Title

Regional Differences in Structure and Cellular Composition within Sub-Regions of the Perinatal Piglet Ventricular-Subventricular Zone

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Regional Differences in Structure and Cellular Composition within Sub-Regions of the Perinatal Piglet Ventricular-Subventricular Zone

by

Kadellyn Sandoval Gutierrez

DISSERTATION

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ABSTRACT

Kadellyn Sandoval Gutierrez

Regional Differences in Structure and Cellular Composition within Sub-Regions of the Perinatal Piglet Ventricular-Subventricular Zone

The ventricular-subventricular zone (V-SVZ) has long been a region of great interest due to the invaluable role that it plays in generating neurons throughout development. Studies in rodents have shown that the quantity and identity of neurons born within the V-SVZ change not only temporally but spatially, with different regions of the mouse V-SVZ giving rise to distinct neuronal subtypes. In the human brain, however, certain regions of the V-SVZ have expanded to accommodate a need for greater cell numbers, including the addition of an outer sub-ventricular zone and the presence of the Arc, a three-tiered structure present during the perinatal period of development that is located at the dorsal horn of the lateral ventricle. Given the novelty of these findings, it remains unclear how these expansions change the local cytoarchitecture of different parts of the V-SVZ and how these changes may affect the local proliferating cell population. To explore these questions, we use the piglet brain as a proxy for the human brain due to its similar developmental trajectory during the perinatal period of development. Using histological and imaging techniques, we identified both spatial and temporal differences in cellular architecture and composition between four sub-regions of the V-SVZ. We additionally discovered significant differences in both the density and distribution of proliferative cells between different sub-regions. Finally, we provide evidence that proliferating cell populations in different sub-regions have distinct identities. These findings suggest that different regions of the V-SVZ in large-gyrified brains may act as distinct niches, giving rise to different neuronal and glial populations.

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Chapter 1 | Introduction

Ventricular-subventricular zone structure and function in the mouse and human brains

The ventricular-subventricular zone (V-SVZ) is a neurogenic niche that lines the walls of the lateral ventricle (LV) in the mammalian brain. The V-SVZ is populated by four major cell types: ependymal cells, astrocytes, intermediate progenitor cells, and neuroblasts (Doestch et al., 1997). V-SVZ neural stem cells (NSCs) are capable of producing both intermediate progenitors and neuroblasts, both of which retain some proliferative potential throughout the lives of some mammalian species, including mice (Doestch et al., 1999, Ponti et al., 2012). In the mouse V-SVZ, ependymal cells form an epithelial layer separating the SVZ from the lateral ventricle, while astrocytes and intermediate progenitor cells surround chains of migrating neuroblasts (Alvarez-Buylla and Garcia-Verdugo, 2002). These chains of migrating neuroblasts persist into adulthood, continually supplying new neurons to the olfactory bulb.

Structural differences between the rodent and human brains

In the human brain, the structure and function of the V-SVZ differ significantly from that observed in the rodent brain. Rather than maintaining production of new neurons throughout life, the number of migrating progenitors drops precipitously early in life. No chains of migrating neurons surrounded by astrocytes are present; instead, a hypocellular gap layer forms in the space previously occupied by migrating progenitors, separating the astrocytic cell bodies from the ependymal cell layer (Sanai et al., 2011). The human V-SVZ has thus been described as a layered structure with layer I referring to the ependymal cell layer, layer II referring to the gap layer populated by GFAP+ processes, layer III referring to GFAP+ cell bodies, and layer IV

referring to the region underlying the striatal brain parenchyma (Quinones-Hinojosa et al., 2005).

In addition to an overall change in V-SVZ structure, the human V-SVZ has also undergone two notable expansions in the form of an outer sub-ventricular zone (oSVZ) and the Arc. The oSVZ is an expansion of the SVZ that contains neural stem cells that differ molecularly from their counterparts in the VZ and are responsible for producing the majority of cortical neurons in the human cortex (Pollen et al., 2015). The Arc is a three-tiered expansion located at the dorsal horn of the lateral ventricle that contains large numbers of migrating inhibitory neuroblasts (Paredes et al., 2016). The discrepancies in the structure of these expanded regions compared to the rodent brain imply that these regions may have needed to expand to meet the higher neuronal number requirements of a much larger cortex.

In many mammalian brains, including mice, rabbits, dogs, and marmosets, neuronal migration through the rostral migratory stream to the olfactory bulb persists for the entirety of the animals' life (Ponti et al., 2006, Fernandes-Flores et al., 2017, Pencea et al., 2001). In the human brain, however, postnatal neuronal migration is largely directed toward various cortical regions, suggesting that the postnatal V-SVZ may play a very different role during this time period. It has been reported that many migrating neuroblasts within the Arc appear to migrate primarily into cortical regions such as the cingulate gyrus and superior frontal gyrus as well as the prefrontal and entorhinal cortices rather than the olfactory bulb (Kim et al., 2025). These migratory neuroblasts almost completely disappear from the Arc by 7 months of age, meaning that postnatal plasticity in the human brain may be restricted to a much shorter period of time than in other mammalian species (Paredes et al., 2016). This early postnatal period therefore represents a period of increased vulnerability in which environmental factors may affect the development of cortical regions that rely on these early postnatal processes for their development.

The piglet brain as a model of perinatal cortical development

While much remains unknown about the dynamics of the final waves of human cortical development, it is very difficult to study the human brain directly, requiring the use of an appropriate model organism. Many recent studies have explored the use of the domestic pig (*sus scrofa domesticus*) as a potential model for human cortical development due to striking similarities in white and grey matter growth, particularly during the perinatal period (Conrad et al., 2012). Contrary to the mouse and marmoset brains, the pig brain is highly gyrified and contains both an oSVZ and an Arc-like structure and can recapitulate environmental disturbances that occur in the early postnatal human brain, making them useful preclinical models (Morton et al., 2017, Costine et al., 2013). The Arc-like structure within the piglet brain also mirrors many of the features observed in the human Arc, including a tiered structure containing cells with similar transcriptomic profiles to those in the Arc in the human brain (Kim et al., 2025). Finally, the cellular architecture of the postnatal piglet V-SVZ is arranged in a similar layered structure like that described in the human V-SVZ (Torrijos-Saiz et al., 2025). These vital morphological features of the piglet V-SVZ make it an ideal model for the changes that occur in the human V-SVZ during the perinatal period.

