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Putative adult neurogenesis in two domestic pigeon breeds (Columba livia domestica): racing homer *versus* utility carneau pigeons

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Abstract

Generation of neurons in the brains of adult birds has been studied extensively in the telencephalon of song birds and few studies are reported on the distribution of PCNA and DCX in the telencephalon of adult non-song learning birds. We report here on adult neurogenesis throughout the brains of two breeds of adult domestic pigeons (Columba livia domestica), the racing homer and utility carneau using endogenous immunohistochemical markers proliferating cell nuclear antigen (PCNA) for proliferating cells and doublecortin (DCX) for immature and migrating neurons. The distribution of PCNA and DCX immunoreactivity was very similar in both pigeon breeds with only a few minor differences. In both pigeons, PCNA and DCX immunoreactivity was observed in the olfactory bulbs, walls of the lateral ventricle, telencephalic subdivisions of the pallium and subpallium, diencephalon, mesencephalon and cerebellum. Generally, the olfactory bulbs and telencephalon had more PCNA and DCX cells than other regions. Two proliferative hotspots were evident in the dorsal and ventral poles of the lateral ventricles. PCNA- and DCX-immunoreactive cells migrated radially from the walls of the lateral ventricle into the parenchyma. In most telencephalic regions, the density of PCNA- and DCX-immunoreactive cells increased from rostral to caudal, except in the mesopallium where the density decreased from rostral to middle levels and then increased caudally. DCX immunoreactivity was more intense in fibres than in cell bodies and DCX-immunoreactive cells included small granular cells, fusiform bipolar cells, large round and or polygonal multipolar cells. The similarity in the distribution of proliferating cells and new neurons in the telencephalon of the two breeds of pigeons may suggest that adult neurogenesis is a conserved trait as an ecological adaptation irrespective of body size.

Key Words: *nerve regeneration; proliferating cell nuclear antigen; doublecortin; immunohistochemistry; avian brain; racing homer; utility carneau; brain evolution; neural regeneration*

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Introduction

The process of adult neurogenesis occurs in both invertebrates and vertebrates, including humans (Eriksson et al., 1998; Bartkowska et al., 2010; Barnea and Pravosudov, 2011). In birds, generation of new neurons is limited to the dorsal and ventral reaches of the subventricular zone of the lateral ventricles (Alvarez-Buylla and Nottebohm, 1988; Alvarez-Buylla et al., 1998). Along these areas of the subventricular zone, proliferating cells form aggregates referred to as proliferative hotspots (Alvarez-Buylla et al., 1990a). From the walls of the lateral ventricles, new neurons migrate to various areas of the telencephalon which includes, but are not limited to, the high vocal centre, area X, the nidopallium caudale in song birds, and the hippocampus in both non-song birds and song birds (Paton and Nottebohm, 1984; Nottebohm, 1985; Alvarez-Buylla et al., 1994; Lipkind et al., 2002; Sherry and Hoshooley, 2010; Melleu et al., 2013).

In adult song birds, such as canaries, and food caching birds, such as the black capped chickadees, seasonal variations in the recruitment of new neurons in the high vocal centre and hippocampus, respectively, have been observed (Kirn and Nottebohm, 1993; Barnea and Nottebohm, 1994). There is evidence that adult neurogenesis varies in the members of the same species from different populations, for example in black capped chickadees (Chancellor et al., 2011) and mice (Kempermann et al., 1997).

Adult neurogenesis has been reported in the telencephalon of song birds including canaries (Alvarez-Buylla and Nottebohm, 1988; Balthazart et al., 2008) and zebra finches (Kim et al., 2006) and non-song birds such as chickens (Mezey et al., 2012), black capped chickadees (Sherry and Hoshooley, 2010), Japanese quails (Balthazart et al., 2010), ring doves (Ling et al., 1997) and rock pigeons (Melleu et al., 2013, 2016). Domestic pigeons (Columba livia domestica) of the order Columbiformes are descendants of the wild rock pigeon through domestication and selective breeding (Levi, 1986; Stringham et al., 2012). The racing homer possesses characteristics such as increased flight speed, large home ranges, improved spatial memory, and larger hippocampal formations and olfactory bulbs than other pigeon breeds (Bingman et al., 2006; Rehkämper et al., 2008; Mehlhorn and Rehkämper, 2009). Utility pigeons were selectively bred for their fast growth and large body size desirable for meat production. These different characteristics in the two breeds of domestic pigeons allow

examining and comparing the process of adult neurogenesis in closely related species with different behaviour repertoires.

Examination of multiple species with phylogenetically diverse traits and also in closely related species, or breeds, in different ecological niches may facilitate our understanding of the functions of adult neurogenesis and the factors contributing to variances in this neural trait amongst species (Jarvis et al., 2005; Amrein and Lipp, 2009; Bartkowska et al., 2010; Ihunwo and Olaleye, 2014). Based on this premise, we examined putative adult neurogenesis in the brains of two breeds of the adult domestic pigeon (Columba livia domestica), the racing homer pigeons and the utility carneau pigeons using the markers proliferating nuclear cell antigen (PCNA) and doublecortin (DCX) which label proliferating cells and immature neurones respectively (Hall et al., 1990; Brown et al., 2003; Melleu et al., 2013).

Materials and Methods

Animals and tissue processing

Four adult male domestic pigeon brains, two of each of racing homer and utility carneau pigeons were purchased from a local breeder and used in this study. The animals were bred in isolated cages according to the breeds in large social groups consisting of both males and females. The animals were supplied with water and food *ad libitum*.

The pigeons were 8 months old and sexually mature (Ling et al., 1997) and sex was determined by inspecting gonads during dissection. The animals were purchased and sacrificed during spring (November 1–8) in 2013. Animals were transported from the aviary to the University of the Witwatersrand Central Animal Services (CAS) in well ventilated, roomy plastic boxes. Animals were housed in the CAS and allowed to acclimatize for a period of 7 days with food and water supplied *ad libitum*. All animals were treated and used according to the guidelines of the University of the Witwatersrand Animal Ethics Committee (approval No. 2013/05/02B), which parallel those of the NIH guide for the care and use of animals in scientific experimentation.

Five minutes prior to being euthanized, all animals were given an intramuscular dose of heparin (0.5 mL) to prevent blood clotting. Animals were euthanized with an intraperitoneal injection of Euthapent (0.5–1 mL/kg) and the average body mass was 316.00 ± 23.33 g for racing homer pigeon and 542.35 ± 7.00 g for utility carneau pigeons. Animals were transcardially perfusion-fixed, initially with a rinse of 0.9% saline solution, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4) solution. Brains were carefully removed from the skull, post fixed overnight in 4% paraformaldehyde in 0.1 M PB. The brains were then weighed 2.20 ± 0.00 g for racing homer pigeons and 2.80 ± 0.14 g for utility carneau pigeons, cryopreserved in 30% sucrose in 0.1 M PB at 4°C for 3 days and then stored in an antifreeze solution at –20°C. Before sectioning, the tissue was allowed to equilibrate in 30% sucrose in 0.1 M PB at 4°C. The brains were frozen in dry ice and sectioned along the coronal plane into 50 µm thick sections, on a sliding microtome (Microm HM 430, Thermo Scientific, Schaumburg, IL, USA). One of three series of sections from each animal were taken and stained for Nissl substance, PCNA and DCX.

