Chemoarchitecture of area prostriata in adult and developing mice: Comparison with presubiculum and parasubiculum

Sheng-Qiang Chen¹,² | Chang-Hui Chen¹,² | Xiao-Jun Xiang¹,² | Shun-Yu Zhang¹,² | Song-Lin Ding¹,²,³

¹Key Laboratory of Neuroscience, School of Basic Medical Sciences, Guangzhou Medical University, Guangzhou, China
²Institute of Neuroscience, The Second Affiliated Hospital, Guangzhou Medical University, Guangzhou, China
³Allen Institute for Brain Science, Seattle, Washington, USA

Correspondence
Song-Lin Ding, Allen Institute for Brain Science, 615 Westlake Ave N, Seattle, WA 98109, USA.
Email: songd@alleninstitute.org

Funding information
National Natural Science Foundation of China, Grant/Award Number: #31771327

Abstract
Retrosplenal area 29e, which was a cortical region described mostly in earlier rodent literature, is often included in the dorsal presubiculum (PrSd) or postsubiculum (PoS) in modern literature and commonly used brain atlases. Recent anatomical and molecular studies have revealed that retrosplenal area 29e belongs to the superficial layers of area prostriata, which in primates is found to be important in fast analysis of quickly moving objects in far peripheral visual field. As in primates, the prostriata in rodents adjoins area 29 (granular retrosplenal area), area 30 (apgranular retrosplenal area), medial visual cortex, PrSd/PoS, parasubiculum (PaS), and postrhinal cortex (PoR). The present study aims to reveal the chemoarchitecture of the prostriata versus PrSd/PoS or PaS by means of a systematic survey of gene expression patterns in adult and developing mouse brains. First, we find many genes that display differential expression across the prostriata, PrSd/PoS, and PaS and that show obvious laminar expression patterns. Second, we reveal subsets of genes that selectively express in the dorsal or ventral parts of the prostriata, suggesting the existence of at least two subdivisions. Third, we detect some genes that show differential expression in the prostriata of postnatal mouse brains from adjoining regions, thus enabling identification of the developing area prostriata. Fourth, gene expression difference of the prostriata from the medial primary visual cortex and PoR is also observed. Finally, molecular and connectional features of the prostriata in rodents and nonhuman primates are discussed and compared.

KEYWORDS
cell types, gene expression, laminar organization, postnatal development, retrosplenal area 29e, subicular cortex, visual cortex

1 | INTRODUCTION

Brodmann appeared to be the first to describe the retrosplenal subarea (area 29e) on the caudal medial aspect of the rabbit brain (Brodmann, 1909). According to him, rabbit area 29e, together with other four retrosplenal subareas (areas 29a–d or RSg), occupies whole medial surface of the occipital lobe. On his map, rabbit area 29e is a large region labeled among areas 29b–d, presubiculum (PrS; or area 27), and areas 19 and 20, whereas an agranular retrosplenal area (area 30 or RSag), which was described in other species, is absent in rodents including rabbits (Brodmann, 1909; rabbits belong to lagomorphs in modern literature). Although Brodmann did not illustrate area 29e in brain sections, a conspicuous triangular field located between the dorsal presubiculum (PrSd)/postsubiculum (PoS) and
parasubiculum (PaS) of the rat and mouse brains was treated as the equivalent of area 29e by later scientists (Blackstad, 1956; Haug, 1976; Preston-Ferrer et al., 2016; Slomianka & Geneser, 1991; Vaz Ferreira, 1951) or included in the PrSd/PrO (e.g., Paxinos & Franklin, 2001; Swanson, 2018). In many other species, this triangular region was not identified likely because it did not display triangular shape in these species. On the other hand, another unique area, region prostriata (or prostriata), was identified at the location immediately adjoining the striate cortex (i.e., primary visual cortex, V1, or area 17) in human and nonhuman primates (Allman & Kaas, 1971; Morecraft et al., 2000; Rockland, 2012; Sanides, 1969; Sousa et al., 1991) but not in rodents. In sequential sections stained with Nissl substance, calcium-binding proteins, and a nonphosphorylated neurofilament protein, the prostriata in human and nonhuman primates was found to adjoin the retrosplenial cortex (RS), PrSd/PrO, PaS, and posterior cingulate cortex (area 23) at the anterior levels and the V1 and V2 (i.e., secondary/association visual cortex, or area 18) at the posterior levels (Ding et al., 2003, 2016).

In a survey of the subicular complex across species, the triangular region in rodent brains was also found to adjoin the RS, PrSd/PrO, PaS, and visual cortex (V1 and V2) (Ding, 2013). This reminds the similar findings from detailed studies of the prostriata in macaque monkeys (Ding et al., 2003; Morecraft et al., 2000). In addition, the triangular region in rodents and the prostriata in nonhuman primates both receive direct projections from the V1 (Ding, 2013; Sousa et al., 1991). These findings strongly suggest that the triangular region (or area 29e) in rodents is the equivalent of part of the prostriata in human and nonhuman primate brains. Our recent studies on this triangular region of the rat and mouse brains have discovered that the triangular region belongs to the superficial layers (layers 2–3) of the prostriata rather than the RS or PrSd/PrO (Chen et al., 2020, 2021; Hu et al., 2020; Lu et al., 2020). These studies have further revealed that rodent prostriata also has deep layers 5 and 6 and a cell-less zone (lima dissecans [Idl], which separates layers 2–3 from 5–6. Although functional studies of area 29e were not carried out previously, our recent findings that the prostriata in rodents receives strong inputs directly from dorsal lateral geniculate nucleus (DLG) and V1 (Chen et al., 2021; Lu et al., 2020) support the functions of the prostriata in fast analysis of rapid moving stimuli in far peripheral visual field observed in human and nonhuman primate brains (Mikellidou et al., 2017; Tamietto & Leopold, 2018; Yu et al., 2012).

In the present study, we aim to systematically investigate the chemomecharchitecture of the prostriata with focus on laminar and regional differences. This type of information is important for future cell type-specific circuit and functional studies of the prostriata. The second aim is to identify gene markers for localization of the prostriata at different developmental stages and for developmental study of this region. Our third aim is to compare expression patterns of the prostriata and adjoining structures such as PrSd/PrO or PaS to further illustrate the difference among these structures.

2 | MATERIALS AND METHODS

2.1 | Animals and in situ hybridization data

In situ hybridization (ISH) raw data were derived from Allen Brain Atlas (https://mouse.brain-map.org) for adult mouse brains; see Lein et al., 2007 and Allen Developing Mouse Brain Atlas (ADMDA https://developingmouse.brain-map.org); see Thompson et al., 2014. The ADMDA covers seven ages including four embryonic (postconception days: E11.5, E13.5, E15.5, and E18.5) and three postnatal ages (P4, P14, and P28 days after birth, where day of birth is P0).
All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Research Ethics Committee. The details for generating these data including probe synthesis, primer design, tissue preparation, condition of hybridization, image processing, and quality control are published online (https://help.brain-map.org/display/mousebrain/Documentation for ABA; https://help.brain-map.org/display/devmouse/Documentation for ADMBA). Briefly, after sectioning, ISH of stains was performed on slides using a semiautomated nonsitopic digoxigenin-labeled colorimetric platform. Riboprobes were labeled with either digoxigenin-UTP or dinitrophenyl-11-UTP (DNP; Perkin Elmer, Waltham, MA, USA). A DNP-labeled probe and a DIG-labeled probe were hybridized simultaneously. Tyramide signal amplification was performed for each probe individually, using either anti-DIG-HRP with tyramide biotin or anti-DNP-HRP with tyramide-DNP for amplification. The sections from embryonic ages were counterstained with nuclear HP Yellow to aid identification of anatomic structures. Finally, it should be mentioned that mRNA expression does not necessarily correlate one-to-one with protein expression but many or at least some of them should correlate. For example, both Calb1 (gene) and calbindin-D28k (the protein coded by Calb1) are strongly expressed in the prostriata, as reported in our previous studies (Chen et al., 2021; Lu et al., 2020).

### 2.2 Data analysis and image capture

From the ABA and ADMBA portals, we first screened enriched gene expression in the region corresponding to the prostriata defined in our recent studies (Hu et al., 2020; Lu et al., 2020) using the gene finder tool of the Anatomic Gene Expression Atlas (AGEA) application. We then manually examined each selected case to confirm the enriched expression and determine their laminar distribution in the prostriata. Those cases with no obvious expression in the prostriata (except for the cases with clear negative expression in layers 2–3 or 5 of the prostriata, which were labeled as negative marker genes) were excluded from further analysis. The same protocols were used for searching enriched genes in the PrS/PoS and PaS. In this study, a total of 250 and 90 cases were examined for adult (P56; C57BL/6J; two to four cases for each gene) and postnatal (P4, P14, and P28; C57BL/6J; one or two cases for each gene/age) mice, respectively. Finally, the images from the regions of interest (with scalable resolution up to 1.0 μm/pixel) were selected and imported into Adobe Photoshop (CS5) for adjustment of brightness and contrast as well as arrangement and anatomical annotation. Since our main aim is to identify differential gene markers of the prostriata from adjoining regions, the present study focuses on obvious or clear differences of gene expression that can be clearly observed without quantitative evaluation. Accordingly, slight difference is still considered as “similar” because it cannot be used as reliable differential gene markers.

