INTRODUCTION

The small family of sengis or elephant shrews (Macroscelidae) was long placed within the order Insectivora, but is now considered to form an order by itself, the Macroscelidea (Rathbun, 2005, 2009). The radiation that gave rise to extant species dates to only ∼11 million years ago (Smit et al., 2011), i.e., at about the time when also the house mouse and rat may have diverged (Michaux et al., 2012). Sengis live in facultative monogamy, a rare trait in mammals, and give birth to highly precocial young.

Based on a phylogenetic analysis of gross characters of the brain, the rufous sengi (Elephantulus rufescens) associated with insectivores (Johnson et al., 1982; Kirsch and Johnson, 1983). However, Stephan and colleagues (Stephan and Spatz, 1962; Stephan and Andy, 1964) noted the prominent development of olfactory and subcortical visual centers. Based on these and other morphological observations and reflecting the later taxonomic changes,
Stephan grouped the dusky footed sengi (Elephantulus fuscipes) and checkered sengi (Rhynchocyon cirnei) as macroproptic insectivores, which clearly derivated from his basal and higher insectivores in the relative size of the entire brain as well as those of major brain divisions. The most notable deviation was found for the hippocampus, which is 3.5 times larger than that of basal insectivores (Stephan, 1961; Stephan and Andy, 1964), larger than that of most primates, and equal in relative size to that of humans (Stephan, 1983). This observation confirmed the rather graphic early description of the appearance of sengi brains, including the eastern rock sengi (Elephantulus myurus) that we investigated, by Le Gros Clark (1928), who found the hippocampus to be an “enormous mass,” “hypertrophied,” and “immense and seemingly bizarre” developed. The few later studies of sengi neuroanatomy can still be comprehensively reviewed. Sanides and Sanides (1974) grouped Elephantulus with insectivores based on the large size of neocortical stellate cells and their extensive dendritic arbors when compared to rodents and primates. Sensory cortices of the cape sengi (Elephantulus vanbuerenii), with emphasis on the somatosensory cortex, were mapped, found to be well-developed, yet without the cytochrome oxidase signature that allows the anatomical identification of these areas in other mammals (Dengler-Crish et al., 2006). Based on the distributions of cortical interneuron types characterized by the presence of calcium-binding proteins, of non-phosphorylated neurofilament containing neurons and glial fibrillary acidic protein (GFAP) expression, sengis, represented by a single specimen of the black and rufous sengi (Rhynchocyon petersii), were grouped with other afrotherian and xenarthran species and suggested to express distributions observed early in mammalian cortical evolution (Sherwood et al., 2009). A survey of the eastern rock sengi serotonergic, catecholaminergic, and cholinergic systems revealed an unusual presence of presumptive cholinergic neurons in the superior and inferior colliculi and cochlear nucleus, while other aspects of the distribution of markers for these systems did not deviate grossly from patterns observed in other eutherian mammals (Pieters et al., 2010). Notably, there have been no studies that followed up on what makes the sengi forebrain stand out among other mammals — their very large hippocampus.

In this study, we provide a general description and discussion of the cytoarchitecture of the hippocampus of the eastern rock sengi, the numbers of its principal neurons and the distribution of interneuron populations characterized by the expression of calcium-binding proteins and somatostatin. Of particular interest to us is how a very large hippocampus impacts on adult hippocampal neurogenesis (AHN). The observations that, in Murinae, a large habitat can be associated with either a large dentate gyrus and a low level of AHN or with a smaller dentate gyrus and a high level of AHN (Amrein et al., 2004a,b) suggest that species of this group may pursue different strategies to satisfy dentate information processing needs. Low levels of AHN in primates (Eriksson et al., 1998; Kornack and Rakic, 1999; Jabès et al., 2010; Amrein et al., 2011; Marlatt et al., 2011) may find their explanation in their large hippocampus (Stephan, 1975, 1983). This idea was put to the test in the sengi dentate gyrus by assessing cell proliferation, neuronal differentiation, and cell death.

MATERIALS AND METHODS

ANIMALS

Seven female (body weight: 44–46 g) and seven male (body weight: 40–56 g) eastern rock sengis (Elephantulus myurus, Figure 1) were caught in Sherman life traps at the Goro Game Reserve, Limpopo Province, South Africa (Permit 0089-CPRM-403-00004, CITES and Permit Management Office, Department of Environmental Affairs, Limpopo Province). Tissues were harvested from animals euthanized under projects in accord with the ethics guidelines of South Africa (University of Pretoria Clearance EC28-07) and the guidelines of the American Society of Mammalogists (Gannon et al., 2007). After trapping, animals were deeply anesthetized with pentobarbital (50 mg/kg), weighed and perfused transcardially using heparinized, cold phosphate buffer saline (PBS, pH 7.4) followed by 0.6% sodium sulfide in phosphate buffer and, finally, cold 4% paraformaldehyde solution in PBS with 15% picric acid (PFA-PA). The brains were removed, weighed, separated into hemispheres and post-fixed overnight. Thereafter all right hemispheres and two left hemispheres were transferred to 30% sucrose solution for cryoprotection. The remaining left hemispheres were conserved in fresh PFA-PA for plastic embedding.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

The left hemispheres of 12 animals were dehydrated and embedded in glycol methacrylate (Technovit 7100, Heraeus Kulzer GmbH, Wehrheim/Ts, Germany) according to the manufacturer’s instructions but with extended infiltration times (Amrein and Slomianka, 2010). Series of every sixth 20 μm thick horizontal section were mounted, one series was Giemsa-stained (Giemsa stock solution 1:2004:50, Merck, Darmstadt, Germany) following the protocol of Iljaz et al. (1985) and one series of two animals was Timm-stained (Danscher and Zimmer, 1978).

Twelve series of 40 μm thick frozen sections were cut from the right hemispheres (12 sagittal, one coronal and one horizontal) and two left hemispheres (one coronal and one horizontal). Sections were collected in cryoprotectant and stored at −20°C until further processing. Details of the immunohistochemical
Table 1 | Antigen specific details of the immunohistochemical procedures.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Antigen</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>Peroxidase block</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyclonal rabbit anti-calbindin IgG</td>
<td>CB-38a, Swant</td>
<td>Recombinant rat calbindin</td>
<td>1:10000</td>
<td>See calbindin</td>
<td>See calbindin</td>
</tr>
<tr>
<td>Monoclonal mouse anti-calretinin IgG</td>
<td>MAB1568, Milipore</td>
<td>Recombinant rat calretinin</td>
<td>1:500</td>
<td>3 x 15 s microwaved in citrate buffer pH 6.0 (Target Retrieval Solution, DAKO)</td>
<td>15 min in 0.6% H2O2 in TBS-Triton</td>
</tr>
<tr>
<td>Polyclonal goat anti-doublecortin IgG</td>
<td>sc-8086, Santa Cruz Biotechnology</td>
<td>Epitope mapping at the C-terminus of human doublecortin</td>
<td>1:5000</td>
<td>See calbindin</td>
<td>See calbindin</td>
</tr>
<tr>
<td>Polyclonal goat anti-NeuroD IgG</td>
<td>sc-1084, Santa Cruz Biotechnology</td>
<td>Epitope mapping to the N-terminus of NeuroD</td>
<td>1:1000</td>
<td>See PCNA</td>
<td>None</td>
</tr>
<tr>
<td>Polyclonal rabbit anti-PSA-NCAM IgG</td>
<td>sc-7907, Santa Cruz Biotechnology</td>
<td>Synthetic full-length human PCNA</td>
<td>1:200</td>
<td>40 min at 95°C in citrate buffer pH6.0 (Target Retrieval Solution, DAKO)</td>
<td>None</td>
</tr>
<tr>
<td>Monoclonal mouse anti-parvalbumin IgG</td>
<td>P-3171, Sigma</td>
<td>Carp muscle parvalbumin</td>
<td>1:20000</td>
<td>None</td>
<td>See calbindin</td>
</tr>
<tr>
<td>Monoclonal mouse anti-somatostatin IgG</td>
<td>MAB8324, Millipore</td>
<td>Viable Meningoococcus group B (strain 35B)</td>
<td>1:10000</td>
<td>None</td>
<td>See calbindin</td>
</tr>
<tr>
<td>Polyclonal rabbit anti-somatostatin IgG</td>
<td>ab103790, abcam</td>
<td>Synthetic peptide corresponding to amino acids 1-14 of human somatostatin</td>
<td>1:500</td>
<td>See calbindin</td>
<td>See calbindin</td>
</tr>
</tbody>
</table>