Nissl staining for cytoarchitecture

The first series of sections for Nissl staining were mounted on 0.5% gelatine coated slides, dried overnight, cleared in a 1:1 mixture of 100% ethanol and 100% chloroform and stained with a 1% cresylviolet solution (Sigma-Aldrich).

Immunohistochemistry for PCNA and DCX

The second and third series of sections from each animal were used for free floating PCNA and DCX immunohistochemistry. The sections were incubated in a solution containing 1.6% H_2O_2, 49.2% methanol and 49.2% 0.1 M PB, for 30 minutes to reduce endogenous peroxidase activity, which was followed by three 10-minute rinses in 0.1 M PB. To block unspecific binding, the sections were then pre-incubated for 2 hours, at room temperature under gentle shaking, in a blocking buffer solution consisting of 3% normal horse serum (for PCNA sections) or 3% normal rabbit serum (for DCX sections), 2% bovine serum albumin, and 0.25% Triton X-100 in 0.1 M PB. Following pre-incubation, the primary antibodies were added to the blocking buffer solution and the sections were incubated for 48 hours at 4°C under gentle shaking (PCNA, 1:500 dilution of mouse anti-PCNA, NCL-L-PCNA Leica Biosystems, Newcastle, United Kingdom; DCX, 1:300 dilution of goat anti-DCX antibody, C-18, Santa Cruz Biotechnology, Dallas, TX, USA) under gentle agitation. The primary antibody incubation was followed by three 10-minute rinses in 0.1 M PB and the sections were then incubated in a secondary antibody solution [PCNA sections, 1:1,000 dilution of biotinylated anti-mouse IgG (BA-2001, Vector Laboratories, Burlingame, CA, USA)] in 3% normal horse serum and 2% bovine serum albumin in 0.1 M PB; DCX sections, 1:1,000 dilution of anti-goat IgG (BA-5000, Vector Laboratories) in 3% normal rabbit serum and 2% bovine serum albumin in 0.1 M PB) for 2 hours at room temperature. This was followed by three 10-minute rinses in 0.1 M PB, after which sections were incubated for 1 hour in an avidinbiotin solution (1:125 in 0.1 M PB); Vector Laboratories), followed by three 10-minute rinses in 0.1 M PB. Sections were then transferred to a solution consisting of 0.05% diaminobenzidinete trahydrochloride in 0.1 M PBs for 5 minutes at room temperature, after which 3.3 µL 30% H_2O_2/mL solution was added. With the aid of a low power objective lens on a stereomicroscope (Leica MZ 7.5, Meyer instruments, Houston, TX, USA), the progression of the staining was visually followed and allowed to continue until a level was reached where the background staining could assist in analysis by matching to architectonic borders from Nissl stained sections without obscuring the immunopositive structures. The tissue was then rinsed twice more in 0.1 M PB before being mounted on glass slides coated with 0.5% gelatine and allowed to dry overnight. Once dry, the slides were placed in a solution of 70% ethanol for 2 hours and then dehydrated, cleared in xylene, and coverslipped with DPX mountant. To test for non-specific staining of the immunohistochemical protocol, the primary and secondary antibodies were omitted from random sections and no specific staining was evident. The observed immunostaining patterns support the specificity of the antibodies and are compatible with observations made in columbiformes and other avian species (Boseret et al., 2007; Charvet and Striedter, 2008; Melleu et al., 2013, 2016).

Data analysis

The Nissl stained sections were examined under a low power

objective lens on a stereomicroscope (Leica MZ 7.5, Meyer instruments) and the architectonic borders were traced using a camera Lucida fitted on the stereomicroscope (Leica MZ 7.5, Meyer instruments). The PCNA and DCX immunostained sections were then matched to the drawings from the Nissl stained sections and the location of immunopositive soma marked on the drawings. Selected drawings were then scanned and redrawn using the Canvas 8 Software (Deneba Software, Miami, FL, USA). The brain regions were identified and named in accordance with the stereotaxic atlas of the brain of the pigeon (Karten and Hodos, 1967) using the nomenclature recommended by the Avian Brain Nomenclature Forum (Reiner et al., 2004). Digital photomicrographs were captured using a digital camera (Axio Cam HRc, Zeiss, South Africa) mounted on the light microscope (Axioskop 2 plus, Zeiss, South Africa) and operating on the ZEN 2010 computer software (Axioskop 2 plus). No pixilation adjustments or manipulation of the captured images were undertaken, except for the adjustment of contrast, brightness, and levels using Adobe Photoshop 7. The distribution of PCNA and DCX-immunoreactive cells was described qualitatively using a low power (×5) objective lens on a light microscope (Axiostar plus, Zeiss, South Africa) and the densities of immunostained cells were visually compared and recorded on a scale ranging from low (+), moderate (++) to high (+++). A second observer was used to eliminate observer bias.

Results

General observations

We examined putative adult neurogenesis throughout the brains of the racing homer and utility carneau pigeon breeds using immunohistochemical techniques for the endogenous markers PCNA and DCX. The distribution of PCNA and DCX immunoreactivity was almost identical in both breeds, but a few minor differences were observed (**Table 1**). Due to this extensive similarity, we depicted only the mapping of the distribution of the PCNA and DCX-immunoreactive cells in the racing homer pigeons (**Figure 1**).

In both pigeons, PCNA and DCX immunoreactivity was observed in the olfactory bulbs, subdivisions of the pallium (hippocampus, hyperpallium apicalle, hyperpallium intercalatum, hyperpallium densocellulare, mesopallium, nidopallium, entopallium, arcopallium, dorsolateral cortical area, piriform cortex and the temporo-parieto-occipital area), subpallium (medial striatum, lateral striatum and the globus pallidus), diencephalon, mesencephalon (pretectum and tectum), and cerebellum. Generally, the telencephalic regions had a higher density of PCNA- and DCX-immunoreactive cells than other brain regions in both breeds of pigeons examined, while the lowest density of cells immunoreactive to both markers was observed in the cerebellum. In the majority of the telencephalic regions, the density of PCNA- and DCX-immunoreactive cells increased from rostral to caudal in both pigeon breeds, except in the mesopallium where the staining density of cells first decreased from rostral to middle levels and then increased at caudal levels. DCX immunoreactivity was more intense in fibres than in cell bodies. DCX-immunoreactive cells included small rounded cells, fusiform bipolar cells, and large round and/or polygonal multipolar cells in terms of cell shapes.

Table 1 Qualitative distribution and density of PCNA and DCX-immunoreactive structures in the brains of the racing homer and utility carneau pigeons

	Racing homer pigeon		Utility carneau pigeon	
Brain region	PCNA	DCX	PCNA	DCX
Subventricular zone	+++	++	+++	++
Olfactory bulb	+++	+++	+++	+++
Telencephalon				
Hippocampus	++	++	++	++
Hyperpallium apicale	+++	++	+++	+++
Hyperpallium intercalatum	++	++	++	++
Hyperpallium densocellulare	+	++	+	++
Mesopallium	++	++	++	++
Nidopalium	++	++	++	++
Entopallim	++	++	+++	+
Arcopallium	+++	++	+++	++
Striatum	++	+++	++	+++
Pallidum	++	++	++	++
Diencephalon	++	++	++	++
Mesencephalon				
Pretectum	++	+	++	++
Tectum	++	++	++	+
Metencephalon				
Cerebellum	+	++	+	+

+, ++, +++: Low, moderate, and high density of DCX-immunoreactive structures, respectively. PCNA: Proliferating cell nuclear antigen; DCX: doublecortin.