### 3 RESULTS

#### 3.1 Borders, topography, and extent of area prostriata and adjoining regions

As in our previous studies, we define the boundaries of the prostriata using a combination of cytoarchitecture and molecular markers. To demonstrate the borders, topography, and extent of the prostriata in both sagittal and coronal sections, here we use Pcsk5 as a gene marker for both sectioning planes so that the lamination, shape, and extent of the prostriata can be directly visualized and compared on the two planes. Generally, it is relatively difficult to distinguish the prostriata from adjoining regions in Nissl preparations. For example, both the prostriata and PaS display similar cell size and packing density (e.g., Figures 1a,b, and 2a). However, some features of the prostriata are still visible such as larger cells in its superficial layers 2–3 compared to adjoining PrSd, RS, and V1 (Figures 1c and 2a and the insets in Figure 1h,i). In Pcsk5-ISH-stained sagittal sections, the triangular layers 2–3 of the prostriata stand out because of their strong Pcsk5 expression, while no or faint expression is observed in the superficial layers of the neighboring regions such as PaS, PrSd, RS, and V1 (Figures 1d–i and 2b,c,e). Layers 5 and 6b of the prostriata are also clearly seen since they are Pcsk5 positive and continue with layers 5 and 6b of adjoining regions, respectively (Figure 2b,c,e). It is noted that the shapes and trajectories of layer 5 of the prostriata vary from lateral (Figure 1d) to medial (Figure 1i) sagittal levels. At the most lateral level, layer 5 is straight down into the deep part of the PaS and larger in length than layers 2–3 (Figure 1d), while at the medial levels layer 5 is slightly curved and smaller in length compared to layers 2–3 in sagittal sections (Figure 1e–i). Compared to surrounding regions, layer 6 of the prostriata is a cell-sparse zone (Figures 1a–c and 2a,d) and negative for Pcsk5 (Figures 1d–i and 2b,c,e). It is worth mentioning that layer 5 of the PrSd displays relatively stronger Pcsk5 expression than that of the prostriata (Figure 2b,c,e).

In Pcsk5-ISH-stained coronal sections (Figure 3), both layers 2–3 and 5 of the prostriata can be identified but they look different from those seen in sagittal sections. Most of the RSg does not adjoin the prostriata (Figure 3a) except its most caudal part (Figure 3b). From the rostral (Figure 3b) to caudal (Figure 3i) levels, the overall sizes of layers 2–3 and 5 change from small (Figure 3d) to large (Figure 3e–g) to small (Figure 3h,i). In coronal sections, the prostriata adjoins the RSg, RStg, and V2M, and V1 dorsally, and the PrSd and PaS ventrally (Figure 3b–i). The most caudal part of the prostriata merges with the postrhinal cortex (PoR) and PaS laterally (Figure 3i). The PoR strongly expresses Nnat, while the prostriata, PaS, and V1 do not (see the inset in Figure 3i). It is also noted that the PaS but not the PrS/PoS directly adjoins the medial entorhinal cortex (MEC; Figure 3f–i).

In Cxcl14-ISH-stained coronal sections, layers 2–3 of the RSg, layer 2 of the PrSd, and layers 2–3 of the prostriata show strong
FIGURE 1 Location, topography, and lamination of area prostriata (Pro) in sagittal sections. Arabic numbers indicate cortical layers. (a–c) Nissl-stained sections from three lateral to medial levels (a–c) showing the location and lamination of the Pro and adjoining regions. The inset in panel (a) shows the locations (indicated by white vertical lines) of the three Nissl-stained sections in panels (a–c) and the six Pcsk5-stained sections from panel (d) to panel (i) on the dorsal aspect of the left hemisphere. Panels (a–c) are closely adjacent sections of panels (d–f), respectively. (d–i) Expression of the gene Pcsk5 in the Pro from lateral (d) to medial (i) sagittal sections. The interval between each section from panel (d) to panel (i) is 160 µm. Layers 2–3 of the Pro is clearly identified with its strong Pcsk5 expression compared to faint expression in adjoining postrhinal cortex (PoR), primary visual cortex (V1), and dorsal presubiculum (PrSd). Pcsk5 is also expressed in layer 5 of the Pro, PoR, V1, and PrSd. The borders of layer 5 of the Pro with these three regions were determined by layer 5-selective/dominant expression of C1ql2 (see Lu et al., 2020), Lypd1, and Efnb3 (see Figure 5) in the Pro. Note the differential shapes and orientation of the layers across lateral (d) to medial (i) levels. The insets in panels (h) and (i) show the location of the Pro in Nissl-stained sections adjacent to panels (h) and (i). Higher magnification views of the Pro in panels (b), (e), (d), (c), and (g) are presented in Figure 2a–e, respectively. DG, dentate gyrus; Sub, subiculum; ProS, prosubiculum; PoS, presubiculum; PoS, postsubiculum; PaS, parasubiculum; MEC, medial entorhinal cortex; RSG, granular retrosplenial cortex (area 29). Scale bar: 210 µm in panel (a) for all panels.

3.2 Layers of area prostriata, presubiculum, and parasubiculum

Cxcl14 expression, whereas the PaS is negative (see the insets in Figure 3a,c,e,g). In fact, layer 2 of the PrSd is composed of densely packed small neurons, whereas the PaS does not have this kind of layer 2 and this can be clearly appreciated in Nissl preparations (see Lu et al., 2020).
such as Rfx3 and Prkca (for layers 2–3; Figure 4b–n), Etv1 and C1ql2 (for layer 5; Figure 4c,d), and Foxp2 (for layers 6 and 6b; Figure 4e). Etv1 and Foxp2 also label layers 5 and 6 of other cortical regions, respectively. Layer-specific gene markers for the PrS are also identified such as Dgkb (for layer 2; Figure 4f), Scn4b (for layer 3; Figure 4g), Lxn (for layer 5; Figure 4h), Deptor (for layer 6; Figure 4i), and St3gal1 (for layers 5–6; Figure 4j). Note that the expression of these genes does not extend into the prostriata. Although it is very difficult to identify layers 5–6 of the PaS in coronal and sagittal sections, its superficial layers 2–3 selectively expresses Wfs1 (see Chen et al., 2020), Fn1, and Lypd1.
FIGURE 3  Location, topography, and lamination of area prostriata (Pro) in coronal sections. Pcsk5 expression in the Pro from rostral (a) to caudal (i) sections. The inset in panel (b) shows the locations (indicated by white horizontal lines) of the nine sections from panels (a–i) on the dorsal aspect of the right hemisphere. The interval between each section from panel (a) to panel (i) is 100 µm. Layers 2–3 (indicated by star) of the Pro is clearly distinguishable with its strong Pcsk5 expression compared to no or faint expression in adjoining V1 and PrSd. Pcsk5 is also expressed in layer 5 of the Pro, RS, V1, and PrSd. The borders of layer 5 with adjoining regions were determined by layer 5-selective/dominant markers such as C1ql2 (Lu et al., 2020), Ociad2, and Slc24a3 (see Figure 8). Note that the overall sizes, shapes, and extent of the Pro vary along rostrocaudal axis with the largest extent at the middle coronal levels (d–g). The insets in panels (a), (c), (e), and (g) show the borders between RSg and PrSd (a), PrSd and PaS (c), Pro and PrSd (e), and Pro and PaS (g), respectively, on matched Cxcl14-ISH-stained sections. Cxcl14 is strongly expressed in layers 2–3 of RSg, layer 2 of PrSd, and layers 2–3 of the Pro, while similar layer 2 is not available in the PaS. The inset in panel (l) is from a Nnat-ISH-stained section caudal to level (l) displaying the location of the postrhinal cortex (PoR), which strongly expresses Nnat in its layers 2–3 (see the inset) and is located caudolateral to the Pro and dorsal to the PaS. Layers 2–3 of the Pro and PaS have faint Nnat expression. RSag, agranular retrosplenial cortex (area 30); RSagl, lateral agranular retrosplenial cortex; V2M and V2L, medial and lateral secondary visual cortex. Scale bar: 350 µm in panel (a) for all panels.
3.3 | Gene expression enriched in distinct layers of area prostriata

Based on the definition and location of the prostriata, the expression of many genes is found to be enriched or restricted in layers 2–3 with no or faint expression in other layers of the prostriata. These genes include Prkca (Figure 4k–o), Differential expression of Prkca is observed in layers 2–3 of the prostriata, PrS, and PaS. Specifically, the PrS shows relatively weaker expression than adjoining prostriata and PaS (Figure 4k–o).

3.4 | Negative gene markers for layers 2–3 or 5–6 of area prostriata

Some genes are not or faintly expressed in layers 2–3 or 5–6 of the prostriata but display strong expression in neighboring regions. This pattern is also helpful in distinguishing the prostriata from adjoining PrSd and V1. Prkcb (Figure 7a–d) and Pcp4 (Figure 7e–h) are the negative marker genes for layers 2–3 and 5 of the prostriata, respectively, in sharp contrast to layers 2–3 and 5 of the PrSd and V1. Other examples for the difference include the expression of Lnx (Figure 4h), Loc434300 (Figure 7i–l), Trib2, Nts, Nr4a2, and Col25a1, which are negative in layer 5 of the prostriata and PrSd, respectively (Table 1). Additional example is the weak expression of Coro6 (see the inset in Figures 6d and 8a), Ier5, and Cdh8 (Figure 8bc) in layer 5 with no expression in layers 2–3 and 6 of the prostriata. Ier5 and Cdh8 are strongly expressed in layers 2–3 of adjoining PrSd and V1 (Figure 8bc). There are genes displaying fainter expression in both layers 5–6 and 2–3 compared to stronger expression in the V1 and PrSd. For example, Arc shows weak expression in layers 2–3 and no or faint expression in layers 5–6 of the prostriata (Figure 8d). Sncn4b (Figure 4g) and Neurod6 (Table 1) show no and weak expression in layers 2–3 and 5–6 of the prostriata, respectively. In contrast, their expression in the PrSd, PaS, and V1 is much stronger (Table 1). Finally, Deptor shows no and strong expression in layer 6 of the prostriata and PrSd, respectively (Figure 4i).

3.5 | Gene expression enriched in both layers 2–3 and 5 of area prostriata

In addition to the layer-specific gene expression, there are other genes expressed in both layers 2–3 and 5. For example, Kcnn2 (Figure 8e) and Gpr123 (Table 1) display higher expression in layer 5 than in layers 2–3, while the reverse is true for Spon1 (Figure 8f). Like Spon1, many more genes show higher expression in layers 2–3 than in layer 5 and these genes include Gria3, Lifr, Sdp2, Syt17, Icam5, Id2, Smpd4, Ociad2, and Tmem150c (Figure 9a–i). Many other genes such as Chst8, Gda, Slc24a3, Chr3, 6430573F11Rik, Mal2, Ctnn4 (Figure 9j–p), Bcl6, Lamp5, Cpe7, and Adrbk2 show similar expression in both layers 2–3 and 5 (Table 1).