Table 2 | Cell numbers of PCNA-positive (PCNA+) proliferating cells and doublecortin-positive (DCX+) type 2b-progenitors and young neurons were estimated in the immunohistochemically stained sagittal series of the right hemispheres. PCNA+ cells were counted exhaustively using area and thickness sampling fractions of 1, but omitting cells in the top focal plane of the sections from the counts. DCX+ cells were counted using a 30 μm × 30 μm unbiased counting frame applied at steps of 350 μm along both the x- and y-axis. The thickness sampling fraction was again 1, omitting cells in the top focal plane from the counts.

The ratio CE2/CV2 (CV: group standard deviation/group mean) was calculated to ascertain the contribution of the estimation procedure to group variances (Tables 2 and 4). To compare AHN of the sengi with other species, we normalized cell counts by dividing them by the number of resident granule cells (Amrein et al., 2011).
FIGURE 2  (A) Macroscopic lateral view of the eastern rock sengi brain. The posterior expansion of the hemisphere largely reflects the shape of the underlying hippocampus. Scale bar: 5 mm. (B–E) Light arrows and light dotted line mark the borders between hippocampal fields. Small dark arrows mark the border between the proximal and distal parts of the subiculum. (B,C,E) 40 μm-thick frozen sections; (D,F) 20 μm-thick plastic-embedded section. Scale bars in (B–F) 0.5 mm. (B) Sagittal section of the septal hippocampus located at the transition from the first to the second hippocampal quarter. (C) Frontal section of the hippocampus located midway along the septotemporal hippocampal axis. (D) Horizontal section of the temporal hippocampus located at the transition from the third to the fourth hippocampal quarter. The dark dotted line marks the tentative border between the reflected blade of CA3 and the mossy cell layer. Asterisk: sectioning artifact. (E) Sagittal section of the temporal pole of the hippocampus. The plane of section corresponds to (B). (F) Timm-stained section corresponding to (D) showing a dense, narrow band of staining below the granule cell layer from which individual strands extend to coalesce into the CA3 mossy fiber zone. At the distal end of CA3, mossy fibers form a distinct end-bulb.

AGE ESTIMATION
Eastern rock sengis are seasonal breeders and pups are born between September and March (Rathbun, 2005). Based on the time of capture (September), we expect that none of the animals in this sample is younger than 6 months or older than 12 months. We measured lens weight and bone lines (Cavegn et al., 2013), calculated the means for each measurement and assigned a rank as a percentage of the means. Ranks were linearly transformed into tentative ages in months.

STATISTICS
The degrees of divergence/convergence of connections within the hippocampal region were calculated by dividing the number of cells in the projecting populations by the number of cells in the target populations for the eastern rock sengi and seven other species for which data sets obtained by design-based stereological techniques were available (see Table 3). The definitions of hippocampal fields in these studies were compatible with those used here. In particular, if a CA4 (a reflected blade of the CA3 pyramidal cell layer, Rosene and van Hoesen, 1987) was present, it was included in the cell counts of the hilus. A one-way ANOVA (SPSS 19, IBM SPSS Statistics) was used to test degrees of divergence/convergence for main species effects, with Bonferroni-corrected post hoc testing for differences between species pairs. P-values less than 0.05 (two-tailed) were considered significant. R (version 2.15.3) was used for the following analysis. The relationship between species and hippocampal cell population sizes was visualized with correspondence analysis (MADE4 R package, Culhane et al., 2005), which is similar to principal components analysis, but uses a weighted Euclidean distance to account for large differences in the absolute size of the neuron populations. Values for each animal were scaled by subtracting the mean of all principal neuron populations of that animal and dividing by their standard deviation. All animals therefore have cell counts with
### Table 2 | Unilateral hippocampal principal cell numbers (rounded to the next 1000) in the eastern rock sengi hippocampus and sampling parameters (males, n = 4; females n = 4).

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean (SD)</th>
<th>Mean CE (m = 0)</th>
<th>CE²/CV²</th>
<th>Counting frame size</th>
<th>Sections assessed</th>
<th>Cells counted</th>
<th>Mean and range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dentate gyrus granule cells</strong> All animals</td>
<td>392,900</td>
<td>85,700</td>
<td>0.10</td>
<td>0.20</td>
<td>7 μm × 7 μm</td>
<td>9.6</td>
<td>235</td>
</tr>
<tr>
<td>Females</td>
<td>361,800</td>
<td>73,300</td>
<td>0.11</td>
<td>0.30</td>
<td>8–11</td>
<td>155–234</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>424,000</td>
<td>96,800</td>
<td>0.08</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>All animals</th>
<th>Females</th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hilus/CA4 cells</td>
<td>39,000</td>
<td>52,000</td>
<td>22,000</td>
<td>1.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>All animals</th>
<th>Females</th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CA3 pyramidal cells</td>
<td>44,000</td>
<td>43,000</td>
<td>50,000</td>
<td>0.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>All animals</th>
<th>Females</th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1 pyramidal cells</td>
<td>124,000</td>
<td>73,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>All animals</th>
<th>Females</th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Subicular cells</td>
<td>33,000</td>
<td>16,200</td>
<td>1,000</td>
<td>0.18</td>
</tr>
</tbody>
</table>

### Table 3 | The degree of convergence or divergence (source cell number/target cell number) along the chain of hippocampal projections from the dentate granule cells to the subiculum. Bold numbers are statistically different from the value observed in the eastern rock sengi (P-values in parenthesis).