Regional heterogeneity in the ventricular-subventricular zone

The V-SVZ does not function as a homogenous germinal zone, but rather as a structure made up of a variety of different sub-regions. During the embryonic period, various domains of the V-SVZ can be subdivided based on combinatorial expression of several transcription factors (Flames et al., 2007). Postnatally, sub-regions of the V-SVZ are derived from distinct embryonic regions: the dorsal wall arises from the pallium, the lateral wall from the lateral ganglionic eminence, and the ventral tip from the medial ganglionic eminence (Fiorelli et al., 2015). Additionally, regional expression of some transcription factors expressed embryonically is

conserved during the perinatal period, making these regions distinguishable from each other during the postnatal period (Chaker et al., 2016). The neural stem cells (NSCs) that populate these distinct regions have also been shown to be a heterogeneous population. In the mouse brain, in which most NSCs differentiate into olfactory bulb interneurons, NSCs from separate V-SVZ sub-regions give rise to different sub-classes of olfactory bulb neurons in different regions of the olfactory bulb (Merkle et al., 2007). While many regions of the V-SVZ are able to produce glia, V-SVZ derived oligodendrocytes are primarily produced in the dorsal portion of the V-SVZ (Azim et al., 2016).

The regional heterogeneity and diverse differentiation patterns of the postnatal human V-SVZ are important to recognize because they provide clues about the processes that govern the final stages of cortical development. However, most of what is known about this region is based on studies primarily using mouse models, which are missing various key features of the human V-SVZ. For example, it remains unknown what embryonic structure gives rise to the Arc as this structure is not present in the mouse brain. In order to determine how the V-SVZ expansions in the human brain affect NSC differentiation and distribution, we must first examine how these expansions affect the structure of V-SVZ sub-regions and what features may differentiate them from each other. By leveraging the use of an animal model that more closely reflects the postnatal processes occurring in the human brain, we can begin the process of addressing the questions that remain about the final stages of human cortical development.

Chapter 2 | Regional Differences in Structure and Cellular Composition within Sub-Regions of the Perinatal Piglet Ventricular-Subventricular Zone

Results

Piglet brain size and V-SVZ sub-region changes during the perinatal period

The domestic pig (*sus scrofa domesticus*) has a gestational period of 115 days and reaches sexual maturity between 5 to 6 months of age. We examined brains from piglets at three distinct perinatal developmental stages, the late embryonic period (E100 - E108), birth (P0 - P2), and the early postnatal period (P16 - P28), to capture the period at which the Arc is most prominently present in the piglet brain (Kim et al., 2025). This period of development encompasses human ages from 36GW to toddlerhood (Costine et al., 2015), the time period at which the Arc is also most robust in the human brain (Paredes et al., 2016) (Figure 2.1A). The lateral ventricle (LV) at each stage was identified and the ventricular-subventricular zone (V-SVZ) was subdivided according to previously established parameters (Torrijos-Saiz et al., 2026). These sub-regions are the Arc, located at the dorsal horn of the LV, the dorsal wall (DW), directly bordering the corpus callosum (CC), the lateral wall (LW), stretching along the length of the striatum, and the ventral tip (VT), an extension surrounding the ventral-most point of the LV (Figure 2.1B). The presence of several anatomical features were used to ensure analysis of equivalent regions, including the cingulate gyrus (CG), superior frontal gyrus (SFG), insular

gyrus (InG), and the striatum (Str) (Figure 2.1C).

