004	D3 agonist, rats	↑
Höglinger et al., 2004	MPTP, mice	↑
Baker et al., 2005	D3 agonist, mice	No effect
Liu et al., 2006	6-OHDA, rats	↓
Winner et al., 2006	6-OHDA, rats	↑
Milosevic et al., 2007	D2/D3 agonist, *in vitro*	No effect
Aponso et al., 2008	6-OHDA, rats	↓
Winner et al., 2009	DA agonist, rats	↑
Van Den Berge et al., 2011	MPTP, mice	No effect
Lao et al., 2013	D3 agonist, mice	↑
Sui et al., 2012	6-OHDA, mice	↑
O'Keeffe et al., 2009	6-OHDA, rats	↑

Studies in green have reported a positive effect of DA on proliferation, studies in red have reported a negative effect and studies in white/grey have reported no effect of DA on SVZ proliferation.

Perhaps more surprising was the finding that depletion of DA innervation of the striatum and adjacent SVZ did not impair SVZ cell proliferation either. This is again in contrast to a number of studies with similar designs that report reductions in SVZ cell proliferation in response to loss of DA innervation (Baker et al., 2004; Höglinger et al., 2004; O'Keeffe, 2009; Sui, Horne, & Stanić, 2012). We noted that in some of these studies, cell-proliferation was assessed at shorter intervals after DA lesioning than the nine-week time-point we originally examined. Thus, to account for the possibility we had missed a transient impact on cell-proliferation we established an additional cohort of animals for histological assessment four weeks after lesioning to match previous study designs. Nonetheless, the results were similar to those observed at nine weeks, with no difference in the number of proliferating Ki67+ cells in both SGZ and SVZ despite near complete lesioning of the DA or NA projection systems.

Comparison of other key parameters in the present experimental design, including method of DA ablation, rodent species or strains, age, and sex does not really identify variables that clearly account for the descrapancy in outcome with similarly designed studies in rodents. The study by Höglinger et al (2004), additionally reports reduced cell proliferation in SGZ and SVZ in patients with PD, relative to aged-matched controls, and it is possible that DA differentially regulates cell proliferation across species. This is supported by findings from Baker, *Baker, and Hagg* (2005) showing that activation of the D3 receptor increases BrdU labelling of SVZ progenitors in rats but not mice.

Nonetheless, while there are a number of now well-cited studies that conclude DA positively regulates SVZ cell proliferation, a detailed review of the literature highlights a lack of consensus in much the same way as has been reported for the SGZ. DA has variously been reported to increase (Baker et al., 2004; Höglinger et al., 2004; Van Kampen, Hagg, Robertson, & Van Kampen, 2004; Lao, Lu, & Chen, 2013; O'Keeffe, 2009; Sui et al., 2012; Winner et al., 2006, 2009), decrease (Aponso, Faull, & Connor, 2008; Liu et al., 2006) and have no impact (Baker et al., 2005; van den Berge et al., 2011; Milosevic et al., 2007) on SVZ cell proliferation (Table 1).

In this study, we were interested in basal neurogenesis to understand the origin of the hippocampal neurogenesis decline observed in PD brains (Camicioli et al., 2003; Laakso et al., 1996). However, hippocampal neurogenesis has been shown to be increased in an enriched environment (Kempermann, Kuhn, & Gage, 1997) or following exercise (van Praag, Shubert, Zhao, & Gage, 2005), and it cannot be excluded that NA and/or DA can facilitate changes in the activity dependent regulation of hippocampal neurogenesis.

Identification of pathophysiological mechanisms underlying non-motor symptoms in PD, including dementia, remains an important strategy for the development of new therapeutic approaches. Here we

sought to functionally link DA or NA depletion to reduced hippocampal neurogenesis as a potential substrate for cognitive decline in PD. The results do not support such a link and contribute to an already highly mixed literature regarding the role of DA for regulation of cell-turnover in the two neurogenic niches in the adult mammalian brain. Dementia linked to neurodegeneration in PD is more likely to be directly related to loss of DA and/or NA signalling in various target structures, while reduced neurogenesis and hippocampal atrophy (Camicioli et al., 2003; Höglinger et al., 2004; Laakso et al., 1996) may well occur in parallel, while still contributing to loss of cognitive function through independent mechanisms. Degeneration of other neurotransmitter systems may also play a role. For example, we have recently reported that both loss of A10 DA neurons of the VTA along with cholinergic dysfunction, including reduced acetylcholine levels in the hippocampus, is associated with dementia in PD (Hall et al., 2014). Studies in rodents have also shown that impairment of certain cognitive functions are insensitive to robust ablation of single transmitter systems but arise through simultaneous imbalance between multiple neurotransmitter systems (Wisman et al., 2008). Mechanisms of neurotransmitter-based regulation of cognition and hippocampal neurogenesis may well overlap under certain conditions but the present results do not suggest a simple relationship associated with the degeneration of the two most prominently affected transmitter systems in PD.

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