<table>
<thead>
<tr>
<th>Region</th>
<th>DG → hilus/CA4</th>
<th>DG → CA3</th>
<th>CA3 → CA1</th>
<th>CA1 → subiculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern rock sengi (Elephantulus myurus)</td>
<td>19.6 (0.965)</td>
<td>24.6 (0.986)</td>
<td>20.9 (1.000)</td>
<td>4.8 (1.000)</td>
</tr>
<tr>
<td>House mouse (Mus musculus)</td>
<td>24.6 (0.986)</td>
<td>24.4 (0.986)</td>
<td>24.3 (1.000)</td>
<td>24.3 (1.000)</td>
</tr>
<tr>
<td>Brown rat (Rattus norvegicus)</td>
<td>20.9 (1.000)</td>
<td>20.9 (1.000)</td>
<td>20.9 (1.000)</td>
<td>20.9 (1.000)</td>
</tr>
<tr>
<td>Harvest mouse (Micromys minutus)</td>
<td>45.5 (0.900)</td>
<td>45.5 (0.900)</td>
<td>45.5 (0.900)</td>
<td>45.5 (0.900)</td>
</tr>
<tr>
<td>Dog (Canis lupus familiaris)</td>
<td>24.4 (0.986)</td>
<td>24.4 (0.986)</td>
<td>24.4 (0.986)</td>
<td>24.4 (0.986)</td>
</tr>
<tr>
<td>Domestic pig (Sus scrofa)</td>
<td>6.0 (0.986)</td>
<td>6.0 (0.986)</td>
<td>6.0 (0.986)</td>
<td>6.0 (0.986)</td>
</tr>
<tr>
<td>Tree shrew (Tupaia glis)</td>
<td>0.73 (0.986)</td>
<td>0.73 (0.986)</td>
<td>0.73 (0.986)</td>
<td>0.73 (0.986)</td>
</tr>
<tr>
<td>Rhesus monkey (Macaca mulatta)</td>
<td>20.7 (1.000)</td>
<td>20.7 (1.000)</td>
<td>20.7 (1.000)</td>
<td>20.7 (1.000)</td>
</tr>
<tr>
<td>Human (Homo sapiens)</td>
<td>11.4 (0.986)</td>
<td>11.4 (0.986)</td>
<td>11.4 (0.986)</td>
<td>11.4 (0.986)</td>
</tr>
</tbody>
</table>
Table 4 | Unilateral dentate gyrus neurogenesis related cell numbers in the eastern rock sengi (rounded to the next 100 for PCNA and doublecortin and the next 10 for apoptotic cells) and sampling parameters (CEs and CE2/CV2 rounded to two decimals).

<table>
<thead>
<tr>
<th>Mean</th>
<th>Mean CE (m = 0)</th>
<th>CE2/CV2</th>
<th>Sections assessed</th>
<th>Cells counted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean and range</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td></td>
<td>Mean and range</td>
</tr>
<tr>
<td><strong>PCNA + proliferating cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All animals</td>
<td>12100</td>
<td>3600</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Females</td>
<td>10700</td>
<td>1100</td>
<td>0.04</td>
<td>0.13</td>
</tr>
<tr>
<td>Males</td>
<td>13700</td>
<td>5000</td>
<td>0.04</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Doublecortin + cell of neuronal lineage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All animals</td>
<td>41600</td>
<td>9600</td>
<td>0.09</td>
<td>0.15</td>
</tr>
<tr>
<td>Females</td>
<td>41900</td>
<td>6900</td>
<td>0.09</td>
<td>0.30</td>
</tr>
<tr>
<td>Males</td>
<td>41200</td>
<td>13100</td>
<td>0.09</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Apoptotic cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All animals</td>
<td>800</td>
<td>210</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Females</td>
<td>760</td>
<td>140</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>Males</td>
<td>850</td>
<td>280</td>
<td>0.05</td>
<td>0.07</td>
</tr>
</tbody>
</table>

All animals n = 6, females n = 3, males n = 3.

RESULTS

All descriptions refer to the typical pattern observed at mid-septotemporal levels of the dentate gyrus, hippocampus, and subiculum. Septotemporal changes or gradients are mentioned after the description of the typical pattern.

**HIPPOCAMPAL CYTOARCHITECTURE**

**Dentate gyrus**

Small, round cells with a preference to be located either superficially or at depth are the most common type of neuron in the cell-sparsely dentate molecular layer (ml). The granule cell layer (gcl) is 12–15 cells wide and has sharp boundaries to both the molecular cell layer and, in particular, the hilus (Figure 3A). The layer only forms distinct crests at the septal pole of the dentate gyrus (Figure 2B). Granule cells are densely packed and tend to form columns that span the gcl. Their large nuclei with one distinct large nucleolus – a characteristic of all neuronal cell populations in the sengi hippocampus – are surrounded by a very narrow rim of cytoplasm. Pyramidal-shaped cells are embedded in the lower margin of the gcl (1, Figure 3A), while ovoid cells slightly larger than granule cells and with a larger cytoplasm are found at the upper gcl border (2, Figure 3A). A narrow hilar plexiform layer (hpl), which contains rare spindle-shaped cells that are oriented parallel to the gcl (3, Figure 3A) and rare ectopic granule cells, delimits the gcl from the hilar polymorphic cell layer (hpcl). Immediately below the hpl, a sparse population of large polygonal cells (4, Figure 3A), with 2 or 3 large primary dendrites in the plane of the section, gradually merges with the dominant cell population of the loosely packed hpcl – slightly smaller ovoid to polygonal cells with 3 or more primary dendrites extending from their soma (5, Figure 3A). A third population is formed by distinctly smaller and darker staining cells of typically triangular appearance (6, Figure 3A) that are scattered throughout the hpcl.

The CA3 pyramid cell layer (CA3pcl) inserts into the hpcl close to the suprapyramidal limit of the hpcl (Figures 2B–E). While the hpcl forms a continuous wide band separtly (Figures 2B,C), it is separated into two tiers temporally (Figures 2D,E), leading us to believe that the dominant cell population in the hpcl represents modified pyramids of a reflected blade of the CA3pcl. This interpretation is supported by the distribution of mossy fiber terminals (Figure 2F) that form a dense narrow band below the gcl thereby delimiting cells embedded in the band from the deeper part of the hpcl.

**Hippocampus, CA3**

The CA3 stratum lacunosum molecular (CA3lm) is characterized by a distinctly higher density of glial cells than in the subjacent layers and frequent very small ovoid neurons. A few larger neurons align with the border between the CA3lm and CA3 stratum radiatum (CA3sr). The CA3pcl forms a typically four cells deep, dense band (Figure 3B) that widens considerably (Figures 2B–D) both at the transition to the hpcl, where the CA3pcl fans out into the hpcl, and to the CA1 pyramidal cell layer (CA1pcl). Few dark

Mean and range
Polymorphic cells are embedded in the deep part of the CA3 pcl.
Proximally, i.e., close to the dentate gyrus, ovoid, spindle-shaped, and small, dark pyramidal neurons are common (Figure 3B) in the adjacent CA3stratum oriens (CA3so) and CA3sr. Their density decreases distally toward the transition to CA1. Distally, large round cells are seen in the CA3so and a group of smaller cell bodies of often spindle-shaped but variable morphologies is scattered close to the border between CA3so and the alveus. Their density decreases toward proximal CA3.

Cytoarchitectural characteristics of CA3 are conserved along the septotemporal axis of CA3. However, in proportion to CA1, CA3 shortens septally along its proximo-distal axis while it elongates temporally (Figures 2B–E).