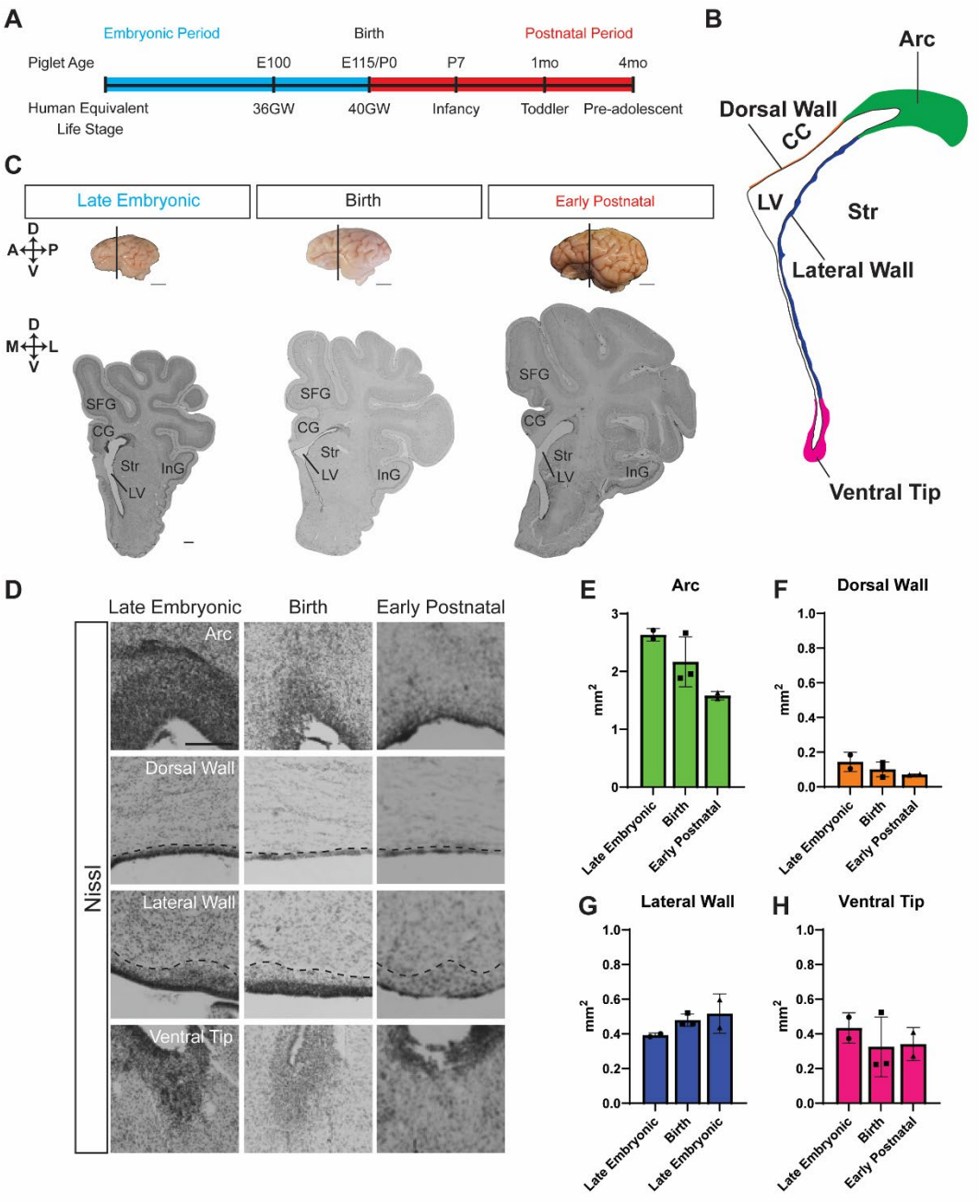

Figure 2.1. Brain structure changes during the perinatal period of piglet development. A) Comparative developmental timeline of the piglet and human perinatal period. B) Schematic representation of V-SVZ sub-regions. C) Nissl-stained sections corresponding to the anatomical level indicated in the gross images. SFG, superior frontal gyrus; CG, cingulate gyrus; Str, striatum; LV, lateral ventricle; InG, insular gyrus. D) Nissl-stained sections demonstrating V-SVZ sub-region boundaries. E – H) Quantification of V-SVZ sub-regions throughout the perinatal period for each developmental stage (n=3).

To determine what global structural changes each V-SVZ sub-region undergoes throughout this time period, we used a cresyl violet stain to identify the cell dense regions around the lateral ventricle that compose the V-SVZ and measured changes in their area (Figure 2.1D). The areas of both the Arc and the dorsal wall appear to decrease throughout the postnatal period and additionally, the Arc appears to lose cell density throughout this time (Figure 2.1D, 2.1E, 2.1F). The areas of the lateral wall and ventral tip, conversely, appear to remain relatively stable throughout this time period, although the ventral tip appears to undergo changes in overall structure (Figure 1D, 1G, 1H). Regardless of any changes occurring, the Arc retains the highest area throughout this entire time period. The apparent changes in different sub-regions during this time period suggest that each sub-region is undergoing dynamic changes independently of each other.

Spatial and temporal changes in cellular architecture during the perinatal period

To assess the structural features of each V-SVZ sub-region, we performed immunohistochemistry on sections from each developmental stage to detect the presence of glial fibrillary acidic protein (GFAP, astrocytes) (Figure 2.2A). In both the Arc and the ventral tip, GFAP fibers extend radially from the lateral ventricle and throughout both the VZ and SVZ in a homogenous pattern. In the dorsal wall, during the late embryonic period, GFAP fibers extend radially throughout the VZ but are oriented tangentially to the ventricular wall in the SVZ. However, the small ribbon of tangentially oriented fibers in the SVZ appear to consolidate with the VZ during birth and into the early postnatal period, making the two regions difficult to distinguish from each other. In the lateral wall, GFAP+ cell bodies are concentrated at the wall of the lateral ventricle and extend their fibers radially throughout the VZ until they reach the SVZ, at which point GFAP+ fibers orient tangentially to the ventricular wall.

Next, we stained for doublecortin (DCX) in order to determine how migrating neuroblasts were organized within each sub-region (Figure 2.2B). In the Arc and ventral tip, DCX+ cells form a dense, honeycomb pattern that extends throughout the entirety of both sub-regions. In the dorsal wall, a sparse number of DCX+ cells are observed within the V-SVZ, with the occasional DCX+ cell observed just outside of the bounds of the dorsal wall. No age-dependent changes in DCX+ cell patterning were observed in any of these three sub-regions. In the lateral wall, however, DCX+ neuroblasts were primarily located in the space created by the radially oriented GFAP+ fibers within the VZ, with only a few cells observed within the SVZ. As this region thinned throughout the early postnatal period, DCX+ neuroblasts continued to be confined to the same region, suggesting that this region may later form the gap layer that has been described in the human V-SVZ (Sanai et al., 2011).

Using a combination of DAPI+ cell density and GFAP+ and DCX+ cellular architecture, we were able to infer potential boundaries between the VZ and SVZ at each sub-region (Figure 2.2C). The Arc's unique cytoarchitecture allows it to be subdivided into three tiers, with tier 1 consisting of the VZ and tiers 2 and 3 making up the SVZ. At all developmental stages, tier 3 accounts for the largest portion of the length of the V-SVZ, with a notable expansion at birth that depletes during the early postnatal period (Figure 2.2D). Tiers 1 and 2 lengths remain constant during this time period. The length of the dorsal wall remains constant through birth and then thins considerably during the early postnatal period (Figure 2.2E). The length of the lateral wall drops steadily throughout the early postnatal period, with reductions in both the VZ and SVZ (Figure 2.2F). During the late embryonic and birth stages, proportions of VZ and SVZ length within the lateral wall are similar, but the length of the VZ thins throughout the postnatal period compared to the SVZ. The thickness of the ventral tip remains constant with the SVZ accounting for the majority of the length (Figure 2.2G). Overall, each V-SVZ sub-region undergoes structural

changes independently of the other regions, suggesting that they may act as independent niches.