3.6 | Subdivisions of area prostriata

Since the prostriata in macaque monkeys was divided into anterior and posterior subareas (Pro-a and Pro-p) based on different cyto- and chemoarchitecture with Pro-p adjoining the V1 (Ding et al., 2003), we have searched the ABA to see if evidence for differential gene expression exists within mouse prostriata. To explore this issue, the borders of the prostriata are first determined based on cytoarchitecture and specific gene markers of the prostriata such as Pck5 (e.g., Figures 1 and 3) and many others described above in this study (Table 1). Layer-specific positive or negative gene markers (e.g., Figures 4 and 8) are very helpful for border determination of layers 2–3 and 5 of the prostriata. For example, the boundary between the prostriata and V1 is determined based on combination of many specific gene expression patterns and is consistent across different cases (e.g., Figures 1, 2, 3, 7, and 8). Within limit of the prostriata, its two subdivisions are defined as the dorsoposterior (Pro-d) and ventroanterior (Pro-v) parts with specific expression of some genes such as Col12a1 and Penk in the dorsal and ventral parts of layers 2–3, respectively (Figure 10).
### TABLE 1  Expression patterns of main marker genes in prostriata (Pro), presubiculum/postsubiculum (PrS/PoS), parasubiculum (PaS), primary visual cortex (V1), and postrhinal cortex (PoR)

| Gene expression patterns at P56 (adult) | Pro (Pro-d, Pro-v) | PrS/PoS | PaSd | PaSv | V1 | PoR |
|----------------------------------------|--------------------|---------|------|------|----|-----|
| | L2–3 | L5 | L6 | L2 | L3 | L5 | L6 | L2–3 | L3 | L5 | L6 | L2–3 | L4 | L5 | L6 | L2–3 | L4 | L5 | L6 |
| 2900026a02rik | ++(d); −(v) | − | − | − | − | + | + | + | − | + | + | − | − | − | + | + | + | + | − | − | − | + |
| 6430573f11rik | ++ | +++ | − | − | ++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Adra1d | ++(d); −(v) | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Adra2a | ++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Adra2c | ++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Adrbk2 | ++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Arc | + | − | − | ++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Bcl6 | ++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| C1ql2 | – | +++ | ++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Cacna1g | +++(d); −(v) | +++ | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Car4 | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Cdh8 | – | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Cdh24 | −(d); ++(v) | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Chm2 | +++(d); −(v) | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Chrm3 | +++ | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Chrm7 | +++(d); −(v) | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Chst8 | ++ | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Cntn4 | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Col5a1 | +++(d); −(v) | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Col6a1 | – | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Col12a1 | – | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Col23a1 | − | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Col25a1 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Coro6 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Cpm7 | +++ | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Crym | − | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Cxcl14 | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Ddit4l | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Deptor | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Dgkb | − | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Efn6 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Evt1 | − | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Fat3 | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Fetz2 | − | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Flr12 | +++(d); −(v) | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Fstl1 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Gabbr3 | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Gdf | +++ | +++ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Gpr88 | +++(d < v) | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | (Continues)
| Gene | Pro (Pro-d, Pro-v) | PrS/PoS | PaSd | PaSv | V1 | PoR |
|------|-------------------|---------|------|------|----|-----|
|      | L2-3 | L5   | L6   | L2   | L3  | L5   | L6   | L2-3 | L3  | L5   | L6   | L2-3 | L4   | L5   | L6 |
| Gpr123 | ++   | +    | -    | +    | -   | +    | ++   | -    | -   | -    | ++   | ++   | +    | -    | ++ |
| Gpr161 | -    | +++  | -    | +    | -   | +    | ++   | -    | -   | -    | ++   | ++   | -    | -    | -  |
| Gria3  | +++  | +    | -    | ++   | +   | +    | ++   | +    | ++  | -    | ++   | ++   | -    | -    | ++ |
| Homer2 | +++  | -    | -    | ++   | -   | -    | -    | +    | -   | +    | +    | ++   | +    | -    | ++ |
| Htr1a  | +++  | +    | -    | +++  | -   | +    | -    | -    | -   | +    | +    | ++   | +    | +    | ++ |
| Htr1b  | +++  | -    | -    | ++   | -   | -    | +    | -    | -   | +    | +    | ++   | -    | -    | -  |
| Htr1f  | +++  | +    | +    | +    | +   | +    | +    | +    | +   | +    | +    | ++   | +    | +    | ++ |
| Icam5  | +++  | +    | -    | ++   | +   | -    | ++   | ++   | +   | +    | ++   | ++   | +    | +    | ++ |
| Id2    | +++  | +    | -    | +++  | -   | +    | +    | ++   | ++  | +    | +    | ++   | +    | +    | ++ |
| Ier5   | -    | +    | -    | +++  | +   | -    | ++   | ++   | +   | +    | ++   | ++   | -    | +    | ++ |
| Igfbp5 | +++(d); -(v) | +    | -    | +++  | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Igsf3  | +++  | -    | -    | ++   | -   | -    | +    | -    | -   | -    | -    | -    | -    | -    | -  |
| Inpp4b | ++   | -    | -    | +    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Kcn2   | +    | ++   | -    | +    | -   | -    | -    | -    | -   | -    | ++   | ++   | -    | -    | +  |
| Kctd   | ++(d); -(v) | -    | -    | -    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Lamp5  | +++(d < v) | +++  | -    | +    | -   | -    | ++   | -    | +   | +++   | +    | ++   | +    | +    | +++ |
| Lfr    | +++  | +    | -    | ++   | -   | -    | +++  | +    | +   | -    | -    | -    | -    | -    | -  |
| Lim1   | -    | +    | -    | -    | -   | +    | +    | -    | -   | +    | +    | ++   | +    | +    | ++ |
| Loc43328 | +++(d); -(v) | -    | -    | -    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | - |
| Loc434300 | +++  | -    | -    | ++   | -   | -    | +    | -    | -   | -    | -    | -    | -    | -    | - |
| Lnx    | -    | -    | -    | -    | -   | +++  | -    | -    | -   | -    | +    | ++   | -    | -    | +  |
| Lypl1  | -    | ++   | -    | -    | -   | -    | -    | -    | -   | -    | -    | -    | +    | +    | +  |
| Mal2   | ++   | ++   | -    | +    | -   | -    | +    | -    | -   | +    | +    | ++   | +    | -    | ++ |
| Meis2  | +++  | +    | -    | +++  | +   | -    | +    | ++   | +   | -    | +    | ++   | -    | -    | +  |
| Neurod6 | -    | +    | +    | +++  | +   | ++   | +++  | +    | +   | +    | +    | ++   | +    | +    | ++ |
| Nnat   | -    | +++  | -    | -    | -   | +    | +++  | +    | +   | -    | -    | -    | -    | -    | -  |
| Nos1ap | -    | ++   | -    | -    | -   | +    | +    | -    | -   | +    | +    | ++   | -    | -    | +  |
| Nr2f1  | +++(d > v) | -    | -    | -    | -   | -    | -    | -    | -   | -    | -    | ++   | -    | -    | ++ |
| Nr4a2  | -    | -    | -    | -    | -   | ++   | +    | -    | -   | -    | -    | -    | -    | -    | -  |
| Ntrg1  | -    | +++  | +    | -    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Nts    | -    | -    | -    | +    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Ntr1   | +++  | +    | -    | -    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Nwd2   | +++(d > v) | -    | -    | -    | -   | -    | -    | -    | -   | -    | -    | ++   | +    | +    | +  |
| Oiad2  | +++  | +    | -    | -    | -   | -    | -    | -    | -   | +    | ++   | ++   | +    | +    | ++ |
| Otfl   | +++  | -    | -    | -    | -   | -    | -    | -    | -   | -    | +    | ++   | -    | -    | +  |
| Palmd  | +++  | -    | -    | -    | -   | -    | -    | -    | -   | -    | +    | ++   | +    | -    | ++ |
| Par1   | -    | +++  | -    | -    | -   | +    | -    | -    | -   | -    | ++   | ++   | -    | -    | +  |
| Pcdh8  | ++   | -    | -    | -    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Pcp4   | + (d) | -    | -    | -    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Pck5   | +++  | +    | -    | -    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Penk   | -(d); +++(v) | -    | -    | -    | -   | -    | -    | -    | -   | -    | -    | -    | -    | -    | -  |
| Pex5f1 | -    | +++  | -    | -    | -   | +    | +++  | +    | +   | +    | ++   | ++   | +    | ++   | ++ |
| Plced2 | +++(d); -(v) | -    | -    | ++   | +   | +    | ++   | -    | -   | -    | -    | -    | -    | -    | -  |