Distribution of PCNA-immunoreactive cells

Olfactory bulbs

The olfactory bulbs contained the highest density of PCNA-immunoreactive cells of all regions of the brain in both pigeon breeds. PCNA-immunoreactive cells were observed at high density in the internal granular layer, mitral cell layer and external plexiform layer, while they were found at a lower density in the glomerular layer and olfactory nerve layer (**Figure 2A** and **C**).

Subventricular zones of the lateral, third, fourth, and tectal ventricles

PCNA-immunoreactive cells at a high density were observed in the walls of the lateral, third, fourth and tectal ventricles, forming an uninterrupted layer, one to four cells thick, of cells with dark-stained nuclei in both pigeon breeds. Proliferative hotspots were observed in both dorsal and ventral poles of the lateral ventricle, mostly in rostral levels of the brain (**Figure 3A** and **C**). PCNA-immunoreactive cells at a high density was observed in the racing homer pigeon associated with the walls of the lateral ventricle abutting the medial striatum, nidopallium, nidopallium caudale and hyperpallium apicale when compared to the utility carneau pigeon.

Hyperpallium, mesopallium, nidopallium, and entopallium

In the pallial regions (hyperpallium apicale, hyperpallium intercalatum, hyerpallium densocellulare, mesopallium and nidopallium), PCNA-immunoreactive cells were observed in high density along the cortical margins and regions adjacent

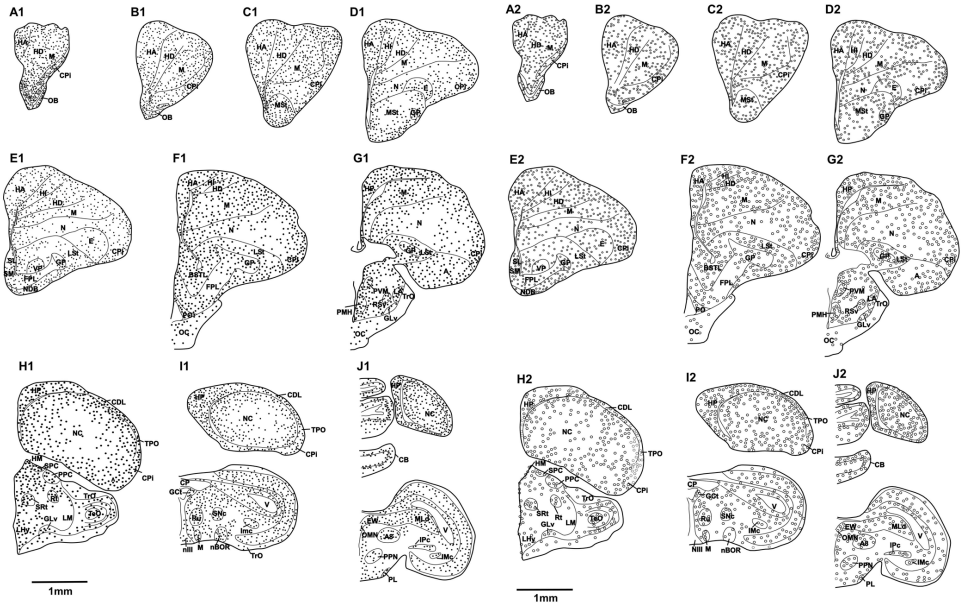

Figure 1 Reconstruction of coronal sections through one half of the brain of the racing homer pigeon showing areas of the detected PCNA-immunoreactive cells and DCX-immunoreactive immature and migrating neurons.

(A1–J1 and A2–J2) Black dots represent proliferating cells (PCNA-immunoreactive cells) and black circles indicate DCX-immunoreactive cells. Each drawing of the coronal section is approximately 1,500 μm apart, with A1 and A2 being the most rostral section and J1 and J2 being the most caudal. All architectonic drawings were traced and drawn from selected Nissl stained sections using camera Lucida mounted on the stereomicroscope (Leica MZ 7.5, Meyer instruments, Houston, TX, USA). The PCNA- and DCX-immunostained sections were then matched to the drawings from the Nissl-stained sections and the location of immunopositive soma marked on the drawings. The drawings were then scanned with a color document scanner (DS-50000, Epson, South Africa) and reproduced using the Canvas 8 software (Deneba Software, Miami, FL, USA). A: arcopallium; BSTL: lateral part of the bed nucleus of the stria terminalis; CB: cerebellum; CDL: dorsolateral cortical area; CP: posterior commissure; CPi: piriform cortex; E: entopallium; EW: nucleus of Edinger-Westphal; FPL: fasciculus prosencephali lateralis; GCt: griseum centrale; GLv: nucleus geniculatus lateralis, pars ventralis; GP: globus pallidus; HA: hyperpallium apicale; HD: hyerpallium densocellulare; HI: hyperpallium intercalatum; HM: nucleus habenularis medialis; HP: hippocampus; IMc: nucleus isthmi, pars magnocellularis; IPc: nucleus isthmi, pars parvocellularis; LA: nucleus lateralis anterior thalami; LHy: lateral hypothalamus; LM: nucleus lentiformis; LSt: lateral striatum; Mm: mammillary region; M: mesopallium; N: nidopallium; MLd: nucleus mesensephalicus lateralis, pars dorsalis; MSt: medial striatum; nBOR: nucleus of theoptic root; NC: nidopalliun caudale; NDB: nucleus diagonalis Broca; nIII: oculomotor nerve; OB: olfactory bulb; OC: optic chaism; OMN: oculomotor nucleus; PL: nucleus pontis lateralis; PMH: nucleus medialis hypothalamic posterioris; PO: preoptic region; PPC: nucleus principalis precommissuralis; PPN: pedunculopontine tegmental nucleus; PVM: nucleus periventicularis magnocellularis; RSv: nucleus reticularis superior, pars ventralis; Rt: nucleus rotundus; Ru: nucleus ruber; SL: lateral septum; SM: medial septum; SNc: substantia nigra, pars compacta; SPC: nucleus superficialis parvocellularis; SRt: nucleus subrotundus; TeO: optic tectum; TPO: temporo-parieto-occipital area; TrO: optic tract; V: ventricle; VP: ventral pallidum.

to the lateral wall of the lateral ventricle, but this density was obviously reduced in the core regions of hyerpallium densocellulare, mesopallium and nidopallium in both pigeon breeds (**Figure 1**). At caudal levels, the nidopallium caudale exhibited an increased density of PCNA-immunoreactive cells at the regions adjacent to the walls of the lateral ventricle and also towards the lateral cortex. The core of the NC had scattered PCNA-immunoreactive cells. In the entopallium, there was a higher density of PCNA-immunoreactive cells rostrally, but the density gradually decreased towards the caudal levels of the telencephalon in all pigeons. In all these telencephalic regions, the density of PCNA-immunoreactive cells appeared greater in the racing homer pigeons than in the utility carneau pigeons.