Hippocampus, CA1

Similar to the CA1slm, the CA1stratum lacunosum moleculare (CA1slm) is glia-rich but small neurons are rare. Spindle-shaped cells are found at the border between the CA1slm and the CA1stratum radiatum (CA1sr) and align with it. The transition from the CA1slm to CA1sr is marked by a sudden decrease of the thickness of the CA1sr that also contains markedly smaller pyramidal cell bodies (Figure 3C). CA1 and CA3 pyramidal cells intermingle for a very short distance. The CA1sr is densely packed and typically four cell bodies deep (Figure 3D) – temporally it tapers from the proximal to the distal end (Figure 2D). Very rare (one or two per section) darkly stained polymorph or pyramidal-shaped cells are embedded in the deep part of the CA1sr. Neurons in the CA1sr and CA1stratum oriens (CA1so) are rare, typically ovoid or spindle-shaped and oriented perpendicular to the CA1sr (Figure 3D). The distribution of neurons along the CA1so/alveus borders mirrors that of CA3 with cells being more frequent in proximal CA1 than in distal CA1. The CA1sr ends abruptly at the subicular border (Figures 2B–D). Although the subicular cell layer (Scl) undercuts CA1 slightly, it does so less than in laboratory mice or rats, and the border is typically very sharp throughout the radial extent of the deep hippocampal layers.

Cytoarchitectural characteristics of CA1 are conserved along most of the septotemporal axis (Figure 2). Only in the temporal one-quarter are scattered larger pyramidal cells located beneath the compact superficial layer (Figures 2E and 3E) – first in the immediate vicinity of the subiculum and later beneath the distal one-third of the CA1sr. CA1 and CA3 pyramidal cells do maintain their distinct difference in size along the entire septotemporal axis of the hippocampus.
Subiculum
Due to oblique borders with CA1 and the presubiculum, the Scl forms a lanceolate-shaped field (Figures 2B–D). It is divided into distal and proximal areas of roughly equal size. Proximally, a condensation of small neurons is seen at the border between the subicular plexiform layer (Spl) and Scl (Figure 3F). Beneath this condensed part of the proximal Scl, a visually homogeneous population of neurons with large oval somata and one large apical dendritic trunk spans most of the Scl (Figure 3F).

Some smaller and darker cells are scattered within this population. The degree of convergence of CA1 pyramidal cells onto subicular neurons by far exceeds the values observed in all other species. The eastern rock sengi most closely resembles the macaque monkey until the subiculum is reached. It is divided into distal and proximal parts of the Scl. This allows multidimensional patterns to be visualized in 5-dimensional space (where each hippocampal field is a separate axis) to two values. The eastern rock sengi most closely resembles the macaque monkey until the subiculum is reached. The eastern rock sengi most closely resembles the macaque monkey until the subiculum is reached.

HIPOCAMPAL CELL NUMBERS
The total number of neurons estimated to be present in the dentate gcl, hilus/CA4, CA3p/c, CA1p/c, and Scl of male and female animals are listed in Table 2. The number of male and female animals are listed in Table 2. C1/C2/CV2 were typically below 0.5, suggesting that only a minor part of the group variances originated from the estimation procedure. Estimates tend to be slightly higher in males (+6% to +17%) than in females (except for the subiculum: −18%). Significant sex differences could, however, not be observed for individual populations or total hippocampal cell number (p = 0.02–0.71) with the group sizes used.

Degrees of convergence and divergence are listed in Table 3 and compared to published data sets of other species that allowed calculating at least two values. The eastern rock sengi most closely resembles the macaque monkey until the subiculum is reached. The degree of convergence of CA1 pyramidal cells onto subicular neurons by far exceeds the values observed in all other species. "fnana-07-00034" — 2013/10/27 — 18:06 — page 8 — #8
FIGURE 4 | Correspondence analysis (A,B) and species profile (C) plots show the relationships between cell counts and hippocampal fields. Species form distinct clusters (A) with phylogenetically similar species, such as the rodents clustering close together. The spatial arrangement of hippocampal fields in (B) can be used to determine which fields are driving the species clusters. For example, the rodents are located in the bottom left quadrant in (A), as is the CA3 region in (B). This means that rodents have relatively high numbers of cells in the CA3 (and relatively few cells in the CA4/Hilus). These relationships can also be seen in the species profile plots (C), where each line represents an individual animal. The y-axes range from the minimum to the maximum value for each hippocampal field, and therefore the most relevant comparisons are across species for a given field (e.g., it can be determined that rodents have relatively more cells in the CA3 than humans). Note that it is not possible to determine from these graphs whether mice have a greater number of cells in the CA3 or dentate gyrus (DG), as each field uses a different scale.
FIGURE 5 | Calretinin and Calbindin in the eastern rock sengi dentate gyrus, hippocampus, and subiculum. (A–B) Calretinin. Asterisks: vessels in the obliterated hippocampal fissure. Scale bars: (A) 50 μm; (B,C) 100 μm; (D) 25 μm. (A) Septal dentate gyrus and border between CA1 stratum lacunosum moleculare (slm) and subicular plexiform layer. (B) Mid-septotemporal dentate gyrus and CA1slm. (C) Temporal dentate gyrus and CA1slm. (D) Calretinin+ cells in the deep pyramidal cell layer of temporal CA3.

(E–L) Calbindin. Scale bars: (E,G,I–L) 50 μm; (F,H) 250 μm.

(E) Heterogeneous distribution of calbindin in dentate granule cells. (F) calbindin+ cells are near absent in septal CA3. (G) Temporal CA3 (H) dense band of calbindin+ cells and processes at the stratum oriens/alveus border, but immunoreactive cells are otherwise near absent in CA1. (I) Calbindin+ neurons deep to the CA1 pyramidal cell layer and fine plexus of fibers in the deep stratum oriens. (J) Calbindin+ deep pyramidal cells in temporal CA1. (K) Calbindin+ cells in CA1/subiculum border.

Calbindin

Dentate gyrus. The ml stains evenly light to moderate. Throughout most of the septotemporal extent of the dentate gyrus, the gcl has a trilaminar appearance: moderate to strongly stained superficial and deep cells are separated by a tier of very light or unstained cells. While the deep cells form a near continuous band, lighter cells scatter between the darkly stained cells of the superficial tier (Figure 5E). A few darker cells are also found in the light middle tier. The hpl appears unstained. The superficial hpcf appears similar to the ml and extends stained strands into the deep hpcf (Figure 5E) that coalesce to form the mossy fiber layer in CA3 (Figure 5F).

Hippocampus and subiculum. CA3 appears, with the exception of the mossy fiber layer, largely unstained (Figures 5E,G). Rare moderately stained cells are found at the CA3so/alveus border, which resemble those seen in CA1 (see below). Very rare (<1 per section) darkly stained cells are located around the CA3slm border. Coinciding with the end of the mossy fiber layer, the
number of cells located at the subalveus border increases in CA1 (Figures 5E, F). They often appear bipolar in sections cut perpendicularly to the hippocampal long axis but multipolar in sections cut parallel to the alveus (Figure 5I) and extend a network of processes that is confined to the narrow band defined by the cell bodies (Figures 5H, L). At the CA1/subiculum border, cell bodies ascend toward the CA1pcl (Figure 5L). Fine processes and a few cell bodies extend into the proximal extreme of the subiculum (Figure 5L).