(Continues)
| Table 1 (Continued) | Pro (Pro-d, Pro-v) | PrS/PoS | PaSd | PaSv | V1 | PoR |
|---------------------|-------------------|---------|------|------|----|------|
|                     | L2–3              | L5      | L6   | L2   | L3  | L5   | L6   | L2–3 | L3  | L5   | L6   | L2–3 | L4   | L5   | L6   |
| Prkca               | +++               | –       | –    | +    | –   | –    | –    | +++  | +    | –    | –    | –    | +    | –    | –    |
| Prkcb               | –                 | –       | –    | +    | ++  | –    | –    | +++  | +    | ++   | –    | +    | +    | +    | +++  |
| Rfx3                | +++               | –       | –    | –    | +   | –    | –    | ++   | –    | +    | –    | –    | ++   | –    | –    |
| Rims3               | +++ (d); –(v)     | –       | –    | +    | ++  | ++   | +    | +++  | –    | +    | –    | +    | –    | –    | –    |
| Rorb                | +++ (d); –(v)     | –       | –    | –    | –   | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Rxfp1               | +++ (d > v)       | +       | –    | +++  | –   | –    | –    | +    | –    | –    | –    | +    | +    | +    | +    |
| Scn4b               | –                 | –       | –    | +    | +   | –    | –    | ++   | –    | +    | –    | –    | –    | –    | –    |
| Scnn1a              | +++ (d); –(v)     | –       | –    | +    | –   | –    | –    | –    | –    | –    | ++   | –    | –    | –    | –    |
| Sdk2                | –                 | ++      | –    | –    | +   | –    | –    | +    | –    | +    | –    | –    | ++   | –    | –    |
| Slc17a6             | +++               | –       | –    | –    | –   | –    | –    | +++  | +    | ++   | –    | –    | –    | –    | –    |
| Slc24a3             | +++               | +++     | +    | –    | –   | –    | –    | +    | –    | –    | –    | ++   | –    | –    | –    |
| Smpd4               | +++               | ++      | –    | ++   | –   | –    | –    | +    | ++   | –    | –    | ++   | –    | –    | –    |
| Smpd5               | +++ (d); –(v)     | –       | –    | –    | –   | –    | –    | ++   | –    | +    | –    | –    | ++   | –    | –    |
| Spn2                | +++               | –       | –    | +    | –   | –    | –    | +    | –    | –    | +    | –    | ++   | –    | –    |
| Spon1               | +++               | +       | –    | +    | –   | –    | –    | +    | –    | +    | ++   | –    | +    | +    | +    |
| Sbap2               | +++               | ++      | –    | –    | –   | –    | –    | +    | –    | +    | ++   | –    | +    | +    | +    |
| St3gal1             | –                 | +       | –    | –    | +   | –    | –    | +    | –    | +    | –    | ++   | –    | –    | –    |
| Syt10               | +++ (d > v)       | +       | –    | +    | –   | –    | –    | +    | –    | +    | –    | ++   | –    | –    | –    |
| Syt17               | +++               | ++      | –    | –    | –   | –    | –    | +    | –    | +    | ++   | –    | +    | ++   | –    |
| Syt12               | +++ (d); –(v)     | –       | –    | +    | ++  | +    | –    | +    | –    | –    | –    | +    | +    | +    | +    |
| Tcerg1l             | +++ (d > v)       | –       | –    |+++  | –   | –    | –    | +    | –    | +    | ++   | –    | +    | ++   | –    |
| Tie4                | –                 | –       | –    | +    | –   | –    | –    | +    | –    | +    | ++   | –    | +    | ++   | –    |
| Tmem150c            | +++ (d > v)       | +       | –    | +    | –   | –    | –    | +    | –    | +    | –    | ++   | –    | –    | –    |
| Trib2               | +++ (d); –(v)     | –       | –    | –    | +   | –    | –    | +    | –    | –    | –    | +    | –    | –    | –    |
| Unc5d               | +++               | –       | –    | +    | –   | –    | –    | +    | –    | +    | –    | ++   | –    | –    | –    |
| Wfs1                | +                 | –       | –    | –    | –   | –    | –    | +    | –    | +    | –    | ++   | –    | –    | –    |
| Whmn                | +++ (d); –(v)     | –       | –    | ++   | ++  | –    | –    | +    | –    | –    | –    | ++   | –    | –    | –    |
| Zmat4               | +++ (d); –(v)     | –       | –    | +++  | +   | –    | –    | +    | –    | +    | –    | ++   | –    | –    | –    |

Gene expression patterns at P4

| Pro (Pro-d, Pro-v) | ProS/PoS | PaSd | PaSv | V1 | PoR |
|-------------------|----------|------|------|----|------|
| L2–3              | L5       | L6   | L2   | L3  | L5   |
| L6                 | L2–3     | L3  |
| L5                 | L2–3     | L3  |
| L6                 | L2–3     | L3  |
| L2                 | L2–3     | L3  |
| L3                 | L2–3     | L3  |
| L5                 | L2–3     | L3  |
| L6                 | L2–3     | L3  |
| L2                 | L2–3     | L3  |
| L3                 | L2–3     | L3  |
| L5                 | L2–3     | L3  |
| L6                 | L2–3     | L3  |
| L2                 | L2–3     | L3  |
| L3                 | L2–3     | L3  |
| L5                 | L2–3     | L3  |
| L6                 | L2–3     | L3  |
| 6430573f11rnik     | +        | –    | –    |
| C1ql2              | –        | –    | –    |
| Cd8                | –        | –    | +    |
| Crym               | –        | +    | –    |
| Ddit4l             | –        | +    | –    |
| Deptor             | –        | –    | –    |
| Etv1               | –        | +++  | –    |
| Fstl1              | +        | –    | –    |
| Grp                | +        | +++  | –    |
| Htr1a              | +        | –    | +    |
| Id2                | +++      | +    | –    |
| Lypd1              | –        | –    | +    |

(Continues)
### TABLE 1 (Continued)

| Gene expression patterns at P4 | Pro | PrS/PoS | PaSd | PaSv | V1 | PoR |
|--------------------------------|-----|---------|------|------|----|-----|
|                                | L2–3 | L5 | L6 | L2 | L3 | L5 | L6 | L2–3 | L2–3 | L2–3 | L4 | L5 | L6 | L2–3 | L4 | L5 | L6 |
| 6430573f11rik                  | ++  | -  | +  | -  | +  | -  | -  | -  | +  | -  | -  | +  | -  | -  | +  | -  | -  | -  |
| C1ql2                          | -   | +  | -  | -  | +  | -  | -  | -  | ++ | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Cdhl8                          | -   | +  | -  | +  | +  | ++ | -  | -  | ++ | -  | +  | ++ | -  | +  | +  | ++ | -  | +  | ++ |
| Crym                           | -   | +++ | -  | +  | ++ | +  | +  | -  | -  | +++ | ++  | +  | ++ | -  | +  | +  | +++ | -  | +  | +++ |
| Ddit4l                         | +   | -  | -  | ++ | +++ | -  | +++ | +  | +++ | -  | +++ | +  | +  | -  | +  | +  | +++ | +  | +  | +++ |
| Deptor                         | +   | -  | -  | -  | -  | +  | -  | -  | -  | ++ | -  | +  | -  | +  | +  | -  | +  | +  | ++ |
| Fstl1                          | ++  | -  | -  | +++ | -  | +  | -  | +  | +  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  |
| Grp                            | -   | +  | -  | -  | -  | +  | -  | +  | ++ | -  | -  | ++ | -  | +  | +  | -  | +  | +  | ++ |
| Htr1a                          | +++ | -  | -  | +++ | -  | +  | -  | -  | +  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  |
| Id2                            | +++ | -  | -  | +  | -  | -  | -  | -  | +  | -  | +  | ++ | -  | +  | ++ | -  | +  | ++ | +++ |
| Lypld                          | -   | ++  | -  | +  | -  | -  | -  | +  | ++ | -  | +  | ++ | -  | +  | ++ | -  | +  | ++ | +++ |
| Neurod6                        | -   | -  | -  | +  | +++ | +  | +++ | +  | +++ | -  | +  | +  | -  | +  | +  | -  | +  | +  | ++ |
| Nnat                           | +++ (d > v) | -  | -  | -  | +  | -  | +  | +  | ++ | -  | +  | ++ | -  | +  | ++ | -  | +  | ++ | +  |
| Nr2f1                          | -   | +  | -  | -  | -  | ++ | -  | -  | -  | +  | -  | +  | -  | +  | +  | -  | +  | +  | ++ |
| Pcp4                           | ++(d); -(v) | +  | +  | -  | +  | -  | +  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Tcerg1l                        | +++ | +  | -  | +++ | -  | +  | -  | -  | -  | +  | -  | +  | -  | +  | ++ | -  | +  | ++ | +++ |
| Wfs1                           | +   | +  | -  | -  | -  | -  | -  | -  | +  | -  | +  | -  | +  | +  | -  | +  | +  | ++ |

### Gene expression patterns at P14

| Gene expression patterns at P28 | Pro | PrS/PoS | PaSd | PaSv | V1 | PoR |
|---------------------------------|-----|---------|------|------|----|-----|
|                                 | L2–3 | L5 | L6 | L2 | L3 | L5 | L6 | L2–3 | L2–3 | L2–3 | L4 | L5 | L6 | L2–3 | L4 | L5 | L6 |
| 6430573f11rik                   | ++  | +  | -  | -  | +  | -  | -  | +  | -  | -  | -  | +  | -  | -  | +  | -  | -  | -  |
| C1ql2                           | -   | +  | -  | -  | +  | -  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Cdhl8                           | -   | +  | -  | +  | +  | ++ | -  | -  | ++ | -  | +  | ++ | -  | +  | +  | ++ | -  | +  | ++ |
| Crym                            | -   | +++ | -  | +  | ++ | +  | +  | -  | -  | +++ | ++  | +  | ++ | -  | +  | +  | +++ | -  | +  | +++ |
| Ddit4l                          | +   | -  | -  | ++ | +++ | -  | +++ | +  | +++ | -  | +++ | +  | +  | -  | +  | +  | +++ | +  | +  | +++ |
| Deptor                          | +   | -  | -  | -  | -  | +  | -  | -  | -  | ++ | -  | +  | -  | +  | +  | -  | +  | ++ | +++ |
| Fstl1                           | ++  | -  | -  | +++ | -  | +  | -  | +  | +  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  |
| Grp                             | -   | +  | -  | -  | -  | +  | -  | +  | ++ | -  | -  | ++ | -  | +  | +  | -  | +  | +  | ++ |
| Htr1a                           | +++ | -  | -  | +++ | -  | +  | -  | -  | +  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  |
| Id2                             | +++ | -  | -  | +  | -  | -  | -  | -  | +  | -  | +  | ++ | -  | +  | ++ | -  | +  | ++ | +++ |
| Lypld                           | -   | ++  | -  | +  | -  | -  | -  | +  | ++ | -  | +  | ++ | -  | +  | ++ | -  | +  | ++ | +++ |
| Neurod6                         | -   | -  | -  | +  | +++ | +  | +++ | +  | +++ | -  | +  | +  | -  | +  | +  | -  | +  | ++ | ++ |

(Continues)
### TABLE 1 (Continued)

| Gene expression patterns at P28 |
|--------------------------------|
| **Nnat** | - | +++ | + | - | - | - | - | - | + | +++ | - | - | ++ | ++ | - | - |
| **Nr2f1** | +++ (d > v) | - | - | - | - | - | - | - | - | - | - | - | + | ++ | - | - |
| **Pcp4** | +(d); -(v) | - | - | - | - | - | - | - | - | - | - | ++ | - | - | ++ | - |
| **Tcerg1I** | +++ | + | - | +++ | - | - | - | - | - | - | - | - | - | - | - | - |
| **Wfs1** | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

Note: Gene expression levels: ++++, strong; ++, moderate; +, weak; -, faint or no. Layer 6b is not included in this table due to its very thin thickness and no clear difference between regions.