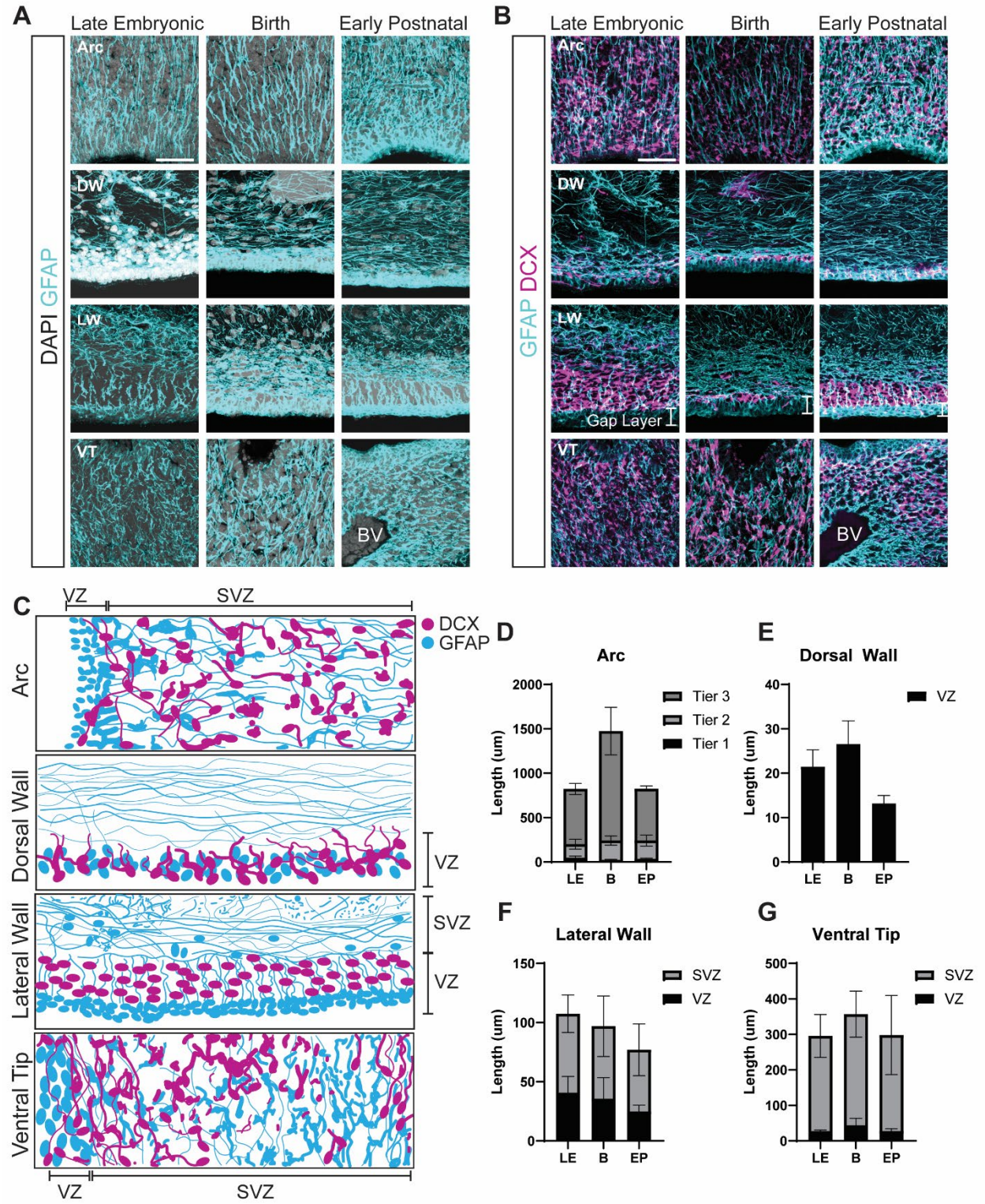

Figure 2.2. Cellular architecture and V-SVZ thickness variations in V-SVZ sub-regions.

A – B) Regional and temporal differences in GFAP+ and DCX+ cell distribution throughout the V-SVZ. DW, dorsal wall; LW, lateral wall; VT, ventral tip; BV, blood vessel. C) Schematic representation of GFAP+ and DCX+ cell distribution in V-SVZ sub-regions. D – E) Quantification of length of tiers, VZ, and SVZ in different sub-regions of the V-SVZ (n=2). LE, late embryonic; B, birth; EP, early postnatal.

Distribution of proliferative cells throughout sub-regions of the ventricular-subventricular zone

To investigate if the observed differences in distribution of neuroblasts between V-SVZ sub-regions also correlate to differences in distribution of the proliferative cell populations during the perinatal period, we performed immunohistochemistry for the proliferation marker Ki67 (Figure 2.3A). In the Arc, Ki67+ cells can be found distributed throughout all three tiers with a bias toward the VZ. In the dorsal wall, Ki67 cells can be found lining the wall throughout the entire region. In the lateral wall, Ki67+ cells are not typically found within the VZ during the late embryonic period, but can be observed in both the VZ and SVZ starting at birth and continuing into the early postnatal period. Similar to the Arc, Ki67+ cells in the ventral tip can be found distributed throughout the region with a slight bias towards the VZ.

To identify any variations in Ki67+ cell density throughout the entirety of the V-SVZ, we used the detect cell function in the neuron tracing software Neurolucida to place a marker over every Ki67+ cell within each V-SVZ sub-region (Figure 2.3B). In the Arc, Ki67+ cells appear more concentrated closer to the ventricular wall (tier 1). Interestingly, there also appeared to be a discrepancy in the density of Ki67+ cells between the dorsal and ventral portions of the Arc (Figure 2.3C). In the dorsal wall and ventral tip, Ki67+ cells appear to be evenly distributed throughout the regions with no apparent variations (Figures 2.3D and 2.3F). In the lateral wall, Ki67+ cells were evenly distributed for the most part; however, outcroppings of Ki67+ cells could be observed in areas with large blood vessels (Figure 2.3E). Overall, with the exception of the

Arc, there did not appear to be large variations in the distribution of Ki67+ cells within the same sub-regions.