Hippocampus, piriform cortex, and dorsolateral cortical area

The hippocampus in the pigeons studied herein was defined according to the descriptions provided by Atoji and Wild (2004). PCNA-immunoreactive cells were sparsely distributed throughout the medial layer of the ventral hippocampus, while a medium density of PCNA-immunoreactive cells was observed in the triangular area (Tr), the lateral layer (**Figure 4A** and **D**), and the dorsomedial region in both pigeons. The dorsolateral region of the hippocampus exhibited the highest density of PCNA-immunoreactive cells in both pigeon breeds. Throughout dorsolateral region of the hippocampus, the PCNA-immunoreactive cells were homogeneously distributed, and these cells were observed to continue into the dorsolateral cortical area. The dorsolateral cortical area exhibited a high density of PCNA-immunoreactive cells, especially in areas adjacent to the dorsolateral wall of the lateral ventricles in the caudal levels of this region. Ventrolaterally from dorsolateral cortical area towards the temporo-parieto-occipital cortex, the density of the PCNA-immunoreactive cells gradually decreased to a moderate density. The piriform cortex exhibited a high density of PCNA-immunoreactive cells throughout the rostro-caudal axis of the telencephalon in both pigeon breeds.

Striatum, septum, and arcopallium

In the striatum of both pigeonbreeds, a moderate density of PCNA-immunoreactive cells was observed in the medial striatum adjacent to the ventrolateral wall of the lateral ventricle, while the lateral striatum exhibited a low density of PCNA-immunoreactive cells. Generally in the striatum, there was a rostro-caudal decline in the density of PCNA-immunoreactive cells. In the racing homer pigeon, the medial septum housed a medium density of PCNA-immunoreactive cells, the density of which lessened towards the most medial margin of this nucleus. The SM of the utility carneau pigeon had very densely packed PCNA-immunoreactive cells with large nuclei, which was prominent at the level of the anterior commissure (**Figure 5A**, **C** and **D**). In the lateral septum of both pigeonbreeds, a higher density of PCNA-immunoreactive cells was

observed when compared to the medial septum, and within this nucleus there was a rostrocaudal decline in PCNA-immunoreactive cellular density. The arcopallium in both pigeon-breeds exhibited a moderate density of PCNA-immunoreactive cells along the cortical margins and towards the ventral parts of the nidopallium caudale, while the central areas had a very low to absent density of PCNA-immunoreactive cells.

Diencephalon, optic chiasm, and anterior commissure
The diencephalon exhibited a high density of PCNA-immunoreactive cells in the various nuclei compared to the surrounding tissues. This was observed in the paraventricular nuclei which include nucleus preopticus medialis, periventricular magnocellular nucleus and nucleus medialis hypothalamic posteriolis medially, the dorsal margin in the nucleus dorsomedialis anterior thalami, nucleus dorsolateralis anterior thalami, pars lateralis nuclei and the lateral margin in the nucleus rotundus and nucleus geniculatus lateralis, pars ventralis. Further laterally the optic tract housed a moderate density of PCNA-immunoreactive cells. In the core regions of the hypothalamus, PCNA-immunoreactive cells were either absent or in a low density. The optic chiasm of both pigeon breeds exhibited a cluster of PCNA-immunoreactive cells which increased slightly in density adjacent to the ventral pole of the subventricular zone of the third ventricle (**Figure 6A** and **C**). There were few scattered PCNA-immunoreactive cells in the anterior commissure.

Pretectum, tectum, posterior commissure, and cerebellum
The pretectum in both pigeon breeds exhibited a high density of PCNA-immunoreactive cells in the dorsal margins and ventrally in the nucleus subpretectalis. Laterally, the layers of the optic tectum had a low density of PCNA-immunoreactive cells, and in the core regions only a few PCNA-immunoreactive cells could be observed. Within the optic tectum, a high density of PCNA-immunoreactive cells was observed in the paraventricular nuclei closer to the fourth ventricle medially, the layers of the optic tectum laterally in areas adjacent to the tectal ventricle and the nucleus of the basal optic tract ventrally in both pigeon breeds. In the utility carneau pigeons, the nucleus principalis precommissuralis near the subventricular zone of the fourth ventricle had a high density of PCNA-immunoreactive cells that exhibited very large cell bodies, but these were not observed in the racing homer pigeons. PCNA-immunoreactive cells were scattered in the central nuclei of the optic tectum, such as the nucleus spiriformislateralis, but were absent in the nucleus subpretectalis. In the posterior commissure of both pigeon breeds, there were a few PCNA-immunoreactive cells that were intercalated with the commissural fibres. In the cerebellum of both pigeon breeds, PCNA-immunoreactive cells were observed in high density in the Purkinje cell layer and the occasional PCNA-immunoreactive cells were observed in the molecular and granule cell layers (**Figure 7A** and **C**).

Distribution of DCX-immunoreactive cells
DCX-immunoreactive cells were identified throughout the brains of the two adult pigeon breeds and were generally small fusiform shaped, bipolar and round multipolar cells with slight variations in size in the different brain regions. Any size and/or shape variations are noted in relevant regions.

Olfactory bulb
The olfactory bulb was the region that contained the highest density of DCX-immunoreactive structures in both pigeon breeds. DCX-immunoreactive cells and fibres were seen in high density in the inner layers of the olfactory bulb, but at a lesser density in the outer two layers (**Figure 2B** and **D**). DCX-immunoreactive cells appeared to be in the process of migrating from the olfactory bulbs into the pallial regions of hyperpallium apicalle and hyperpallium densocellulare.

Subventricular zone
The subventricular zone of the lateral and third ventricles evinced 3 to 4 layers of DCX-immunoreactive cells in both breeds, which intermittently formed cell clusters of intensely stained cells at high density. This arrangement was more apparent on the lateral wall of the lateral ventricle than on the medial wall. The DCX-immunoreactive fibres emanating from the subventricular zone were oriented either parallel or orthogonal to the ventricular wall (**Figure 3B** and **D**).The fourth and tectal ventricles of the racing homer pigeon exhibited a continuous layer of DCX-immunoreactive cells without cell clustering, whereas in the utility carneau pigeon, the tectal ventricles had more DCX-immunoreactive fibres rather than cells.

Hyperpallium, mesopallium, nidopallium, and entopalliuma
There was a moderate density of DCX-immunoreactive cells in the divisions of the hyperpallium (hyperpallium apicale, hyperpallium intercalatum and hyperpallium densocellulare), with these cells and fibres being of higher density at the periphery and very low density in central areas (**Figure 1**). At more rostral levels, the hyperpallium densocellulare appeared to receive migrating neuroblasts from the olfactory bulb. A similar group of apparently migrating neuroblasts was observed at caudal levels, towards the anterior commissure, where they appeared to migrate from the walls of the lateral ventricle into the hyperpallium apicalle. The mesopallium and nidopallium exhibited a moderate density of DCX-immunoreactive cells and fibres, with higher densities of DCX-immunoreactive structures observed in the peripheral areas adjacent to the lateral wall of the lateral ventricle medially, the dorsoventral wall of the lateral ventricle dorsolaterally and piriform cortex ventrolaterally (**Figure 1**). At the caudal level, posterior to the anterior commissure, the nidopallium caudale exhibited a similar density of DCX-immunoreactive cells and fibres as seen in the nidopallium and mesopallium. Higher densities of DCX-immunoreactive cells and fibres were observed near the walls of the lateral ventricle both medially and dorsolaterally in the nidopalliun caudale, the piriform cortex ventrolaterally and the arcopallium ventrocaudally. The core areas of mesopallium, nidopallium and nidopalliun caudale had a low to absent density of DCX-immunoreactive structures, but the DCX-immunoreactive cells in these areas were characterised by large round or polygonal somata and thick branching fibres. The entopallium contained a moderate density DCX-immuoreactive cells and fibres in the racing homer pigeon but very low density of DCX-immunoreactive cells and fibres in the

utility carneau pigeon. Generally, there was a rostro-caudal increase in the density and morphological complexity of the DCX-immunoreactive cells in the nidopalliun caudale.