### 3.7 Postnatal development of area prostriata

To identify the prostriata in developing mouse brains, we have explored the AD MBA and found that the prostriata is not recognizable in the prenatal brains at E11.5, E13.5, E15.5, and E18.5. However, some genes are clearly expressed in the postnatal prostriata versus PrSd and PaS from P4 to P28. As early as P4, for example, weaker expression of 6430573f11rik and Tcerg1I is observed in layers 2–3 and 5 of the prostriata, while strong expression of 6430573f11rik and Tcerg1I is seen in layers 5 and 2 of the PrSd, respectively (Figures 12a–d, 13a–c). In contrast, genes **Nr2f1** (Figures 12e–h and 13b), Htr1a, and Fst1 (Table 1) display strong and weaker expression in layers 2–3 of the prostriata and PrSd, respectively. Expression difference of **Nr2f1**, Tcerg1I, Etv1, Grp, Crym, Cdh8, Htr1a, Lypd1, Neurod6, and Pcp4 is also obvious between the prostriata and PaS or V1 (Table 1). For instance, **Nr2f1** shows strong and weaker expression in layers 2–3 of the prostriata and V1, respectively (Figures 12e–h and 13b). In contrast, weak and strong **Tcerg1I** expression can be found in layer 5 of the prostriata and V1, respectively (Figures 12i–l and 13c). In addition, stronger expression of Etv1, Grp, and Crym in layer 5 of the prostriata versus V1 and PrSd also makes the prostriata stand out at P4 (Figure 14a–f). Differentially expressed genes between the prostriata and the PaS include Crym, Lypd1, Neurod6, and Nnat with much stronger expression in layers 2–3 of the PaS at P4 (Table 1; e.g., Figure 14c for Crym). Finally, it is worth mentioning that layer 5 of the prostriata at P4 does not express C1ql2, which is strongly expressed in adult prostriata (see Lu et al., 2020).

At P14, strong 6430573f11rik expression is detected in layers 2–3 and 5 of the prostriata, while in the PrSd its expression is contained in layer 5 with weak expression in layer 2 (Figure 15a–d) and this pattern remains from P14 onward to adult (Figure 9n). Strong C1ql2 expression is detected and restricted in layer 5 of the prostriata with no expression in adjoining V1, PaS, and PrSd at P14 (Figure 15e–g) and P28 (e.g., Figure 15h). Tcerg1I expression is mostly found in layers 2–3 of the prostriata, layer 2 of the PrSd, and layer 5 of the V1 from P14 (Figure 15i–l) to adult (Table 1). Crym expression in layer 5 of the prostriata remains stronger than the PrSd and V1 at P14 (Table 1), although the difference is reduced at P28 (Figure 14g–i), as in adult (Figure 6). The reduced difference at P28 is mainly the result of the increased Crym expression in layer 5 of the PrSd and V1. Finally, **Nr2f1** is strongly expressed in layers 2–3 of the prostriata with no or faint expression in the PrSd from P14 onward (Table 1).

The negative/faint expression of Cdh8 in layers 2–3 of the prostriata also makes the prostriata identifiable from the neighboring regions such as the PrSd, V1, and PaS at P4 (Figure 16a–d for sagittal; 16e–h for coronal sections), P14 (Figure 16i–l for coronal sections), and P28 (Table 1). Another example of negatively expressed genes in the prostriata is **Neurod6**, which is negative in layers 2–3 of the prostriata with strong expression in adjoining PrSd and V1 at P4 (Figure 17a), P14 (Figure 17b), and P28 (Figure 17c). Taken together, the prostriata can be reliably identified in postnatal mouse brains using both positive and negative gene markers such as those described above.

### 3.8 Molecular markers for presubiculum, parasubiculum, and postrhinal cortex of postnatal mice

Molecular markers for the PrS and PaS in postnatal mice were further explored. At P4, P14, and P28, some genes such as Ddit4l, Nnat, and Wfs1 display differential expression in the PrS/PoS versus PaS (Figure 18). Specifically, **Ddit4l** is strongly expressed in layer 3 of the PrSd with faint expression in the PaS and layer 2 of the PrSd at P4 (Figure 18a,b). At P14 and P28, **Ddit4l** expression in layer 3 of the PrSd is reduced, while that in the dorsal PaS is greatly increased (e.g., Figure 18c for P28; Table 1). Nnat expression at P4 is strong in layers 2 and 5–6 of the PrSd and in both dorsal and ventral parts of the PaS (Figure 18d,e). Weak Nnat expression is also seen in the prostriata at the lateral and medial levels (Figure 18d,e). At P14 and P28, the Nnat expression in the PrSd almost disappears, whereas in the dorsal PaS is greatly decreased (e.g., Figure 18f for P28; Table 1). In contrast, strong Nnat expression appears in layer 5 of the prostriata at P28 (Figure 18f). As for gene Wfs1, faint expression is detected in the PaS at P4 (Figure 18g), whereas very strong Wfs1 expression is seen in both dorsal and ventral parts of the PaS with weak expression in layers 2–3 and 5 of the prostriata at P14 (Figure 18h). At P28, Wfs1 expression in the PaS is slightly reduced, while that in layer 5 of the prostriata disappears (Figure 18i). It is also noted that some other genes such as Grp, Fst1, and Lypd1 are strongly expressed in the PaS across P4, P14, and P28 (e.g., Figure 19a,d for Lypd1; Table 1).
FIGURE 4  Molecular markers for the prostriata, presubiculum, and parasubiculum. Gene names are indicated on the top of each penal. The sections in panels (a–e) are at level (f) of Figure 3, while the sections in panels (f–j) are at level (a) or (b) of Figure 3. The five sections in the third row are at levels (a, c, e, g, i) of Figure 3 with an interval of 200 µm. (a) Cytoarchitecture of the Pro and PaS. Layers 2–3 of the Pro and the dorsal PaS are relatively darkly stained compared to surrounding regions. (b–e) Examples of molecular markers for layers 2–3 (b), 5 (c, d), and 6 (e) of the Pro. Note that Etv1 is expressed in layer 5 of the Pro and adjoining regions, while C1ql2 expression is restricted in layer 5 of the Pro. (f–j) Examples of molecular markers for layers 1 (f), 2 (g), 5 (h), 6 (i), and 5–6 (j) of the PrSd. Note that the expression of these genes does not extend into the Pro. (k–o) Differential expression of Prkca in the Pro, PrSd, and PaS from rostral (k) to caudal (o) coronal levels. The PrS shows relatively weaker expression than adjoining Pro and PaS, which displays strong expression. Strong Prkca expression is also seen in layer 2 of the medial entorhinal cortex (MEC). Scale bar: 350 µm in panel (a) for all panels.

Since the PoR also adjoins the prostriata and PaS in adult mice (e.g., Figures 1a and 3i), molecular markers for the PoR at postnatal ages were also investigated. For example, Lypd1 is not expressed in the PoR at P4, but strong Lypd1 expression is observed in layers 2–3 of the PoR from P14 onward. Strong Lypd1 expression is also detected in the PaS from P4 to adult (Figure 19a,d,g; Table 1). Nnat are strongly expressed in the PoR and PaS but not in the prostriata at P4, P14, and P28 (Figure 19b,e,h; Table 1). In addition, stronger Deptor and Pcp4 expression is seen in layer 5 of the PoR versus the prostriata at P14 and P28 (Table 1). Finally, weak Cdh8 expression is seen in layers 2–5 at P4 (Figure 19c,f), but strong expression of Cdh8 is obvious in layers 2–5 of the PoR from P14 to adult (Figure 19i; Table 1). In the
FIGURE 5  Gene expression in layers 2–3 of the prostriata (Pro) in coronal sections. The sections in each panel are at level (f) of Figure 3, where the Pro dorsally adjoins the PaS. (a–t) Examples of 20 genes enriched in layers 2–3 of the Pro. Gene names are indicated on top-right corners of each panel. It is clear that Igsf3, Inpp4b, Nwd2, Rxfp1, Ntsr1, Homer2, Tcerg1l, Fstl1, Cxcl14, Nr2f1, Cdc42ep3, Otof, Unc5d, Syt10, Gabrb3, Car4, Spns2, Palmd, and Fat3 (a–s) are enriched in layers 2–3 of the Pro but not the PaS. Strong expression of Inpp4b, Nwd2, and Ntsr1 in layer 2 of medial entorhinal cortex (MEC; b, c, e), Rxfp1 in layer 3 of the MEC (d), Homer2 in layers 2–3 of the PaS (f), and Cux2 in layers 2–4 of the neocortex (NCx; t) are also observed. Scale bar: 210 µm in panel (a) for all panels.

prostriata, Cdh8 expression is negative in layers 2–3 and weak in layer 5 (Figure 16; Table 1). In addition, Cdh8 expression in the PaS is fainter at P4 and weaker from P14 to adult compared to the PoR. All these gene expression patterns are helpful in distinguishing the PoR from the prostriata and PaS (Table 1).