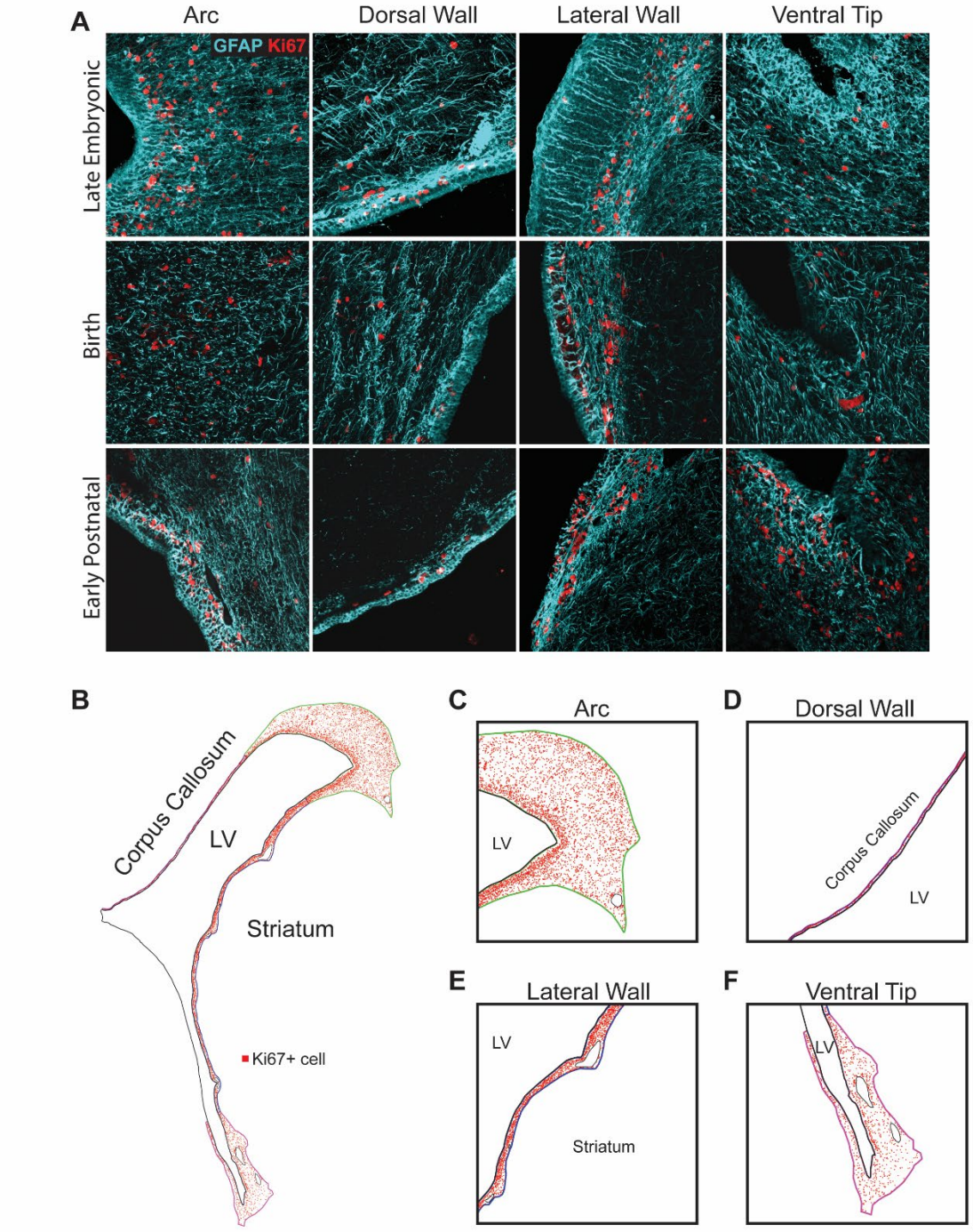

Figure 2.3. Ki67+ cell distribution throughout the perinatal piglet V-SVZ. A) Regional and temporal differences in GFAP+ and Ki67+ cells in V-SVZ sub-regions. B) Schematic representation of Ki67+ cell distribution throughout the V-SVZ. C – F) Representative V-SVZ sub-region Ki67+ cell distribution.

Spatial and temporal variations in Ki67+ cell density in the perinatal ventricular-subventricular zone

While observing the distribution of Ki67+ cells, we noted that there appeared to be variations in the density of Ki67+ cells between V-SVZ sub-regions. To explore this possibility, we calculated the average Ki67+ cell density of three ROIs per sub-region in three slices from three individuals per developmental stage (Figure 2.4A). During the late embryonic period, the lateral wall has the highest Ki67+ cell density compared to the Arc and dorsal wall (Figure 2.4B). At birth, the discrepancy in density between the Ki67+ cell density in the lateral wall and Arc/dorsal wall persists, but the density of the ventral tip is also significantly higher than the Arc and dorsal wall (Figure 2.4C). During the early postnatal period, the Ki67+ cell density in the lateral wall remains higher than in the Arc or dorsal wall (Figure 2.4D). Overall, a similar pattern emerges at each developmental stage in which the lateral wall and ventral tip have markedly higher Ki67+ cell densities than the Arc or dorsal wall.

Earlier in our study, we noted that there were significant differences in the areas of each V-SVZ sub-region (Figure 2.1). To account for potential differences in total Ki67+ cell number arising from density and area differences, we calculated the predicted absolute cell number of each region by multiplying the density of each sub-region by the area of that same region. During the late embryonic period and at birth, the predicted absolute cell number of the Arc was significantly higher than any other region (Figure 2.4E – 2.4F). We found that during the early postnatal period, as the Arc area shrinks, the predicted absolute Ki67+ cell number is comparable to the lateral wall, whose area does not significantly change during this time period and whose cell density is consistently higher than all other regions (Figure 2.4G). This data suggests that the V-SVZ expansion that led to the formation of the Arc and is not only meant to accommodate greater number of migrating neuroblasts, but also a persistent population of proliferating cells that continue to populate the Arc during the early postnatal period.