Generally, stained cells are very rare in the remaining layers of CA1 (Figure 5H). A fine plexus of fibers is seen in the deep part of CA1so (Figure 5B). Rare strongly stained pyramidal or multipolar cells are seen in the CA1pcl (Figure 5). Small moderately stained cells are seen rarely in the CA1sr and CA1slm. Well-stained fibers and fine particles stain the CA1slm.

Temporally, an increasing number of moderately stained cells is evenly distributed in the CA1sr and CA1som (Figure 5G) and at the CA1swl border. The temporal one-third of the CA1pcl is characterized by the gradual appearance of moderate to strongly stained deep pyramidal cells. Initially, they scatter as individual cells along the deep border of the CA1pcl (Figure 5F), but distally and further temporally, they form a continuous deep band of the CA1pcl (Figure 5K).

**Parvalbumin**

**Dentate gyrus.** Stained cells or terminal-like staining are not seen in the ml (Figure 6A). Only at the border between the ml and gcl, a dense band of fine granules extends for a short distance into the layers forming the border (Figures 6A, B). Lower density, fine granules scatter also over the remainder of the gcl. Pyramidal-shaped somata are found between the deepest granule cells with a preference for the suprapyramidal blade of the gcl (Figures 6A, B) and extending beaded dendrites into the ml. The deep part of the hplc harbors a dense network of coarse and fine beaded processes, a few of which reach into the otherwise unstaed hplc. Parvalbumin+ multipolar cell bodies in the hplc are located mainly beneath the suprapyramidal gcl.

Septally, pyramidal-shaped cells (Figure 6B) are more common and more evenly distributed. Horizontal fibers run through the hplc and superficial hplc. Horizontal fibers are also present. Cell bodies in the hplc become rare septally. In the temporal quarter, stained structures become rare until even fine-grained staining remains over the gcl at the temporal pole.

**Hippocampus and subiculum.** In CA3, most cells are either located in CA3so or associated with the CA3pcl (Figures 6C, D). In CA3so, their dendrites form an irregular network, while most apical dendrites ascend straight through the CA3sr (Figure 6D). They rarely enter CA3slm. Coarse and dense terminal-like staining is seen in CA3slm and at the deep and superficial borders of CA3pcl (Figure 6D). In CA1, cells are less frequent than in CA3. The vast majority is either associated with the CA1pcl or found at the CA1subalveus border. They extend beaded dendrites into the adjacent layers (Figures 6E, F), but dendrites rarely cross into the CA1slm (Figure 6K). Parvalbumin+ cell morphologies at the CA1subalveus border correspond to those of cadbindin+ cells. Pyramidal-shaped cells are most common in association with the CA1pcl, but multipolar and horizontal cells are also present. Rare cells of CA1so and CA1sr are often bipolar and oriented perpendicularly to the CA1pcl (Figure 6F). A light and fine punctuate stain fills the CA1slm. Dense and coarse particles together with fine fibers are seen throughout CA1sr and so, but concentrate above and below the CA1pcl (Figures 6E, F). Subicular parvalbumin+ cells are often pyramidal in the proximal Scl, but ovoid or multipolar in the distal subiculum. In both parts, they are located mainly in the deep Scl (Figure 6G). Dendrites extend to the pial surface proximally only, while immunoreactive cells are more frequent distally (Figure 6G). Coarse terminal-like staining fills the deep Spl and entire Scl (Figures 6G, I).

No gross changes in the staining pattern are seen along the septotemporal axis of CA3. Septally, cells associated with the CA1pcl become fewer, while terminal- and fiber-like staining is unchanged. Temporally, cells become rare in the distal subiculum (Figure 6I).

**Somatostatin.**

**Dentate gyrus.** Immunoreactive cells are absent from the ml, gcl, and hplc. 30–50 large, bi- or multipolar cells scatter evenly throughout the superficial hplc (Figure 6I). There is a clear septotemporal low to high gradient in cell density. Temporally, cells appear smaller and scatter evenly throughout the hplc. Septally, cell morphology remains unchanged but staining intensity decreases.

**Hippocampus and subiculum.** Immunoreactive cells are scattered evenly along the CA3subalveus border (Figure 6J). From there, they also extend in between CA3 and its reflected blade forming part of the hplc. They stain weaker than in the dentate gyrus and their morphology is therefore difficult to determine. Both bipolar cells and cell with a prominent apical dendrite are among them. 5–10 per section are found scattered throughout the remaining layers of CA3, with a slight preference for the CA3sr (Figure 6I). In CA1, immunoreactive cell are almost exclusively found at the CA1subalveus border (Figure 6K). Cell density is highest in the proximal part of CA1, and many cells have a bipolar appearance. Rare cells outside this zone (1 or 2 per section) are often located close to the deep CA1pcl (Figure 6K). The deep band of cells follows the CA1/subiculum border to the junction between CA1pcl and Scl. From there, they scatter in the deep one-half of the Scl without a proximal–distal gradient (Figure 6L).

Cell density increases from the septal to the temporal CA3, while it appears stable in CA1. In CA3, this increase is most pronounced in CA3so and sr, and it is accompanied by a dispersal of the cell band at the subalveus border.

**Neurogenesis.**

The appearances of PCNA+ proliferating cell, DCX+, PSA-NCAM+, and NeuroD+ young neurons, and apoptotic cells are illustrated in Figure 7. Both proliferating cells (Figure 7A) and young neurons (Figures 7B–D) are distributed rather evenly along the lower margin of the gcl. Young neurons are rarely positioned further superficially than in the deep 2–3 cell tiers of the gcl. A decline in their numbers along the septotemporal axis of the gcl is noticeable but not pronounced. DCX did define cell bodies and dendrites more clearly than PSA-NCAM (Figures 7B, D), while PSA-NCAM defined the axonal fields of the cells in the hilus and...
FIGURE 6 | Parvalbumin and Somatostatin in the eastern rock sengi dentate gyrus, hippocampus and subiculum. (A–H) Parvalbumin. Scale bars: (A,C,G,H) 250 μm; (B,D–F) 50 μm. (A) Mid-septotemporal dentate gyrus. (B) Septal granule cell and hilar plexiform layers. (C) Septal CA3. (D) Mid-septotemporal CA3 containing unusually many parvalbumin+ cells that reflect their morphological heterogeneity. (E,F) Mid-septotemporal CA1. (G) Septal subiculum. Beaded dendrites reach the pial surface in the proximal subiculum only (asterisk). (H) Temporal subiculum. (I–L) Somatostatin. Scale bars: (I,J) 50 μm. (I) Dentate gyrus. Immunoreactive cells are only found in the hilar polymorphic cell layer. (J) CA3. (K) CA1. (L) Proximal subiculum (asterisk marks the end of the CA1 pyramidal cell layer).

CA3 better than DCX (Figures 7D,E). Based on the distribution of PSA-NCAM, the axons of young neurons are evenly distributed within the terminal fields of the mossy fibers (compare Figures 2F and 7E).