3.9  Comparison of area prostriata in the marmosets and mice

To explore if the prostriata has some conservative gene expression between mice and marmoset monkeys, we have carried out a survey on the Riken marmoset database (https://gene-atlas.brainminds.riken.jp), which mainly contains the ISH data from postnatal stages (mostly at P0; see Kita et al., 2021; Shimogori et al., 2018). As expected, some genes indeed show similar expression patterns between the two species. For example, in the neonate marmosets (P0), weak and strong Id2 expression is observed in layers 2–3 of area 29 (A29 in Figure 20a,b) and the prostriata (Figure 20b,c), respectively. In addition, the superficial layers of areas 30, 23, and V1 also display strong Id2 expression (Figure 20a–d). The V1 in marmosets has a very thick layer 4 (negative/faint for Id2; see Figure 20c,d), making it distinguishable from the prostriata, where almost no granular layer 4 is detected. Another gene Grp is strongly expressed in layers 2–3 of A29 (Figure 20e,f) and layer 5 of many cortical regions including the prostriata (Figure 20e–h). The Grp expression in layer 5 is stronger in the prostriata compared to
FIGURE 6  Gene expression in layers 5–6 of the prostriata (Pro). Gene names are indicated on top-right corners of each panel; layer 5 is outlined by dashed lines. The four sagittal sections in each row are at levels (e–h) of Figure 1 with an interval of 160 μm. (a–g) Examples of seven genes enriched in layer 5 of the Pro in sagittal sections. It is clear that Parm1, Nnat, Fezf2, Lypd1, Col6a1, and Pex5l (a–g) are enriched in layer 5 of the Pro but not the PrSd. Note the strong expression of these genes in layer 5 of the overlying V1 with exception of Efnb3, which has much fewer expression in V1 (e). Inset in panel (a) shows Parm1 expression in layer 5 of the Pro in a coronal section. Inset in panel (d) shows faint and strong Coro6 expression in the Pro and PrSd, respectively, in a matched sagittal section. (h–l) Examples of five genes enriched in layer 6b of the Pro in sagittal sections. Strong expression of Nos1ap (h) and Crym (j) is also seen in layer 5 of the Pro. Inset in panel (l) shows Limch1 expression in layer 6 of the overlying V1 in a coronal section. Note that layer 6 of the Pro is a cell-sparse zone with less gene expression. Scale bars: 210 μm in panel (b) for all panels; 350 μm in the inset in panel (a) for all insets.

adjoining regions (Figure 20f,g). In the neonate mice (P4), strong Id2 expression exists in layers 2–3 of A29 and A30 (Figure 20i) and the prostriata (Figure 20j) and in layer 5 of the PrSd (Figure 20k), and this pattern remains at P14. However, weak and strong Id2 expression (similar pattern as in the marmosets; see above) is detected in layers 2–3 of A29 and A30 at P28, respectively (inset in Figure 20i). The Grp expression pattern in the neonate mice (P4) is generally similar to that in the marmosets at P0. Specifically, Grp is strongly and faintly expressed in layers 2–3 of A29 and A30, respectively (Figure 20k). Stronger Grp expression is also found in layer 5 of the prostriata compared to layer 5 of the V1 and PrSd (Figure 20l). Similar patterns of Grp expression are observed in the marmosets at P0 (Figure 20o–h). Finally, as in mice, the prostriata in the marmosets also displays strong Adcyap1 and Meis2 expression in its layers 2–3, and Etv1, Crym, and Fezf2 expression in its layer 5 (https://gene-atlas.brainminds.riken.jp).

DISCUSSION

The present study has revealed clear differential expression of many genes in mouse prostriata and adjoining structures such as PrS, PaS, V1, and PoR (Table 1). As demonstrated above, layer-specific genes of the prostriata contain Pcsk5, Rfx3, Lgfs3, Inpp4b, Nwd2, Rxfp1, Ntsr1, Tcerg1l, Fstl1, Cxcl14, Nr2f1, Otof, Car4 (for layers 2–3), C1ql2, Etv1, Parm1, Nnat, Lypd1, Efnb3, Pex5l, and Crym (for layer 5) (see Table 1). Layer-specific genes of the PrS are also identified and these include Dgkb (for layer 2),
FIGURE 7 Negative/Faint gene expression in layers 2–3 and 5 of the prostriata (Pro) in sagittal sections. The four sections in first two rows are at levels (e–h) of Figure 1, while the four sections in the third row (i–l) are at levels (d–g) of Figure 1 with an interval of 160 µm. Layers 2–3 of the Pro are indicated by the stars. (a–d) Negative Prkcb expression in layers 2–3 of the Pro from lateral (a) to medial (d) levels. Note the surrounding regions with strong Prkcb expression. (e–h) Faint Pcp4 expression in layer 5 of the Pro from lateral (e) to medial (h) levels. In contrast to the Pro, layer 5 of the adjoining PrSd and V1 strongly expresses Pcp4. Note the weaker Pcp4 expression in the dorsal part of layers 2–3 of the Pro. (i–l) Loc434300 expression in the Pro from lateral (i) to medial (l) levels. Negative and strong Loc434300 expression is found in layers 5 and 2–3 of the Pro, respectively. Note that layer 5 in both PrSd and V1 expresses this gene. Strong expression is also visible in the PaS and layer 2 of the PrSd. Scale bar: 210 µm in panel (a) for all panels.

Scn4b (for layer 3), Lxn (for layer 5), Deptor (for layer 6), and St3gal1 (for layers 5–6) (Figure 4f–j). Among the genes enriched in the PaS (layers 2–3) are C1ql2, Pex5l, Tmem150c, Gpr161, Gpr88, Scn4b, Fn1, and Wfs1 (Table 1). The genes strongly expressed in certain layers of the V1 versus prostriata (Table 1) include Arc, Cdh8, Dgkb, Ier5, Lypd1, Nrr4a2, Penk, Prkcb, Wfs1 (for layers 2–3), Arc, Cdh8, Ier5 (for layer 4), Adra1d, Pcp4, Loc434300, Trib2, Scn4b, Prkcb, Gpr88, Deptor, Cdh8, Coro6, and Neurod6 (for layer 5). Finally, Lypd1, Nnat, and Cdh8 are enriched in layers 2–3 of the PoR (Figure 19) but not of the prostriata from P14 onward (Figures 16 and 18; Table 1). The distinct gene expression revealed in this study strongly supports the prostriata as an independent anatomical entity (Ding, 2013; Lu et al., 2020) rather than as part of RSg (area 29e) (Blackstad, 1956; Haug, 1976; Slomianka & Geneser, 1991; Vaz Ferreira, 1951) or part of PrS/PoS (Paxinos & Franklin, 2001; Swanson, 2018). The similar topographic relationship of the prostriata with neighboring regions (see Figures 1 and 3) compared to nonhuman primates (Ding et al., 2003; Morecraft et al., 2000; Figure 20) further supports its nature as the equivalent of the primate prostriata (Allman & Kaas, 1971; Ding et al., 2003; Morecraft et al., 2000; Paxinos et al., 2012; Rockland, 2012; Sanides, 1969; Sousa et al., 1991). Similar expression patterns of some conservative genes such as Id2 and Grp in the prostriata of the neonate mice and marmosets (Figure 20) also confirm the rodent homolog of the prostriata. In this study, we have discovered many layer-specific gene expression or cell types in the prostriata.
and subdivided the prostriata into two main parts based on differential molecular signature. We have also delineated the location and extent of the prostriata in postnatal mouse brains. All these findings would provide a fundamental base for future cell-type-related structural, functional, and developmental exploration of this unique region.

### 4.1 Laminar organization and cell types of area prostriata

Like in human and nonhuman primate brains, the prostriata in rodent brains is a limbic cortex characterized by lack of a clear granular layer...
Gene expression in both layers 2–3 and 5 of the prostriata (Pro) in coronal sections. The sections in each panel are at level (f) of Figure 3, where the Pro dorsally adjoins the PaS. Gene names are indicated on top-right corners of each panel. (a–i) Examples of nine genes with strong expression in layers 2–3 and relatively weaker expression in layer 5 of the Pro. These genes are Gria3, Lifr, Ssbp2, Syt17, Icam5, Id2, Smad4, Ociad2, and Tmem150c (a–i). Note also the strong expression of Gria3 (a), Lifr (b), Icam5 (e), and Tmem150c (i) in the PaS. (j–p) Examples of seven genes with similar expression intensity in both layers 2–3 and 5 of the Pro. These genes are Chst8, Gda, Slc24a3, Chrm3, 6430573f11Rik, Mal2, and Cntn4 (j–p). Scale bar: 210 µm in panel (a) for all panels.

In the present study, we have revealed layer-specific expression of many genes in the prostriata. These include specific expression in layers 2–3 or layer 5 or both (Table 1), indicating the existence of different cell types in the prostriata. These findings are consistent with our recent connectional studies of the prostriata in the rat and mouse brains (Chen et al., 2020, 2021; Hu et al., 2020). For example, layers 2–3 of the prostriata project mainly to contralateral layers 2–3 (Chen et al., 2020) and ipsilateral PrS/PoS (Chen et al., 2021), while layer 5 projects mainly to the lateral dorsal thalamic nucleus (LD), ventral lateral geniculate nucleus (VLG), lateral-posterior thalamic...
FIGURE 10 Identification of two subdivisions of the prostriata (Pro) in sequential sagittal sections. Lateral (top) to medial (bottom) sections are arranged along each column and the interval between each section/level in each column is 160 µm. For general location of each section on the whole hemisphere, see the inset in Figure 1a and the corresponding sections in Figure 1d–i. The borders of the Pro with V1 and PrSd are determined based on cytoarchitecture and combination of multiple gene expression patterns, as shown in Figure 8. (a–e) Locations of the Pro in sequential Nissl-stained sections. The two subdivisions (Pro-d and Pro-v) of the Pro are not well distinguishable in these sections and a granular layer 4 does not appear to exist in both subdivisions. (f–j) Col12a1 expression in layers 2–3 of the Pro-d in sequential sections adjacent to (a–e), respectively. (k–o) Penk expression in layers 2–3 of the Pro-v in sequential sections. Note also the strong Penk expression in layer 2 of the medial PrSd (n). (p–t) Scnn1a expression in deep layer 3 of the Pro-d in sequential sections. Note that Scnn1a expression in the Pro is only seen at the lateral levels (p, q) rather than the medial levels (r, s). Examples of Col12a1 and Scnn1a expression in the Pro-d are also shown in coronal sections in Figures 11a and 11e, respectively, to compare the location of the Pro-d in both sagittal and coronal sections with the same gene markers. Scale bars: 300 µm in panel (a) for panels (a–t).