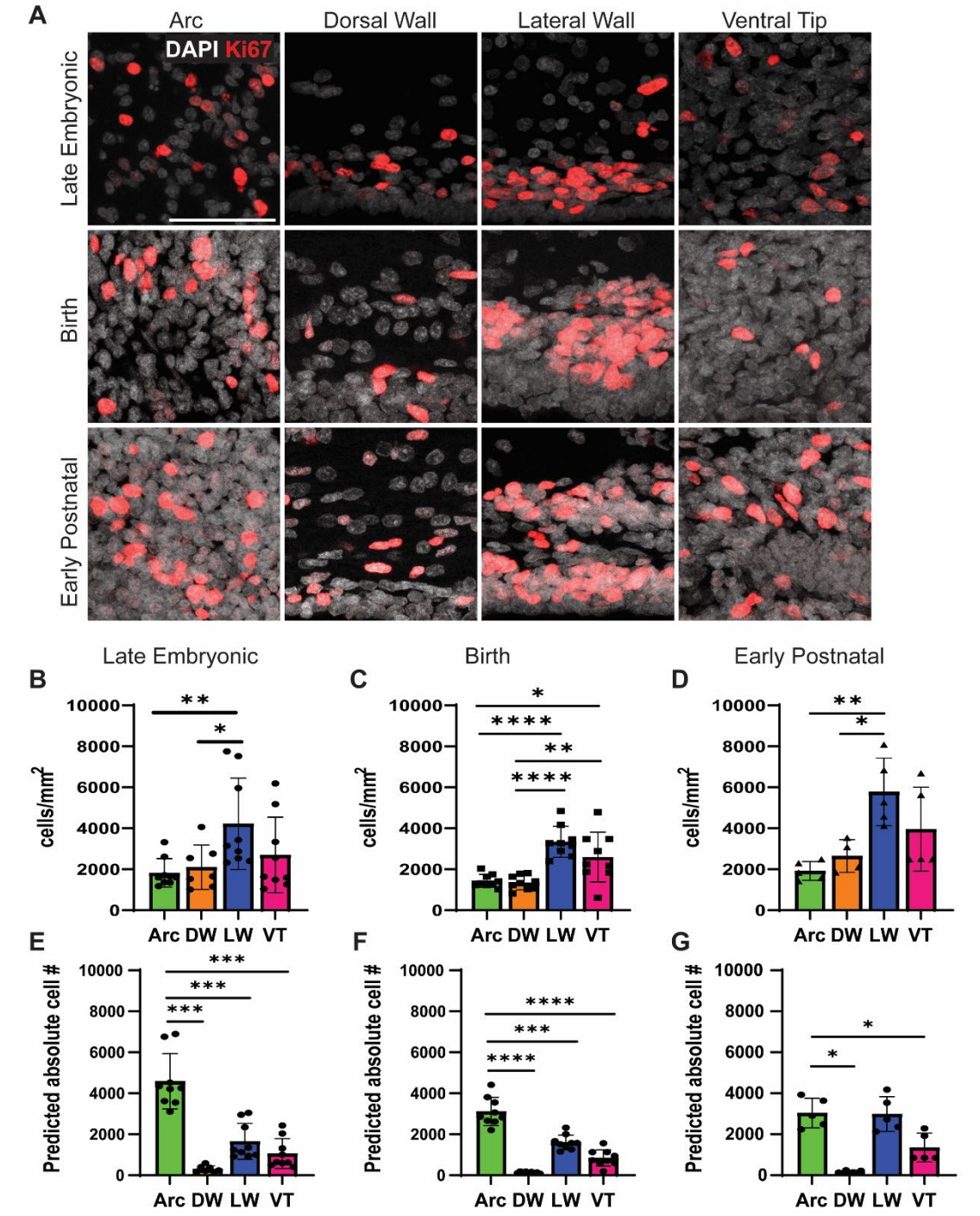

Figure 2.4. Spatial and temporal variations in Ki67+ cell distribution and proportion.

A) Regional and temporal differences in Ki67+ cell populations in the V-SVZ. B) Quantification of Ki67+ cell density during the late embryonic period (n=3) Mann-Whtiney test (**P=0.002756, *P=0.041783). C) Quantification of Ki67+ cell density at birth (n=3) Mann-Whtiney test (*P=0.007775, **P=0.00399, ****P<0.0001). D) Quantification of Ki67+ cell density during the early postnatal period (n=3) Mann-Whtiney test (**P=0.0007937, *P=0.015873) DW, dorsal wall; LW, lateral wall; VT, ventral tip. E) Predicted absolute Ki67+ cell count during the late embryonic period (V-SVZ cell area x Ki67+ cell density) Mann-Whitney test (***P=0.000175). F) Predicted absolute Ki67+ cell count at birth (V-SVZ cell area x Ki67+ cell density) Mann-Whitney test (***P=0.000082, ****P<0.0001). G) Predicted absolute Ki67+ cell count during the early postnatal period (V-SVZ cell area x Ki67+ cell density) Mann-Whitney test (*P=0.015873).

The majority of Ki67+ cells express the intermediate progenitor marker Dlx2

Following the observation that the expansion of the Arc led to higher projected numbers of Ki67+ cells within that region, we questioned which potential cell types are being produced to account for this increase in proliferation. We began to investigate this question by determining if the increase can be attributed to an increase in glial production. We performed immunohistochemistry for the microglial marker Iba1 and the mature astrocyte marker ALDH1L1 and found that neither of these markers co-localized with Ki67 in any V-SVZ sub-region (data not shown). However, we did observe instances of Ki67+Olig2+ cells within each sub-region (Figure 2.5A). Using a quantification strategy similar to the one used for Ki67+ cell density, we measured the density of Ki67+Olig2+ cells in each sub-region at birth and found that the density of these double positive cells was much higher in the lateral wall than the Arc (Figure 2.5B). However, when we determined the proportion of double positive cells that account for the total Ki67+ cell population, we observed that the Ki67+Olig2+ cell population accounted for less than 20% of the total Ki67+ cell population, the dorsal wall having the highest proportion of double positive cells at approximately 15% (Figure 2.5C).

In Kim et al., 2025, it was reported that a large proportion of the neuroblasts within the Arc are young, inhibitory neurons. We therefore performed immunohistochemistry for the inhibitory

neuron intermediate progenitor marker Dlx2 (Figure 2.5D). We immediately noted that there was a much higher density of Ki67+Dlx2+ cells in all sub-regions of the V-SVZ, with the highest density observed in the lateral wall (Figure 2.5E). As expected, the Ki67+Dlx2+ cell population accounted for a much higher proportion of the total Ki67+ cell population, accounting for greater than 75% of the Ki67+ cell population in all regions excluding the dorsal wall, in which the double positive population accounted for only approximately 65% of the total Ki67+ cell population. These results support the theory that V-SVZ expansion, especially during the perinatal period, within large, gyrified brains occurred due to the need for an increased number of neurons.