Number estimates corresponding to those presented for the principal cell populations are listed in Table 4. We did not observe any gender differences between the numbers of PCNA+ cells, DCX+ young neurons and apoptotic cells with [PCNA: F(1,9) = 1.22, p = 0.30; DCX: F(1,9) = 0.24, p = 0.64; Apo: F(1,9) = 1.21, p = 0.30] or without [PCNA: F(1, 10) = 0.92, p = 0.36; DCX: F(1, 10) = 0.25, p = 0.63; Apo F(1,10) = 0.24; p = 0.64] tentative age as a covariate. CE2/CV2 ratios were well below 0.5, suggesting that only a very small part of the group variances originated from the estimation procedure.

The number of PCNA+ proliferating cell was highly correlated with both the numbers of DCX+ young neurons ($r = 0.64, p = 0.007$) and apoptotic cells ($r = 0.72, p = 0.004$). There was no correlation between the number of DCX+ young neurons and apoptotic cells ($r = 0.35, p = 0.13$). No correlation was found between tentative age and the numbers of PCNA+ proliferating cell ($r = 0.055, p = 0.43$) and apoptotic cells ($r = -0.15, p = 0.32$) while a trend toward a negative correlation was seen for DCX+ young neurons ($r = -0.395, p = 0.10$).

The normalized numbers of PCNA+ cells in sengis fall within the range defined by all four species of murine rodents that had been captured in the same location. However, the normalized numbers of DCX+ cells were lower than in three of the murine species (Figure 8).
FIGURE 7 | Adult hippocampal neurogenesis in the eastern rock sengi dentate gyrus. Images were taken in the septal hippocampus corresponding to Figure 2B. (A) PCNA+ cells. (B) DCX+ cells. (C) NeuroD+ cells. (D) PSA-NCAM+ cells and neuropil. (E) PSA-NCAM+ cells and mossy fiber terminal fields. (F,G) Pyknotic cells (arrows). Scale bars: (A–D) 50 µm; (E) 250 µm; (F,G) 10 µm.

DISCUSSION

The eastern rock sengi hippocampus does show individual characters that associate it with any one of the phylogenetic groups that it has been related with. Their combination is, however, unique and, as will be discussed below, forms a distinct data point in the matrix of hippocampal characters that represent species with different phylogenetic and ecological backgrounds. Results will be discussed in the order in which they have been described.

HIPPOCAMPAL CYTOARCHITECTURE AND CELL NUMBERS

Cytoarchitecturally, the eastern rock sengi hippocampus may, grossly speaking, be described as having a primate like dentate gyrus and CA3, to which a CA1 and subiculum that resemble those found in the rabbit or guinea pig (Geneser-Jensen et al., 1974; Geneser, 1987a) have been added. Cell numbers also reflect the qualitative similarity of the dentate gyrus and CA3 to that of the thalamic monkey. A high degree of convergence from granule cells to CA3 pyramids is shared by the two species. Recalling early comments on the size of the sengi hippocampus, the total number of granule cells in the sengi (3.9 million unilateral, bodyweight $\sim 50$ g) is higher than in marmoset monkeys (3.6 million unilateral, bodyweight $\sim 250–500$ g; Kozorovitskiy et al., 2005) and more than half of that in adult rhesus monkeys (7.7 million unilateral, bodyweight $\sim 7$ kg; Keuker et al., 2003a). The large sengi dentate gyrus is not accompanied by a large entorhinal cortex (Stephan, 1961).

Comparative aspects of the junction between the dentate gyrus and CA3 have been subject of early and sustained discussions (Lorente de Nó, 1934; Rosene and van Hoesen, 1987; Keuker et al., 2003b). It is generally accepted that primates show a reflected blade of the hippocampal pyramidal cell layer, a CA4 as defined by Rosene and van Hoesen (1987), that is not found in laboratory mice and rats, in which the deep hilus forms a polymorphic cell mass connectionally and cytoarchitecturally unrelated to CA3 pyramidal cells (Blackstad, 1956; Amaral, 1978). The definition of CA4 by Rosene and van Hoesen (1987) excludes the hilar polymorphic cells that Lorente de Nó (1934) for “didactic” rather than connectional or cytoarchitectural reasons included as a second reflected blade in his definition of CA4. A reflected blade of the hippocampal pyramidal cell layer is, with minor morphological variations on the theme found in primates, present in fox (Amrein and Slomianka, 2010), pig (Holen and West, 1994), guinea pig (Geneser-Jensen, 1972), and rabbit (Geneser, 1987b) – to name but a few species. Despite the close relation between CA4 and CA3, CA4 pyramidal cells have typically been grouped with hilar polymorphic cells in quantitative studies, presumably because a reliable border between CA3 and CA4 is often easier to define than the border between the hippocampal pyramidal cell layer and CA4. Unexpectedly, this apparently spurious assignment does result in very similar degrees
FIGURE 8 | Neurogenesis in the eastern rock sengi in comparison to southern African rodents from the same temperate climate, compared with neurogenesis in four murids from a northern cold climate (adapted from Cavegn et al., 2013). Neurogenesis data of sengis and spiny mice, both precocial species with exceptionally high numbers of granule cells, cluster with the species from the cold climate. For species comparisons, the numbers of proliferating cells (PCNA+ or Ki67+) and young cells of the neuronal lineage (DCX+) were normalized to total granule cell number.

of convergence from dentate granule cells to hilar/CA4 cells in species with and without a CA4 (laboratory mouse, laboratory rat, dog, rhesus monkey, and sengi). Only pigs and humans, both with a CA4, show a much smaller convergence. In contrast, more variation is seen in the convergence from the anatomically “cleanly” defined dentate granule cells to CA3 cells. The unexpected association of rats and mice with dog, rhesus monkey and sengi may be explained by septotemporal changes in dentate histotarchitecture. An increase in the cell number of the temporal hilus (Gaarskjaer, 1978) of rats and mice that cannot be accounted for by an increase in the number of mossy cells alone (Fujise and Kosaka, 1999) and concomitant changes in the conformation of the dentate-CA3 junction (Haug, 1974) suggest that a CA4-like cell population may be present temporally. Beyond the mossy fiber projection, back projections from proximal/ventral CA3 to the dentate gyrus (Li et al., 1994; Scharfman, 2007) may create a functional link (e.g., in dentate pattern separation, Myers and Scharfman, 2011) close enough to mediate a concerted phylogenetic development of CA4 cell numbers and dentate granule cell numbers.

Although the cytoarchitecture of the sengi CA1 is strikingly different from that of primates, dog and pig, the degree of divergence from CA3 to CA1 pyramids of all these species is similar and larger than in rat, mice and tree shrew. The correspondence analysis does identify CA1 as a region particularly large in humans, but it does not group humans with rhesus monkeys, which confirms earlier observations (Seres, 1988). Although the size of CA1 expands in primates (Stephan, 1983), this expansion does not set the rhesus monkeys apart from other taxonomic groups represented by pig and sengi.