Many afferent projections to the prostriata also display obvious laminar distribution. For instance, layer 1 of the prostriata receives inputs mainly from sensory cortices and anterior thalamic nuclei, while layers 2–3 receive innervations from the PrS/PoS, Sub, V1, midline thalamic nuclei, and contralateral prostriata. Layer 5 of the prostriata is mainly innervated by the MEC, RSG, and medial orbitofrontal cortex (OFCm), whereas its layer 6 innervated mainly by the RSG and ectorhinal cortex (Chen et al., 2021; Hu et al., 2020). Finally, lamina dissecans of the prostriata receives moderate inputs from the rostral part of the DLG (Chen et al., 2021). These findings indicate that different inputs innervate distinct layers or cell types in the prostriata. Interesting future studies would be functional correlation of these layer- and/or cell type-specific connections via generation of cell type-specific Cre-mouse lines using the gene markers revealed in the present study.
4.2 Subdivisions of area prostriata

Based on gene expression difference within the prostriata, we have divided the prostriata into the dorsal and ventral parts (Pro-d and Pro-v; see Figures 10 and 11). Pro-d adjoins the RS and visual cortex (V2M and V1) dorsally, while Pro-v abuts the PrSd/PrSb and PaS ventrally. Pro-d displays no or incipient granular layer 4 and thus corresponds to Pro-p (i.e., posterior part) of the prostriata in macaque monkeys (Ding et al., 2003). Pro-v shows a clear lamina dissecans and thus corresponds to Pro-a (i.e., anterior part) of the macaque prostriata. In this study, many genes are observed to express in layers 2–3 of the Pro-d but not Pro-v (Figure 11; Table 1). Interestingly, afferent projections from the rostral midline thalamic region and rostral DLG heavily innervate layers 2–3 of the Pro-d but not Pro-v in rodent (Chen et al., 2021; Hu et al., 2020). Cortical projections from the V1 also tend to target the Pro-d more heavily than the Pro-v (Lu et al., 2020). In contrast to Pro-d, we find only a limited number of genes that express in layers 2–3 of the Pro-v (e.g., Penk and Cdh24), although many genes are enriched in both Pro-d and Pro-v (Table 1). Detailed circuit and functional correlation of these two prostriata subareas remains to be investigated.
FIGURE 12 Identification of the prostriata (Pro) in neonate mice (P4). All panels are from sagittal sections. Lateral (top) to medial (bottom) sections at levels 1–4 are arranged along each column and the locations of the sections at levels 1–4 roughly correspond to panels (e–h) of Figure 1. The interval between each section/level in each column is 160 µm. (a–d) Weak 6430573f11rik expression in both layers 5 (arrows) and 2–3 (stars) of the Pro. In contrast, strong expression is detected in dorsal subiculum (Sd) and layer 5 of the PrSd. Note that the expression of this gene is not yet detected in layer 5 of the V1 at P4. (e–h) Strong Nr2f1 expression in layers 2–3 of the Pro (stars) with weaker expression in layer 5 (arrows). Note that this gene is expressed in dorsal lateral geniculate nucleus (DLG) but not in medial geniculate nucleus (MG). (i–l) Weak Tcerg1l expression in layers 2–3 and 5 of the Pro. Note the strong expression in layer 2 of PrSd and layer 5 of the V1. Higher magnification images of panels (d), (h), and (i) are presented in Figure 13a–c, respectively, to show the weak expression in the Pro. It should be mentioned that no expression of C1ql2 was observed in layer 5 of the Pro at P4 and thus not shown here. Scale bar: 220 µm in panel (a) for panels (a–l).

4.3 Development of area prostriata

No studies were previously carried out on the development of the prostriata in any species. To localize the prostriata in developing mouse brains, we have searched the large data set, ADMBA (see Thompson et al., 2014), and find that the mouse prostriata is not identifiable prenatally. However, starting from P4 onward, the prostriata can be reliably identified using some positive gene markers such as 6430573f11rik, Nr2f1, Tcerg1l, and Id2, as well as some negative markers such as Cdh8, Prkcb, Neurod6, and Pcp4 (Table 1). Additionally, our recent pilot study on the connectivity of the prostriata in postnatal rats has revealed the projections from the prostriata to medial V1 at P7 and 14 (unpublished data). It worth noting that, in a recently published prenatal human brain atlas, the prostriata is also not recognizable at PCW 15 and 21 (Ding et al., 2022), although it is possible to identify it at later stages due to the long period of prenatal development. Our localization of the prostriata in the postnatal mouse brains would provide an anatomical base for future studies related to prostriata development and functional maturation. As for developmental expression of specific marker genes in the prostriata, it appears that the expression of many
marker genes at P4 is obviously different from that at P14 (Table 1).

By P14, however, the expression patterns of many genes are generally similar to those at P28 and P56 (Table 1). For example, 6430575f11rik and C1ql2 are weakly or not expressed in layer 5 of the prostriata at P4 but their expression is strong from P14 onward (Figures 13 and 15; Table 1). Tcerg1l expression in layers 2–3 of the prostriata is weak and strong at P4 and P14–P56, respectively (Figures 13 and 15; Table 1). These findings may suggest that the point time of functional maturation of the prostriata in mice could occur at P14. It would be interesting to know in future studies that how specific molecular markers for the prostriata are changing in correlation with other gene expression using genetically engineered mice targeting some specific genes.

4.4 Functional consideration of area prostriata

Although the prostriata region in rodent brains has been treated as a part of area 29e or PrS/PoS since Brodmann (1909), its function has not been investigated and has long been considered as a mystery. On the other hand, the prostriata in nonhuman primates, first described in later 60s (see Sanides, 1969), was reported to be responsive to visual stimuli (Cuénod et al., 1965; Rosa et al., 1997), but no detailed studies have been carried out until recently. A relatively recent study of the marmoset monkeys has revealed that prostriata neurons have enormous receptive fields and short response latencies and are selective to stimulus speed rather than direction of motion (Yu et al., 2012). A more recent study has found, using population receptive field mapping, that the prostriata in human brains responds strongly to very fast motion in the far peripheral field (Mikellidou et al., 2017; Tanietto & Leopold, 2018). These findings suggest that the prostriata is very important in monitoring far peripheral visual field for unexpected fast-moving objects, particularly dangerous ones. However, the underlying neural circuits and mechanisms for these functions remain to be explored (Rockland, 2012). The discovery of rodent equivalent of the prostriata enables direct investigation of brain-wide circuits of the prostriata (Ding, 2013; Lu et al., 2020). Our recent efforts in mouse and rat brains have revealed that the prostriata receives direct projections from rostral DLG and medial V1 and projects directly to the effectors responsible for visuomotor behaviors such as LD, PTN, PN, VLG, LP-Pul, and ZI (Chen et al., 2021; Lu et al., 2020). Such short visual pathways avoid multiple synaptic delay and would provide the neural substrates for fast processing of rapid moving stimuli in the far peripheral visual field. Additionally, strong commissural connections between two sides of the prostriata (Chen et al., 2020) would enable quick integration of related information from two sides of peripheral visual fields. Furthermore, as mentioned above, the prostriata directly receives visual, auditory, olfactory, and limbic inputs and thus could serve as a hub for multisensory integration (see Chen et al., 2021; Hu et al., 2020).

4.5 Comparison of area prostriata with presubiculum and parasubiculum

Although the rodent prostriata has strong reciprocal connections with the PrS/PoS (Chen et al., 2020, 2021; Ding, 2013), many major connectional differences exist between the two structures in addition to the molecular differences described in the present study (see Table 1). First, the PrS/PoS receives moderate projections directly from dorsal CA1 (see table S2 of Ding et al., 2020 for mouse; van Groen & Wyss, 1990b for rat; but fewer in Cenquizca & Swanson, 2007 for rat), while the prostriata does not (Chen et al., 2021; Hu et al., 2020). Second, the prostriata but not the PrS/PoS receives strong projections directly from the V1 and DLG (Chen et al., 2021; Ding, 2013; Lu et al., 2020). Third, the PrS/PoS but not the prostriata receives strong projections from the posterior parietal cortex or parietal association cortex (see figure 7i of Ding, 2013), and this is consistent with the results from macaque monkeys (Ding et al., 2000; Seltzer & Pandya, 1984). Fourth,
**FIGURE 14** Examples of strong gene expression in layer 5 of the Pro at P4 and P28. All panels are from sagittal sections. (a, b) Strong expression of *Etv1* (a) and *Grp* (b) in layer 5 of the Pro in the lateral sagittal sections at P4. Layer 5 of the V1, PoR, and PrSd displays relatively weaker expression, while dorsal subiculum (Sd) contains strong expression. (c–f) Strong *Crym* expression in layer 5 of the Pro across lateral (c) to medial (f) sections at P4. The locations of the sections in panels (c–f) roughly correspond to panels (d–g) of Figure 1. The interval between each section is 160 µm. Strong *Crym* expression is seen in the PaS (c) and layer 6 of the PoR and V1 (c–f), while layer 5 of the V1 and layers 5–6 of the PrSd contain relatively weaker *Crym* expression. Faint *Crym* expression is also detected in layer 3 of the PrSd. It worth mentioning that the expression pattern of *Crym* at P14 is similar to that at P4. (g–i) *Crym* expression in the Pro across lateral (g) to medial (i) sagittal sections at P28. The locations of the sections in (g, h, i) roughly correspond to panels (d, f, g) of Figure 1, respectively. At P28, *Crym* expression in layer 5 of the Pro is slightly stronger than that of the V1 and PrSd (g–i) with weaker expression in layer 5 of the PoR (g). Scale bars: 220 µm in panel (a) for panels (a–f); 230 µm in panel (g) for panels (g–i).
FIGURE 15 Identification of the prostriata (Pro) at P14 and P28. All panels are from sagittal sections. Lateral (top) to medial (bottom) sections at levels 1–4 are arranged along each column and the locations of the sections at levels 1–4 of each column roughly correspond to panels (d–g) of Figure 1. The interval between each section/level in each column is 160 µm. (a–d) 6430573f11rik expression in both layers 5 (arrows) and 2–3 (stars) of the Pro at P14. This gene is also expressed in dorsal subiculum (Sd) and layer 5 of the V1 and PrSd. (e–h) C1ql2 expression in layer 5 of the Pro (arrows) at P14 (e–g) and P28 (h). Note that layer 5 in neighboring regions is negative for C1ql2. The locations of layer 5 of the Pro identified with C1ql2 are consistent with the strong expression of Etv1 and Crym in layer 5 of the Pro shown in Figures 4c and 4d and 14g–i. (i–l) Tcerg1l expression in layers 2–3 of the Pro (stars) at P14. Note the strong expression in layer 2 of the PrSd (k, l). Strong and weaker expression is seen in layer 5 of the V1 and Pro, respectively. Scale bars: 210 µm in panel (a) for panels (a–h); 220 µm in panel (i) for panels (i–l).