Hippocampus, piriform cortex, and dorsolateral cortical area
The medial region of the ventral hippocampus had sparsely distributed DCX-immunoreactive cells and fibres, while the triangular and the lateral regions exhibited a medium density of DCX-immunoreactive cells and fibres in both pigeon breeds (**Figure 4B**, **C**, **E** and **F**). In the dorsomedial region of the hippocampus, DCX-immunoreactive cells were observed at a higher density in the central area and towards the dorsomedial wall of the lateral ventricle. There were low-density DCX-immunoreactive cells and fibres in the dorsolateral region of the hippocampus, close to the border with dorsomedial region medially. The density of DCX-immunoreactive cells in the dorsolateral region gradually increased towards the dorsolateral cortical region.

In the dorsolateral cortical region, there were moderate-density DCX-immunoreactive cells and fibres. At the caudal level, the dorsolateral cortical region had higher density DCX-immunoreactive cells and fibres in areas adjacent to the dorsolateral margin of the lateral ventricle. The piriform cortex had higher density intensely stained DCX-immunoreactive cells and fibres in both pigeon breeds.

Striatum, septum, and arcopallium
In the striatum, the medial striatum exhibited a medium density of DCX-immunoreactive structures with a higher density of stained structures in areas abutting the ventral parts of the lateral wall of the lateral ventricle, while the density of immunoreactive structures was less in the core regions. Ventral to the striatum, there was a high density of DCX-immunoreactive cells in the olfactory tubercle and nucleus of the fascicurius diagonalis Brocae. The lateral striatum had moderate to low density of DCX structures in both pigeon breeds, but a high density of DCX-immunoreactive structures was observed in the globus pallidus and interpeduncular nucleus at levels caudal to the anterior commissure.

In the racing homer pigeon, the septal nuclear complex exhibited a low density of DCX-immunoreactive structures in the medial septum, whereas the lateral septum had a moderate density of DCX-immunoreactive structures, although this density was reduced at the caudal level. In the utility carneau pigeon, the medial septum exhibited a moderate density of DCX-immunoreactive cells and fibres (**Figure 5B**). There was a moderate density of DCX-immunoreactive cells and fibres in the arcopallium, especially along the ventral margins in both pigeon breeds, but the core areas had less dense staining in the utility carneau pigeon.

Diencephalon, optic chiasm, and anterior commissure
The diencephalon exhibited a moderate density of DCX-immunoreactive cells and fibres in the paraventricular regions, near the third ventricle, in the nucleus preotpicus medialis, and the nucleus medialis hypothalamic posterioris (**Figure 1**). Dorsally, a moderate density of DCX-immunoreactive structures were observed in the stria medullaris and some dorsal thalamic nuclei, including the nucleus dorsolateralis anterior, pars medialis and nucleus dorsolateralis, pars lateralis, and laterally in the thalamus, a low density of DCX-immunoreactive structures were evident in the nucleus geniculatus lateralis, pars ventralis, nucleus lateralis anterior thalami and nucleus rotundus. The subthalamic nuclei nuclei were devoid of DCX-immunoreactive. The optic chiasm of both pigeon breeds exhibited a cluster of DCX-immunoreactive cells and fibres that spread lateral to the optic tract (**Figure 6B** and **D**). In the utility carneau pigeon, the anterior commissure exhibited a few scattered DCX-immunoreactive cells and fibres, but this was not evident in the racing homer pigeon.

Pretectum, tectum, posterior commissure, and cerebellum
In the mesencephalic pretectum, a moderate density of DCX-immunoreactive structures were observed in the tegmentum in the supraoptic decussation. In the tectal region of the mesencephalon, a moderate density of DCX-immunoreactive structures were observed medially in the nucleus ruber, griseum centralis, oculomotor nucleus and the Edinger-Westphal nucleus, dorsally in the nucleus harbenularis medialis (HM) and nucleus spiriformis lateralis, and ventrally in the nucleus tuberis, pedunculopontine tegmental nucleus and the nucleus of the basal optic root. The Edinger-Westphal nuclei and nucleus ruber contained large round DCX-immunoreactive cells. DCX-immunoreactive structures were scattered in the nucleus rotundus and in the layers of the optic tectum. The posterior commissure exhibited a few DCX-immunoreactive structures especially on margins adjacent to the walls of the cerebral aqueduct. In the cerebellum, the granule cell layer of the utility carneau pigeon had a moderate density of DCX-immunoreactive structures, while in both pigeon breeds, the Purkinje cell layer had a high density of DCX-immunoreactive structures. The dendrites of the DCX-immunoreactive cells projected into the molecular layer of the cerebellum (**Figure 7B** and **D**).

Discussion

General considerations regarding PCNA- and DCX-immunoreactivity in pigeons

In the current study, we observed potential adult neurogenesis in the brains of the two breeds of the adult pigeons, the racing homer and the utility carneau. Putative adult neurogenesis was demonstrated through the expression of the PCNA and DCX molecules, revealed using immunohistochemical techniques, throughout the brains of both pigeon breeds. The two antibodies were localized in similar brain regions of the pigeon breeds with putative adult neurogenesis including the olfactory bulb, telencephalic subdivisions (hyperpallium apicale, hyperpallium intercalatum, hyerpallium densocellulare, mesopallium, nidopallium, nidopalliun caudale, entopallium, medial striatum, lateral striatum, lateral septum, medial septum), the subventricular zone, the diencephalon, the mesencephalon and the cerebellum.

PCNA is a marker for proliferating cells that is essential for DNA replication and repair. It is synthesized during the early G1 and S phases of the cell cycle, abundant during the S phase and declines during G2/M phase (Kurki et al., 1988; Hall et al., 1990). In the two pigeon breeds studied, the density of PCNA-immunoreactive cells was higher in the olfactory bulbs, subventricular zone, nidopalliun caudale and the piriform

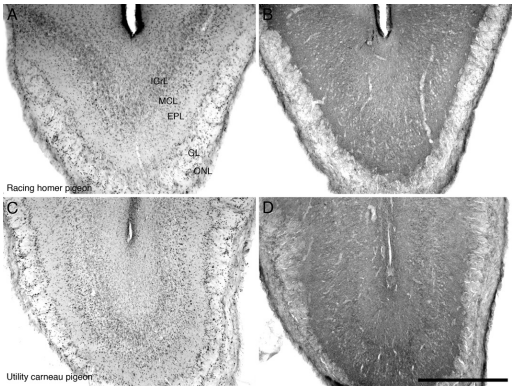

Figure 2 Photomicrographs showing the olfactory bulbs of the racing homer pigeon (A, B) and the utility carneau pigeon (C, D).
The distribution of PCNA immunoreactivity is shown in the cellular layers of the olfactory bulb of the racing homer pigeon (A) and utility carneau pigeon (C). The distribution of DCX immunoreactivity is shown in the olfactory bulbs of the racing homer pigeon (B) and utility carneau pigeon (D). Scale bar: 500 μm. Digital photomicrographs were captured using a digital camera (Axio Cam HRc, Zeiss, South Africa) mounted on the light microscope (Axioskop 2 plus, Ziess, South Africa) and operating on the ZEN 2010 computer software (Zeiss, South Africa). IGrL: Internal granular layer; MCL: mitral cell layer; EPL: external plexiform layer; GL: glomerular layer; ONL: olfactory nerve layer.