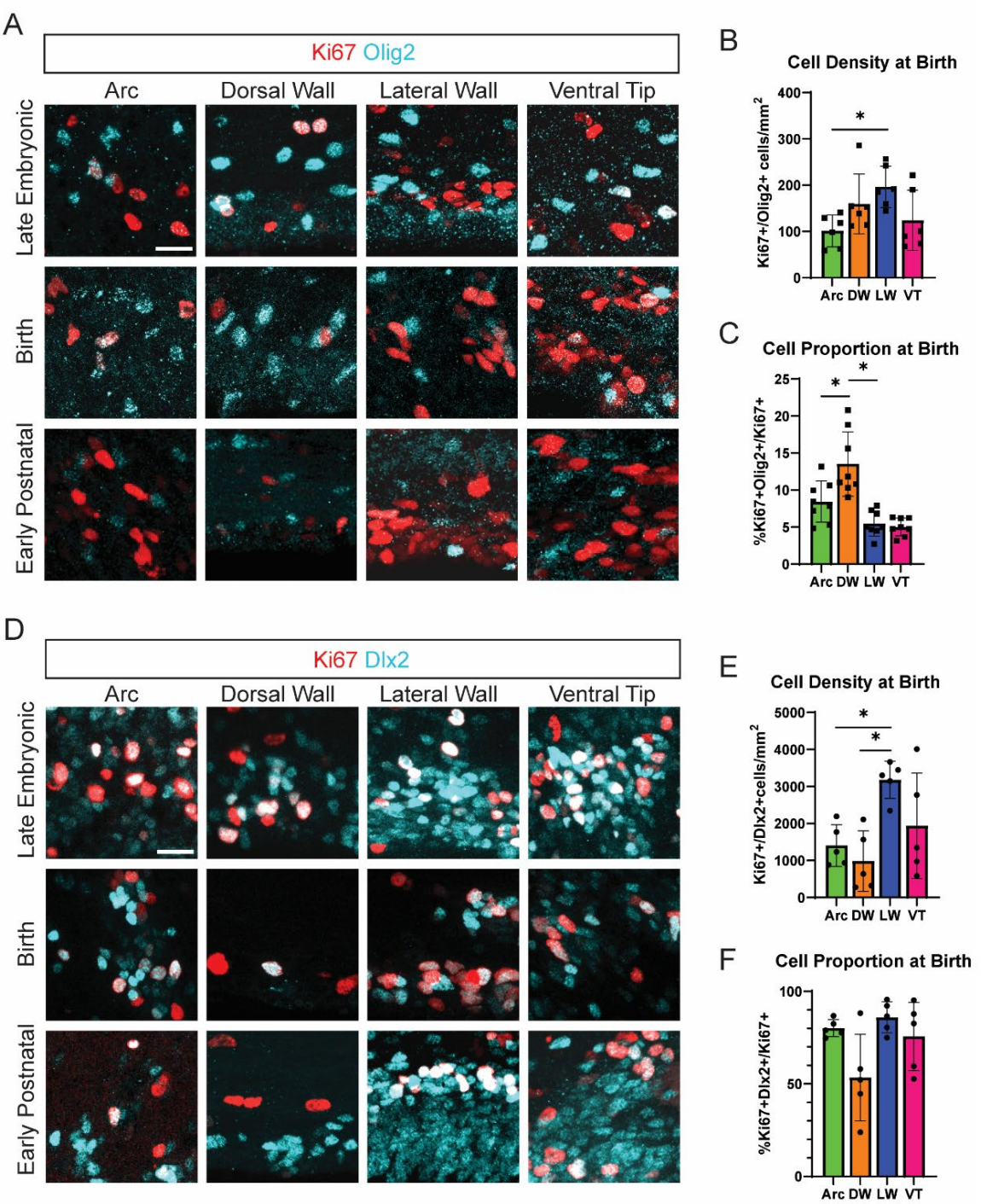

Figure 2.5. Spatial variations in Ki67+ cell subpopulations during birth. A) Colocalization of Ki67+ (red) and Olig2+ (cyan) cells in the V-SVZ. B) Quantification of Ki67+Olig2+ cell density at birth (n=3) Mann-Whitney test (*P=0.002165). C) Proportion of Ki67+ cells that co-express Olig2 (n=3) Mann-Whitney test (*P=0.010412). D) Colocalization of Ki67+ (red) and Dlx2+ (cyan) cells in the V-SVZ. E) Quantification of Ki67+Dlx2+ cell density at birth (n=3) Mann-Whitney test (*P=.007937). F) Proportion of Ki67+ cells that co-express Dlx2 (n=3).

Discussion

We demonstrate that there is considerable heterogeneity in the V-SVZ in both structure and cellular composition. The structure of the lateral wall is the most consistent with previous reports of V-SVZ structure in the human brain, but the other three sub-regions have their own unique structure. These sub-regions also contain varying numbers of dividing cells, although they all seem to be primarily producing inhibitory interneurons.

What regions are these interneurons migrating to? In the mouse brain, interneurons born during the postnatal period mainly migrate to the olfactory bulb, but more recent work on the human and piglet brain has shown that migrating neuroblasts, particularly those in the Arc, migrate to several cortical regions (Paredes et al., 2016, Kim et al., 2025). While the postnatal development of the piglet brain broadly matches that of the human brain, adult pigs rely on olfaction as a sensory modality much more than adult humans do, suggesting that neurons born within the postnatal piglet V-SVZ may show a bias toward olfactory interneuron production. The heterogeneity found within sub-regions of the piglet V-SVZ and the maintenance of interneuron production in the lateral wall and ventral tip as opposed to the Arc support the idea that different regions of the V-SVZ may produce neurons with different target destinations.

We also show variations in the Ki67+ cell density within V-SVZ sub-regions. While it is tempting to infer that this dividing cell population is responsible for the large number of neuroblasts observed within the same region, we have not yet been able to show a direct connection. The possibility remains that the dividing cells within the piglet V-SVZ are unrelated to the neuroblasts, meaning that the postnatal V-SVZ may be able to function as both a neurogenic niche and a migratory corridor.

Methods

Tissue Collection

Pig brains (*Sus Scrofa*) from E100, E108, postnatal day (P) 0–2, P16, and P28 were collected at the Swine Teaching and Research Center at the University of California, Davis. All animal procedures conformed to the requirements of the Animal Welfare Act and were carried out under the Association of Assessment and Accreditation of Laboratory Animal Care International approved conditions with protocols approved before implementation by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Davis (UC Davis IACUC protocol 23682). All brains were fixed in 4% paraformaldehyde (PFA) in PBS for 2 days at 4 °C. Tissue specimens were immersed in 30% sucrose in PBS at 4 °C, then were cryopreserved after tissue specimens had sunk to the bottom of the vial. Tissue specimens were cut into coronal blocks and frozen in an optimal cutting temperature compound. Blocks were cut at ~30 µm on a cryostat and mounted on glass slides for experimental analysis.