The proximal and distal subicular divisions and their changes along the septotemporal axis in the sengi subiculum are similar to those in rats and mice, in which they correspond to subicular compartments with distinct connectional and physiological properties (O’Mara et al., 2001; Witter, 2006; Ohara et al., 2009). In sengis, subicular cell number is very small when compared to that of CA1, the main source of hippocampal subicular afferents. Extrahippocampal limbic system cell numbers are only available for two species (rats and humans) precluding an extended correspondence analysis of cell numbers. However, size indices based on volumes for the sengi retrohippocampal cortical (peri- and entorhinal cortex) and subcortical (septum) CA1 target areas are smaller than those for the hippocampus itself (Stephan, 1961), which would argue that they do not function as alternative, unusually large targets for CA1 projections. This is similar to the size relations between the entorhinal cortex and the dentate gyrus at the entrance to hippocampal circuitry and raises the question of the computational advantage of a large hippocampus when no commensurate changes are apparent in the sources and targets of hippocampal connections.

What is brought out by the comparative quantitative data is that generalized models of hippocampal function will have to be...
robust to up to fivefold differences in ratios between the numbers of interconnected cell populations. Furthermore, interspecies differences encompass opposing trends in the degrees of divergence and convergence both across the entire tri-synaptic loop as well as at specific steps along this path. Lastly, while similarities exist between sengi and taxonomically disparate groups in the quantitative composition of hippocampal principal neurons, sengis form a distinct cluster in the data space defined by quantitative relations between the major brain divisions that is clearly separated from, e.g., primates (de Winter and Oxnard, 2001; Oxnard, 2004). Similarly composed hippocampi have thus been embedded into quantitatively different higher level networks.

MARKERS OF NEURONAL FUNCTION

Calbindin

Based on cellular morphology, location and staining gradients in the 
\( \text{cito} \) of the dentate hilus, calretinin+/ cells in the hilus are likely to represent mossy cells with projections that ascend beyond the location of the cell body, but with only sparse projections reaching the septal pole of the dentate gyrus. Cytosarchitecturally, the cell type that is calbindin+/ in the temporal hilus is calretinin in the septal hilus. A similar distribution of calretinin in mossy cells was seen in the mouse, hamster, gerbil, laboratory shrew, primate, and humans (Liu et al., 1996; Blasco-Ibáñez and Freund, 1997; Fujise et al., 1998; Murakawa and Kosaka, 1999; Jinno and Kosaka, 2006; Serses et al., 2008). Calbindin+/ CA1 and CA3 interneurons that are present in the above mentioned species in rodent development (Jiang and Swann, 1997), monkey (Serses et al., 1993), and human (Nitsch and Ohm, 1995) are, with very rare exceptions, absent in the sengi. In contrast, small calbindin+/ cells in the sengi dentate and CA3 molecular layers are rarely seen in adult individuals of other species. Their distribution in the adult sengi and gerbil (Murakawa and Kosaka, 1999) resembles that of calretinin+/ Cajal–Retzius cells in the developing mouse hippocampus (Soriano et al., 1994; Berger and Alvarez, 1996; Jiang and Swann, 1997) and adult human hippocampus (Abraham and Meyer, 2003).

Calbindin+/ CA3 hippocampal pyramidal cells have been reported during murine development (Jiang and Swann, 1997), but not in adult laboratory rats. A few calbindin+/ proximal CA3 pyramidal cells were seen in laboratory mice (Fujise and Kosaka, 1999). They are present throughout CA3 in a subpopulation of pyramidal cells of the Guzira spiny rat (Fubone et al., 2001), and we also reported the presence of another calcium-binding protein, calabin, in deep CA3 pyramidal cells in naked mole-rats (Slomianka et al., 2011). Lastly, calbindin is not present in cells of the deep gcl or in the bpl (including the subgranular zone, see discussion of neurogenesis).

Calbindin in principal cells

We recently noted a heterogeneous distribution of calbindin in the granule cells of three sympatric African murine rodent species (Cavegn et al., 2013) that in two of the species appears similar to that in the sengi, i.e., calbindin+ cells in superficial and deep cells of the gcl separated by a tier of very light or unstained cells. A morphological and functional heterogeneity defining a population at the superficial limit of the rat gcl has also been described (Williams et al., 2000; Larimer and Strowbridge, 2010). Notably, a preferential expression of calbindin in superficial granule cells is also seen in \( \text{FKHR1} \) knockout mice (Real et al., 2011), a gene encoding FMRP (fragile mental retardation protein). A morphologically heterogeneous population of granule cells is present in the fox as well (Amrein and Slomianka, 2010). It has been suggested that connections between developmentally matched groups within the hippocampal principal cell populations form distinct information streams (Deguchi et al., 2011), and differential expressions of calcium-binding proteins add to the morphological and functional differences that may be reflections of such streams.

Superficial calbindin+/ CA1 pyramidal cells are found in mouse, rat, fox, and primates (reviewed in Slomianka et al., 2011). This marker is not expressed by superficial pyramids in guinea pig and rabbit and is absent in the sengi too. In mice, the condensation of pyramidal cells into a compact layer and the expression of CA1 specific markers, including calbindin, are developmentally regulated by the expression of \( \text{Zfhb2} \) (Nielsen et al., 2007, 2010; Xie et al., 2010; Rosenthal et al., 2012). In contrast, the absence of calbindin from superficial pyramidal cells in spite of a pronounced condensation of the cell layer suggests an independent development of these characteristics in the sengi, guinea pig and rabbit CA1.

Mechanisms related to age- (Poitier et al., 1994; Villa et al., 1994; de Jong et al., 1996), activity- (Lowenstein et al., 1991; Maglóczky et al., 1997), or hormone-dependent (Krüger et al., 1995; Trejo et al., 2000) changes of calbindin content in hippocampal principal cells may mediate species-specific expression patterns. Deep calbindin+/ pyramidal cells are present in CA1 of fox and primates and in the distal and temporal CA1 of mouse and rat. In these species, they form the deepest tier of pyramids in a temporally and distally wide and trilaminar CA1pdl. Deep calbindin+/ pyramids in the sengi share the distal and temporal location, but they still form a compact band together with calbindin– superficial pyramidal cells.

Calbindin in interneurons

There is a very marked shift in the distribution of calbindin+ putative interneurons to the stratum oriens/alveus border in the sengi when compared to mouse (Jinno and Kosaka, 2006), rat (Shlovier, 1989; Toff and Freund, 1992), dog (Hof et al., 1996), or primates (Seress et al., 1991; Slvovitter, 1991). Cell morphologies in this zone correspond to those described in primates. The distinct plexus of fibers in the CA1tho suggest an origin from O-LM inhibitory neurons (Freund and Buzsáki, 1996; Wouterlood et al., 2001) which is supported by the appearance of the dendritic arbors of many calbindin+ cells at the CA1stratum/alveus border. Interneurons with selective axonal projections to the CA1tho have not been described (Freund and Buzsáki, 1996). Although O-LM cell axons do arborize in the CA1tho, this is a minor projection compared to the CA1strat in laboratory mice and rats. Cell morphologies and location of calbindin+ cells also correspond to Septally projecting type II cells of Gulyás and Freund (1996). Outside the stratum oriens/alveus border, calbindin+ interneurons are rare or absent in most of the sengi dentate gyrus and hippocampus – a feature that the sengi shares
AHN. We have recently shown (Cavegn et al., 2013) that Muridae of calbindin, closely the pattern described in mice (Fujise et al., 2003), distribution of parvalbumin in the subiculum follows, like that of basket cells (Freund and Buzsáki, 1996; Sik et al., 1997). The distribution of axons of parvalbumin+ terminal staining reflects, like in other species, the distribution of axons of parvalbumin+ chandelier and pyramidal basket cells (Freund and Buzsáki, 1996; Sik et al., 1997). The distribution of parvalbumin in the subiculum follows, like that of calbindin, closely the pattern described in mice (Fujise et al., 1995). While parvalbumin appears more resilient to phylogenetic change than calretinin or calbindin in eutherian mammals, the distribution is restricted to fewer cell types in prototherian echidna (Hassiotis et al., 2005).