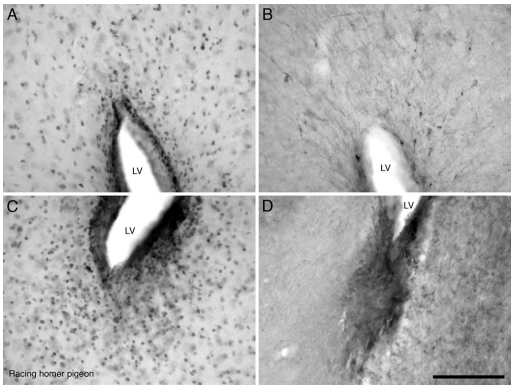

Figure 3 Photomicrographs showing the distribution of PCNA (A, C) and DCX (B, D) immunoreactivity in the dorsal and ventral reaches of the subventricular zone of the lateral ventricle (LV) in the racing homer pigeon.
Note the intense PCNA staining in the subventricular zone of the lateral ventricle and also migrating DCX-immunoreactive cells from the subventricular zone of the lateral ventricle into the parenchyma of the telencephalon. Scale bar: 100 μm. Digital photomicrographs were captured using a digital camera (Axio Cam HRc, Zeiss, South Africa) mounted on the light microscope (Axioskop 2 plus, Ziess) and operating on the ZEN 2010 computer software (Zeiss).

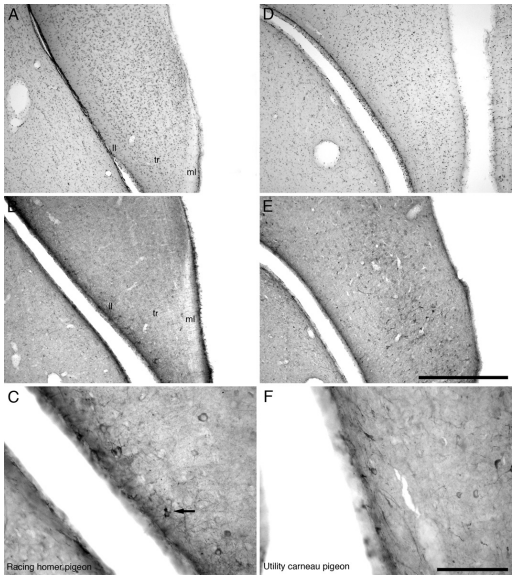

Figure 4 Photomicrographs showing the distribution of proliferating cell nuclear antigen (PCNA) and doublecortin (DCX) in the hippocampus of the racing homer and utility carneau pigeons.
The distribution of PCNA immunoreactivity in the ventral subdivisions of the hippocampus showing mild PCNA immunoreactivity in the medial layer (ml), moderate density in the lateral layer (ll) and dense reactivity in the triangular area (tr) in the racing homer (A) and the utility carneau pigeons (D). The distribution of DCX immunoreactivity in the ventral division of the hippocampus showing a moderate concentration of DCX-immunoreactive cells and fibres in the ll and tr and mild distribution in the ml of the ventral hippocampus of the racing homer (B) and utility carneau pigeons (E). A higher magnification showing DCX-immunoreactive fibres and round unipolar cells in the ll and triangular area of the ventral hippocampus in the racing homer (C) and utility carneau pigeons (F); arrow shows a DCX-immunoreactive cell. Scale bar in E, valid for A, B, D, E: 500 μm. Scale bar in F, valid for C and F: 100 μm. Digital photomicrographs were captured using a digital camera (Axio Cam HRc, Zeiss) mounted on the light microscope (Axioskop 2 plus, Ziess) and operating on the ZEN 2010 computer software (Zeiss).

cortex, but was generally less dense in the potential adult neurogenic sites such as the diencephalon, mesencephalon and the cerebellum. This pattern of cellular staining is similar to that seen in other non-mammalian vertebrates including teleost fish (Zupanc et al., 2005), amphibians (Cerri et al., 2009), and chickens (Hannan et al., 1999). However, BrdU-positive cells in the subtelencephalic regions in canaries did not co-express neuron specific marker Hu protein suggesting that the proliferating cells in this region are of glial cell lineage (Vellema et al., 2010). In the subventricular zone of the lateral ventricles, a high density of PCNA-immunoreactive cells was observed in the ventral and dorsal borders of the ventricles. In these regions, the putative proliferating cells formed intermittent clusters that were consistent with the proliferative 'hotspots' identified by Alvarez-Buylla et al. (1990b).

Proliferating cells were also observed throughout the telencephalon and four possible reasons can be inferred for this: (1) these putative proliferating cells may have migrated from the subventricular zone throughout the parenchyma (Almli and Wilczynski, 2007); (2) resident progenitors may be proliferating (Alvarez-Buylla et al., 1990a, b; Doetsch et al., 1999; Zupanc et al., 2005); (3) putative proliferating cells may be resident glial cells (Cerri et al., 2009); and (4) putative proliferative cells may be both neuronal and glial cells undergoing DNA replication and repair (Hall et al., 1990).

DCX is a microtubule associated protein that plays a key role in the migration of neurons during development and in post-mitotic neurons undergoing migration, remodelling of their dendritic processes and synaptogenesis in adulthood (Gleeson et al., 1999; Capes-Davis et al., 2005; Couillard-Despres et al., 2005). In this study, we observed that DCX expression was widespread and heterogeneous in the brains of the two breeds of domestic pigeons (Columba livia domestica). The density of DCX-immunoreactive cells and fibres was most intense in the olfactory bulbs, associated olfactory cortices, telencephalic pallial structures including the hippocampus, the striatum and the nidopalliun caudale, but less dense in subtelencephalic structures such as the diencephalon, mesencephalon and cerebellum. This widespread expression of DCX

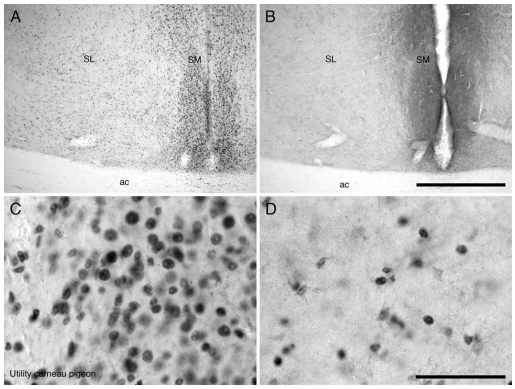

Figure 5 Photomicrographs showing distribution of proliferating cell nuclear antigen (PCNA)- and doublecortin (DCX)-immunoreactive cells in the septum of the utility carneau pigeon.
(A) Distribution of PCNA-immunoreactive cells in the septum. (B) Distribution of DCX-immunoreactive cells and fibres in the septum. (C) A higher magnification of the medial septum (SM) showing dense PCNA-immunoreactive cells. (D) A higher magnification of the lateral septum (SL) showing sparse PCNA-immunoreactive cells. Scale bar in B, valid for A and B: 500 μm. Scale bar in D, valid for C and D: 50 μm. Digital photomicrographs were captured using a digital camera (Axio Cam HRc, Zeiss, South Africa) mounted on the light microscope (Axioskop 2 plus, Ziess, South Africa) and operating on the ZEN 2010 computer software (Zeiss).