Cresyl staining (Nissl)

Frozen slides were allowed to thaw and equilibrate at room temperature overnight. Slides were baked for 30 minutes at 60 °C and incubated in cresyl violet solution (Sigma-Aldrich, V5265) for 30 minutes. Slides were washed with distilled water twice and sequentially incubated in 50%, 70%, 95% and 100% ethanol in distilled water for 1 minute each, followed by incubation in xylene solution for 3 minutes. Slides were mounted and cured overnight and imaged using a Leica Aperio Versa 200 slide scanner microscope (Octopus).

Immunohistochemistry

Frozen slides were thawed overnight at 4 °C and allowed to equilibrate at room temperature for 3 hours. For antigen retrieval, slides were boiled at 95–100 °C in 10 mM sodium citrate buffer

(pH 6.0) for 10 minutes and then cooled at room temperature. Samples were permeabilized with 0.05 % Triton X-100 in PBS for 10 minutes, then incubated in 1% H_2O_2 in PBS for 1 hour and blocked with Tris-NaCl-blocking buffer (TNB) (0.1 M Tris–HCl (pH 7.5), 0.15 M NaCl and 0.5% blocking reagent from PerkinElmer, FP1012) for 1 hour. Slides were incubated with primary antibodies in TNB overnight at 4 °C, followed by incubation with biotinylated secondary antibodies, diluted 1:250 in TNB solution for 2.5 h at room temperature. Next, slides were incubated with horseradish peroxidase-conjugated streptavidin, diluted 1:200 in TNB for 30 minutes, before incubation with tyramide-conjugated fluorophores (Akoya), diluted 1:100, in amplification buffer (PerkinElmer) for 5 minutes. Images were acquired on a Stellaris confocal microscope using ×10 (0.4 NA) and ×40 (1.30 oil) objectives.

Image Analysis

V-SVZ sub-region area and length measurements

Sub-region area was measured using tiled images of the lateral ventricle and surrounding regions. V-SVZ boundaries were determined using expression of DAPI, GFAP, and DCX. Boundaries between regions were determined using a combination of anatomical landmarks and differential expression of Pax6 and Nkx2.1. VZ and SVZ boundaries were determined using distribution of DAPI and GFAP fiber orientation.

Mapping of Ki67+ cells

Slides were stained with anti-Ki67 primary antibody to visualize Ki67+ cell distribution in each V-SVZ sub-region. Using Neurolucida software (MBF Bioscience), a contour surrounding each individual sub-region was drawn and the detect cell tool was used to place a marker over each Ki67+ cell.

Ki67+ cell density measurements

Three regions of interest (ROIs) were chosen from each V-SVZ sub-region at each developmental stage to be imaged on a Stellaris confocal microscope using a ×40 (1.30 oil) objective. Images were analyzed using the detect cell tool in Neurolucida software (MBF Bioscience). False positive and false negative detections were then manually corrected by eye. The resulting Ki67+ cell number is then divided by the V-SVZ area visible within the ROI to generate cell density. Cell densities are then multiplied by the total area of the corresponding V-SVZ sub-region to generate the predicted absolute cell number.

Conclusion

Structural differences in ventricular-subventricular zone sub-regions

The Arc, dorsal wall, lateral wall, and ventral tip regions of the V-SVZ each possess unique structural differences throughout the perinatal period. The Arc is the largest region by area, but its area declines rapidly throughout the perinatal period. The lateral wall and ventral tip, however, have fairly constant areas throughout the same time, suggesting that the role of the Arc is much more transient than that of the other regions. GFAP+ and DCX+ cell architecture provided further insights into the structural differences between sub-regions. The GFAP+ fiber pattern of the lateral wall closely mirrored the layered architecture previously described in the human V-SVZ, including the fiber rich area that eventually becomes the gap layer but remains populated with DCX+ neuroblasts during the early postnatal period. GFAP+ fibers in the Arc and ventral tip, however, do not adopt this layered architecture. Rather, GFAP+ fibers extend radially throughout the area, with DCX+ cells evenly distributed among these fibers. This difference in cellular architecture implies that these regions may serve different purposes during the final stages of development.

Regional heterogeneity in the proliferative population of the ventricular-subventricular zone

Regional heterogeneity extended to the proliferative (Ki67+) cell population, with each region containing different densities of dividing cells. At all developmental stages, the lateral wall contains the highest density of Ki67+ cells. Interestingly, the spatial differences in Ki67+ cell density between regions remained the same at all developmental stages: the lateral wall

consistently contained the highest density. However, when accounting for the differences in area observed earlier to estimate the total number of dividing cells per region, the patterns change drastically. The region with the largest area, the Arc, contains the largest projected number of Ki67+ cells by a wide margin during the early postnatal period and at birth. As the area of the Arc drops, the total projected cell number drops to close to that of the lateral wall, whose area and density both remain constant during this time period. Taken together, this data shows that the Ki67+ cell populations in each region function independently, implying that the dividing cell population within each region is regulated by different factors.

Dividing cell populations within the ventricular-subventricular zone are primarily neurogenic

Previous studies have shown that neural stem cells in the postnatal V-SVZ primarily produce oligodendrocytes and interneurons. Using a combination of a proliferative cells marker (Ki67), an oligodendrocyte precursor marker (Olig2) and an interneuron intermediate progenitor marker (Dlx2), we were able to infer that the vast majority of Ki67+ cells in the perinatal piglet brains are destined to become interneurons. At all developmental stages, >80% of the Ki67+ cell population expressed Dlx2 with the notable exception of the dorsal wall, in which only approximately 65% of Ki67+ cells expressed Dlx2. The dorsal wall also contained the highest proportion of Ki67+Olig2+ cells, with about 15% of Ki67+ cells expressing Olig2. This is consistent with previous studies that show that oligodendrocyte production primarily occurs in the dorsal wall. While this data appears to be consistent with reported findings in the mouse brain, it remains unknown what proportion of the interneurons produced in the perinatal piglet brain are destined for the olfactory bulb versus cortically bound.

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