**Parvalbumin**

The laminar distribution of parvalbumin in sengi interneurons corresponds to that in the dentate gyrus and hippocampus of rat (Nomura et al., 1997), mouse (Jinno and Kosaka, 2006), dog (Heo et al., 1996), tree shrew (Keuker et al., 2003b), or monkey (Seress et al., 1991). They still appear, to us, less frequent than in these species, although the difference is much less pronounced that that for calbindin and calretinin and would require robust quantitation to draw firm conclusions. Much of the dense parvalbumin+ terminal staining reflects, like in other species, the distribution of axons of parvalbumin+ chandelier and pyramidal basket cells (Freund and Buzsáki, 1996; Sik et al., 1997). The distribution of parvalbumin in the subiculum follows, like that of calbindin, closely the pattern described in mice (Fujise et al., 1995). While parvalbumin appears more resilient to phylogenetic change than calretinin or calbindin in eutherian mammals, the distribution is restricted to fewer cell types in prototherian echidna (Hassiotis et al., 2005).

**Somatostatin**

The cellular distribution of somatostatin in the sengi hippocampus is similar to that of the rat and mouse (Kohler and Chan-Palay, 1982; Bakot et al., 1986; Buckmaster et al., 1994), guinea pig and rabbit (Buckmaster et al., 1994), pig (Holm et al., 1992), and human (Chan-Palay, 1987), although neurons are generally more common in the stratum oriens of these species than in the sengi. In the dentate gyrus, they are likely to correspond to cells with axonal arbors in the perforant path zones (HIPP cells, Freund and Buzsaki, 1996). We have not been able to show a plexus of somatostatin containing fibers that is present in the dentate ml or CA1ml of mouse and rabbit (Buckmaster et al., 1994). Somatostatin containing cells at the ammunstral border of CA3 and CA1 are likely to represent LM- and O-LM neurons, respectively (Freund and Buzsaki, 1996). They have also been found to co-localize with calbindin (Wouterlood et al., 2001).

**Neurogenesis**

Home ranges of eastern rock sengi females are typically smaller than those of males (Biddle and Perrin, 2003). In that AHN did not differ between males and females, spatial requirements associated with the absolute size of the home range are unlikely to be related to AHN. We have recently shown (Cavegn et al., 2013) that Muridae trapped in the same habitat (Köppen-Geiger climate classification Coa, Peel et al., 2007) as the sengis did differ in proliferation (lower) and neuronal differentiation (higher) from Muridae that were collected in a different, European habitat (Köppen-Geiger climate classification Dbf). This difference was consistent across multiple species that belonged to different taxonomic subunits and that also differed in, e.g., habitat use and social organization. The sengi does not group with most of the sympatric Muridae, but instead associates with European Muridae. Only southern African spiny mice showed AHN related cell counts close to those of the sengi. Spiny mice and sengi share the traits of exceptionally high numbers of granule cells (Cavegn et al., 2013) and precaudality. Current data cannot address how much of the observed variation in AHN of sengis and spiny mice could be related to phylogeny, to anatomical context of AHN or to demands on hippocampal information processing mediated by the young neurons that relate to their ecology. Also, the regulation of markers that are expressed during specific stages of neuronal differentiation differs in the sengi from that seen in other species. Calretinin is expressed during the late post-mitotic differentiation of granule cells in mice (Brandt et al., 2003; Kempermann et al., 2004). Calretinin+ cells are not seen in numbers or morphologies that correspond to those of DCX+ or PSA-NCAM+ cells in the dentate subgranular zone and deep gc. The expression of calretinin is apparently not part of neuronal differentiation in the sengi.

Similar size progressions of the hippocampus and dentate gyrus in sengis and primates do not relate to AHN in similar ways. AHN is not only low in primates, but also characterized by an extended period of differentiation of the newly formed cells of neuronal lineage (Ngovenya et al., 2006; Kohler et al., 2014). Both the numbers of proliferating and differentiating cells and their ratio in the sengi fall within the ranges seen in murine rodents. Clearly, a large hippocampus relative to body size does not necessarily obviate AHN.

**Conclusion**

The distinct positions of species, including a clustering of mice and rats, in the correspondence analysis point toward the existence of distinct patterns of quantitative relations between hippocampal principal cell populations. If these patterns relate to the phylogenetic history of the species and/or to demands on hippocampal information processing related to species ecology remains an open question. de Winter and Oxnard (2001) showed that quantitative relations between even major brain divisions could group species by lifestyle despite different phylogenetic histories. This is likely to be more common as anatomical resolution and, thereby, functional specificity increases. In our data, this is brought out by the separation of the human and rhesus monkey hippocampus and the association of rhesus monkey, pig, and sengi despite their disparate phylogenetic histories. The scarcity of calbindin+ and calretinin+ interneurons in, in particular, CA1 of the sengi hippocampus represent an additional tier of specialization that separates the sengi from species which appear similar based in quantitative relations between principal cells.

In the context of sengi hippocampal specializations, “normal, mouse-like” quantitative data on sengi AHN are an almost surprising relief. However, the sengi affiliates with our sample of European mice instead of most of the sympatric mouse species, which again raises the question if differences relate to anatomical context and/or selective pressures on AHN related to sengi ecology.
The most intriguing question remaining is why the sengi hippocampus is so large when the cortical and subcortical areas that are hippocampal sources of afferents and targets of efferents are not. We pointed out that generalized models of hippocampal function need to be robust to large differences in convergence and divergence along the intrahippocampal pathways. In addition, such models may have to enlarge hippocampi with intrinsic computational abilities which provide selective advantages that, based on our current knowledge, are independent of the number of available input and output channels.

ACKNOWLEDGMENTS

We thank Inger Drescher and Rosmarie Lang for expert technical assistance in this project. Funding for this work was given by the Swiss-South Africa Joint Research Project IP 09, and the Swiss National Science Foundation Grant Nr 31003A_141244/1.


Zbtb20 in mice. J. Comp. Neurol. 49, 775–796. doi: 10.1002/hipo.20403


Zbtb20 in mice. J. Comp. Neurol. 49, 775–796. doi: 10.1002/hipo.20403


Zbtb20 in mice. J. Comp. Neurol. 49, 775–796. doi: 10.1002/hipo.20403


Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 May 2013; paper pending published: 25 June 2013; accepted: 26 September 2013; published online: 29 October 2013.

Copyright © 2013 Slomianka, Drenth, Cavegn, Menges, Latic, Phalanndwa, Chimimba and Amrein. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.