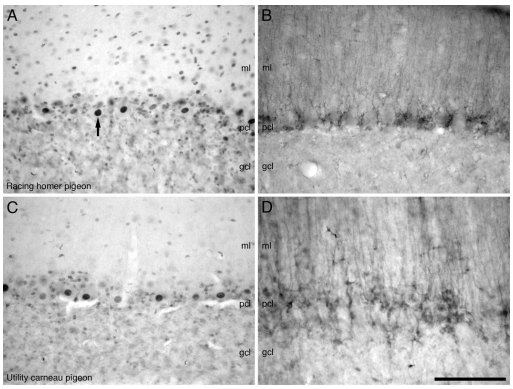

Figure 7 Photomicrographs of the cerebellar cortex of the racing homer (A, B) and utility carneau pigeons (C, D).
The distribution of PCNA-immunoreactive cells in the granule cell layer (gcl), Purkinje cell layer (pcl) and the molecular cell layer (ml) of the cerebella cortex in the racing homer (A) and utility carneau pigeons (C); arrow shows a PCNA-immunoreactive cell. The distribution of DCX-immunoreactive cells and fibres in the layers of the cerebellar cortex in the racing homer pigeon (B) and utility carneau pigeon (D). Note the large and prominent PCNA immunoreactivity cells in the pcl of both pigeon breeds. Scale bar: 100 μm. Digital photomicrographs were captured using a digital camera (Axio Cam HRc, Zeiss, South Africa) mounted on the light microscope (Axioskop 2 plus, Ziess) and operating on the ZEN 2010 computer software (Zeiss). PCNA: Proliferating cell nuclear antigen; DCX: doublecortin.

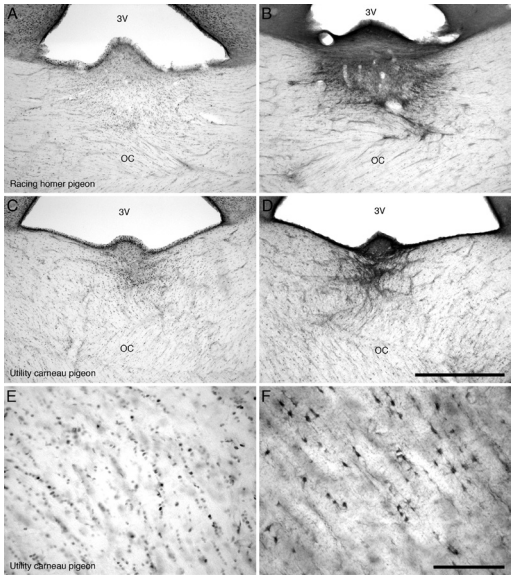

Figure 6 Photomicrographs of proliferating cell nuclear antigen (PCNA) (A, C, E) and doublecortin (DCX) (B, D, E) immunostained sections through the dorsal aspect of the optic chiasm (OC), at its border with the floor of the third ventricle (3V) in racing homer (A, B) and utility carneau (C, D, E, F) pigeons.
These images depict a potential neurogenic zone, or bulge, at the dorsal midline of the optic chiasm that appears to produce newly generated cells (shown by PCNA immunostaining) and appears to migrate (as shown by DCX immunostaining) into the optic chiasm, optic nerve (E, F) and optic tract of both pigeon types. Scale bar in D, valid for A–D: 500 μm; scale bar in F, valid for E and F: 100 μm. Digital photomicrographs were captured using a digital camera (Axio Cam HRc, Zeiss, South Africa) mounted on the light microscope (Axioskop 2 plus, Ziess, South Africa) and operating on the ZEN 2010 computer software (Zeiss).

was consistent with observations made in other non-song birds such as the developing domestic chick brain (Capes-Davis et al., 2005), in song birds such as adult canaries (Boseret et al., 2007; Vellema et al., 2014) and in subadult zebra finches (Kim et al., 2006). Moderate intensity DCX expression in the prosencephalon has been reported in numerous studies (Kim et al., 2006; Boseret et al., 2007; Vellema et al., 2010, 2014; Mezey et al., 2012; Melleu et al., 2013). The majority of pioneering studies on adult neurogenesis in birds reported that the process was only limited to the prosencephalon (Alvarez-Buylla and Nottebohm, 1988; Kirn et al., 1994). Few studies reported the presence of DCX expression in the subtelencephalic regions in pathologically normal adult species (Boseret et al., 2007; Balthazart et al., 2008; Vellema et al., 2014). However, increased levels of adult neurogenesis have been reported in the subtelencephalic regions under injury and pathological conditions (Cao et al., 2002; Chen et al., 2006).

We also noted that that the areas with the densest expression of DCX-immunoreactive structures were adjacent to the walls of the ventricles in all brain regions, suggesting that new neurons were generated in the subventricular zone and migrated to their target regions. Migration of newly born neurons follows well defined routes in mammalian species, where they migrate from the subgranular zone of the dentate gyrus to the granule cell layer, and the subventricular zone of the lateral ventricle to the olfactory bulbs through the rostral migratory stream (Lois and Alvarez-Buylla, 1994; Chawana et al., 2013; Patzke et al., 2013). In birds studied to date, including the current study, the migratory routes appear diffuse, with many regions of the brain appearing to incorporate new neurons. Despite this, as in mammals, a rostral migratory-like stream has been identified in rock pigeons, seen to stretch from the caudal nidopallium to the rostral mesopallium, hyperpallium apicale and densocellulare (Melleu et al., 2013). New neurons migrate from the subventricular zone into the brain parenchyma through radial glia scaffolding (Alvarez-Buylla and Nottebohm, 1988; Alvarez-Buylla et al., 1988). This phenomenon led to the identification of DCX-immunoreactive neuroblasts with the morphology typical of migrating cells (small round and fusiform bipolar cells) (Alvarez-Buylla

and Nottebohm, 1988; Alvarez-Buylla et al., 1988; Melleu et al., 2013) in the parenchyma away from the subventricular zone. However, DCX immunoreactivity was also observed in post-migratory cells of the Purkinje cell layer of the cerebellum in the current study (Capes-Davis et al., 2005) and in areas of the telencephalic structures where large round and polygonal multipolar cells with elaborate dendritic processes were also seen to express DCX. In the rock pigeons, these mature-like DCX-immunoreactive cells did not co-express neuron specific marker NeuN (Melleu et al., 2013), suggesting that these cells were immature neurons establishing synaptic contacts in local circuits (Gleeson et al., 1999). Despite this, DCX-immunoreactive cells outside the subventricular zone in the mouse brain were found to express NeuN (Yang et al., 2004), indicating that DCX is also expressed in mature neurons that undergo dendritic arborization and axonal growth (Nacher et al., 2001; Brown et al., 2003).

DCX expression has been found to persist for more than 20 days in cells in the adult canary brain (Balthazart et al., 2008), explaining its presence in morphologically distinct neurons in the cores of the hippocampus and other pallial structures as observed in the current study, and in rock pigeons (Melleu et al., 2013). This suggests that DCX-immunoreactive cells in deeper areas of the telencephalic parenchyma represent earlier generations of new-born neurons that completed migration prior to the sacrifice of the animal. In addition, the concept also explains the abundance of DCX-immunoreactive cells observed in the telencephalon of avian species in previous studies (Kim et al., 2006; Melleu et al., 2013; Vellema et al., 2014).

Olfactory neurogenesis in pigeons

The olfactory bulbs are known to continuously incorporate new neurons in adulthood in various mammalian species (Kempermann, 2012). We found that the olfactory bulbs and associated brain areas such as the piriform cortex, olfactory tubercles and dorsolateral cortical areas exhibited high densities of PCNA- and DCX-immunoreactive structures. Similar findings were reported in other bird species such as domestic chicks and rock pigeons (Mezey et al., 2012; Melleu et al., 2013). In contrast to olfactory neurogenesis in mammals where new neurons migrate long distances from the subventricular zone, the presence of PCNA- and DCX-immunoreactive cells in the walls of the olfactory ventricle and adjacent olfactory bulb layers suggests that new neurons are generated and incorporated in the olfactory bulbs locally rather than by migration. Melleu et al. (2013) also found conspicuous BrdU labelled cells in the walls of the olfactory ventricles. Retrograde tracing studies in pigeons have shown that olfactory bulb neuronal fibres project to the piriform cortex, medial septum (Reiner and Karten, 1985) and the hyperpallium densocellulare (Patzke et al., 2011). Similar to a report by Melleu et al. (2013) in rock pigeons, we also observed a stream of DCX-immunoreactive cells that appeared to be migrating from the olfactory bulbs to the hyperpallium densocellurare. Processing of the olfactory sense varies greatly in different orders of birds studied to date (Balthazart and Taziaux, 2009). In homing pigeons, olfactory processing is used during navigation from unfamiliar places to home lofts (Patzke et al., 2010) by following familiar airborne odours (Papi et al., 1972). In other species of the order columbiformes, olfactory cues were associated with parental behavior in ring doves (Bonadonna et al., 2003), social and reproductive behaviors in rock pigeons (Patzke et al., 2010), feeding behaviors in domestic chicken (Jones and Roper, 1997), and food localization in vultures (Graves, 1992) and sea birds (Grubb, 1972). Thus, process of adult neurogenesis and neural plasticity in birds might aid in replenishing neurons and renewing circuits to facilitate adaptation into natural environments using the olfactory sense.

Hippocampal neurogenesis

The density of PCNA- and DCX-immunoreactive structures was lower in the hippocampus of domestic pigeons especially in the medial layer of the ventral hippocampus than in the surrounding brain parenchyma. The triangular region and the lateral layers exhibited a moderate density of PCNA- and DCX-immunoreactive structures. The dorsolateral regions of the hippocampus had a higher density of PCNA- and DCX-immunoreactive structures than the dorsomedial region. Similar differential densities were also reported in rock pigeons (Melleu et al., 2013), but domestic pigeons have been found to have a significantly larger hippocampus than the ancestral wild rock pigeons (Rehkämper et al., 2008), indicating that the similarity in the extent of adult hippocampal neurogenesis between rock and domestic (homing) pigeons may be best ascertained by quantitative methods.

In birds, adult hippocampal neurogenesis has been associated with the integration of new experiences, such as new songs, and the clearance of old memories (Nottebohm, 1981; Wilbrecht and Kirn, 2004; Kempermann, 2008), thus, the process may serve to prevent interference between old and new memories, especially in food caching birds that need to recall both old and new food caches accurately (Barnea et al., 2006; Wiskott et al., 2006; Pravosudov and Smulders, 2010). Kempermann (2008) also suggested that AHN may contribute to a neurogenic reserve that can be incorporated only when there is a need for new learning. Generally, AHN decreases with advancing age in both mammals and non-mammalian vertebrates (Barnea and Nottebohm, 1994; Kempermann et al., 1997; Eriksson et al., 1998; Kim et al., 2006); however, in some reptiles and fish, AHN appears to contribute, in part, to the continuous growth of the nervous system (Zupanc and Horschke, 1995; Ngwenya et al., 2013). Barnea and Nottebohm (1994) found that AHN varies seasonally, with more neuronal accumulation occurring in the autumn in black capped chickadees. The hippocampal formation has also been associated with spatial memory dependent tasks related to learning and acquisition of a spatial map and its operation in homing pigeons (Bingman et al., 2005), migration and food caching (Bingman and Cheng, 2005; Hoshooley and Sherry, 2007).

Neurogenesis in other brain areas

Our observation of wide spread DCX immunoreactivity throughout the brains of domestic pigeons is similar to that reported for adult chicken, canaries and zebra finches (Capes-Davis et al., 2005; Kim et al., 2006; Boseret et al., 2007; Balthazart et al., 2008). In the pallial structures, PCNA and DCX-immunoreactive structures were apparent in the margins of the hyperpallium apicale, mesopallium, nidopallium and nidopalliun caudale rather than their core areas. In addition, we observed PCNA and DCX immunoreactivity in

the septum, striatum and the entopallium in both pigeons. Expression of the DCX antibody in the entopallium was comparatively denser in racing homer pigeon than in utility carneau pigeon. Mezey et al. (2012) reported similar findings but mild DCX expression in the entopallium of chicken. The striatum complex and the entopalium participate in the processing of visual information which is vital during navigating long distances particularly by homing pigeons. Birds are heavily visual and auditory reliant during feeding, reproduction, socialisation and many other daily functions (Grubb, 1972; Papi et al., 1972; Graves, 1992; Bonadonna et al., 2003; Patzke et al., 2011), but the cores of the telencephalon that process visual and auditory information exhibited very low to absent densities of PCNA- and DCX-immunoreactive structures in the domestic pigeon breeds studied. In contrast, the diencephalic and mesencephalic regions associated with these senses exhibited moderate densities of PCNA- and DCX-immunoreactive structures for example the dorsal nuclei of the thalamus which connects with the medial striatum (MSt) and also the tectum which connects with the lateral striatum (Kuenzel et al., 2011; Shanahan et al., 2013). We also observed PCNA and DCX immunoreactivity in the optic chiasma, anterior and posterior commissures and suggested that glial cells and neuronal axons may be proliferating and remodelling respectively.

In conclusion, adult neurogenesis appears to be a conserved process in the pigeon brain and may help in continuous reinforcement and remodelling of neuronal circuits and behavior.

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Conflicts of interest: *None declared.*
Research ethics: *All animals were treated and used according to the guidelines of the University of the Witwatersrand Animal Ethics Committee (approval No. 2013/05/02B), which parallel those of the NIH Guide for the Care and Use of Animals in scientific experimentation and "Consensus Author Guidelines on Animal Ethics and Welfare" produced by the International Association of Veterinary Editors (IAVE). The article was prepared in accordance with the "Animal Research: Reporting of In Vivo Experiments Guidelines" (ARRIVE Guidelines).*

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