the PrS/PS has strong projections to lateral mammillary nucleus (for mouse: Ding, 2013; for rat: Shibata, 1989; Swanson & Cowan, 1977; Yoder & Taube, 2011), whereas the prostriata does not project to this nucleus (Chen et al., 2021). Fifth, the PrS/PS rather than the prostriata projects strongly to both sides of the MEC (for mouse: Chen et al., 2020; for rat: Caballero-Bleda & Witter, 1993; Honda & Ishizuka, 2004; Swanson & Cowan, 1977; van Groen & Wyss, 1990a). Sixth, the prostriata projects strongly to the PTN, PN, VLG, LP-Pul, and ZI (Chen et al., 2021), while the PrS/PS was not reported (Ding, 2013; Preston-Ferrer et al., 2016; van Groen & Wyss, 1990a). Seventh, the PrS/PS gives off moderate projections to the RS (https://connectivity.brain-map.org for mouse; Swanson & Cowan, 1977 for rat), whereas the prostriata provides only weak inputs to the RS (Chen et al., 2021). Eighth, the PrS/PS was reported to project to LD and anterodorsal (AD) thalamic nuclei (van Groen & Wyss, 1990c). In contrast, the prostriata projects to LD but not to AD (Chen et al., 2021). Ninth, terminal distribution of the projections from LD to the prostriata ends in layer 1 (Hu et al., 2020), while the terminals to the PrSd/PS distribute in layers 1 and 4–5 (van Groen & Wyss, 1992). Finally, although the RS projects to both prostriata and PrSd, the laminar distribution of these inputs is
different. For example, the RSg projects predominantly to layers 1, 3, 5, and 6 of the PrS, while the RSag mainly innervates layers 1 and 3 of the PrS (Shibata, 1994). In contrast, the RSg and RSag projections to the prostriata mostly terminate in layers 5 and 6, respectively (Hu et al., 2020).

In addition to distinct gene expression patterns shown in the present study (see Table 1), the PaS also displays differential connections from the prostriata. The PaS but not the prostriata receives strong inputs from basolateral nucleus of amygdala (for mouse: Ding, 2013; for rat: van Groen & Wyss, 1990a) and mediodorsal and parafascicular–centromedian thalamic nuclei (https://connectivity.brain-map.org). On the other hand, the PaS does not receive projections from the visual and auditory cortices and DLG (van Groen & Wyss, 1990a), while the prostriata does (Chen et al., 2021). In addition, the RS predominantly innervates layers 1–3 of the PaS (https://connectivity.brain-map.org) and layers 5–6 of the prostriata (Hu et al., 2020). As for the efferent projections, the PaS projects strongly to the superficial layers of the MEC bilaterally (for mouse: Chen et al., 2020; Hu et al., 2020; for rat: Caballero-Bleda & Witter, 1993; van Groen & Wyss, 1990a), whereas the prostriata displays weak projections to the deep layers of the MEC (Chen et al., 2021). Additionally, as mentioned above, the PaS was not reported to project to the PTN, PN, VLG, LP-Pul, and ZI (Ding, 2013; Tang et al., 2016; van Groen & Wyss, 1990a), which are the major targets of the prostriata (Chen et al., 2021).
4.6 Comparison of area prostriata in rodents and monkeys

Although limited data are available about the chemoarchitecture of nonhuman primates, some previous work showed enriched expression of calbindin-D28k in layers 2–3 of the prostriata in macaque and marmoset monkeys (Ding et al., 2003; Paxinos et al., 2012). Similar findings were reported for the prostriata of the mice and rats (Chen et al., 2021; Lu et al., 2020). In both neonate marmosets and mice, strong Id2 and Grp expression is observed in layers 2–3 and layer 5 of the prostriata, respectively (see Figure 20). These findings indicate the existence of some conservative molecular markers for the prostriata across species. As for the connectivity, the rodent prostriata was found to project to cortical regions such as visual and auditory association cortex (V2 and A2), limbic cortex (OFCm, RS, PoR, ectorhinal, and entorhinal cortices), and subcortical regions such as caudal putamen and claustrum, although these projections are usually weak (Chen et al., 2021; unpublished data). Consistent with this, the prostriata in nonhuman primates was also reported to have projections to the V1 (Sousa et al., 1991), secondary auditory cortex (Falchier et al., 2010), and OFCm (Barbas, 1993). On the other hand, the primate prostriata was reported to project to middle temporal area (MT; Palmer & Rosa, 2006; Rosa et al., 1993), frontal pole (Burman et al., 2011), rostral cingulate motor cortex (Morecraft et al., 2000), and dorsal prefrontal cortex (Reser et al., 2013). Although these regions are not yet clearly defined in rodent brains, some of these regions (e.g., MT) may be contained in lateral V2 (V2L) of rodent brains since retrograde tracer injections in many parts of the V2L resulted in some labeled neurons in layer 5 of the prostriata in mice (https://connectivity.brain-map.org). The remaining three regions seem not to receive inputs from the prostriata in rodents (Chen et al., 2021; https://connectivity.brain-map.org), suggesting the existence of some species difference in efferent projections of the prostriata. In addition, our recent study has revealed that the prostriata in rodents mainly projects to the PrS/PoS, LD, PTN, PN, VLG, LP-Pul, and ZI (Chen et al., 2021). Unfortunately, these projections in nonhuman primates have not yet explored. Finally, it should be noted that data are not yet available for afferent connections of the prostriata in nonhuman primates. In human brains, one tractography study indicates the existence of direct projections from the DLG to the prostriata (Kurzawski et al., 2020), and this finding has been confirmed in rats and mice with direct tract-tracing methods (Chen et al., 2021). Therefore, detailed data on afferent projections of the prostriata are only available in rats and mice (Chen et al., 2021; Hu et al., 2020).

Previous studies in nonhuman primates showed that cingulate cortical area 23 (A23) also adjoins the prostriata (Ding et al., 2003; Paxinos et al., 2012). This indicates that A23 (including A23v; see Figure 20) does not belong to the two subdivisions of the prostriata (Ding et al., 2003), although one study (Palmer & Rosa, 2006) suggested that A23v in the marmosets could be equated to one of the two subdivisions proposed by Ding et al. (2003). Whether rodents have a homologous A23 remains a mystery because A23 has not been reported so far in rodents. In our recent studies, we have found that the three subdivisions of the prostriata in rodents shows many different anatomical, molecular, and functional features from the PrS/PoS and PaS and thus should be treated as a distinct entity as in human and nonhuman primates.

Figure 17: Neurod6 expression in the prostriata (Pro) and adjoining regions. All panels are from sagittal sections at the level (g) of Figure 1. Faint or no Neurod6 expression is observed in layers 2–3 of the Pro, while strong expression is seen in layer 3 of the PrSd at P4 (a), P14 (b), and P28 (c). Note that Neurod6 expression in layer 5 of the V1 and PrSd is slightly stronger compared to the Pro. Neurod6 expression in layer 6 of the Pro appears similar to that of the PrSd and slightly stronger than that of the V1. Scale bars: 220 μm in panel (a); 230 μm in panels (b) and (c).

Functionally, the head direction cells, grid cells, and border cells were reported to exist in the PrS/PoS (Boccara et al., 2010; Preston-Ferrer et al., 2016; Taube et al., 1990) as well as in the PaS (Tang et al., 2016). These results indicate that the PrS/PoS and PaS are mainly involved in spatial processing and navigation (Dalton & Maguire, 2017; Preston-Ferrer et al., 2016; Sanguinetti-Scheck & Brecht, 2020). However, it is currently not known if those cells or other specialized cells exist in the region corresponding to the prostriata. Taken together, the prostriata in rodents shows many different anatomical, molecular, and functional features from the PrS/PoS and PaS and thus should be treated as a distinct entity as in human and nonhuman primates.
FIGURE 18  Molecular markers for the PrS and PaS versus the prostriata in postnatal mice. All panels are from sagittal sections. The location of the sections in (b, e) is at level (g) of Figure 1, while that in other panels is at level (d) or (e) of Figure 1. (a–c) Ddit4l expression at P4 (a, b) and P28 (c). At P4, Ddit4l expression is mostly concentrated in layer 3 of the PrSd with faint expression in layer 2 of the PrSd and PaS; almost no expression is seen in the Pro at the lateral (a) and medial (b) levels. At P14 (see Table 1) and P28 (c), Ddit4l expression in layer 3 of the PrSd is reduced, while that in the dorsal PaS is greatly increased (c). (d–f) Nnat expression at P4 (d, e) and P28 (f). At P4, strong Nnat expression is detected in layers 2 and 5–6 of the PrSd and in both dorsal and ventral parts of the PaS (d). Weak Nnat expression is seen in the Pro at the lateral (d) and medial (e) levels. At P14 (see Table 1) and P28 (f), Nnat expression in the PrSd almost disappears, while that in the dorsal PaS is greatly decreased (f). In contrast, strong Nnat expression appears in layer 5 of the Pro (f). (g–i) Wfs1 expression at P4 (g), P14 (h), and P28 (i). At P4, only faint Wfs1 expression is detected in the PaS (g). At P14, very strong Wfs1 expression is seen in both dorsal and ventral parts of the PaS with weak expression in layers 2–3 and 5 of the Pro (h). At P28, Wfs1 expression in the PaS is slightly reduced, while that in layer 5 of the Pro disappears (i). Scale bars: 220 µm in panel (a) for panels (a, b, d, e, and g); 230 µm in panel (c) for panels (c, f, h, and i).
FIGURE 19  Gene expression in postrhinal cortex (PoR) and nearby regions at P4 and P14. All panels are from sagittal sections lateral to panel (a) of Figure 1. (a–c) Three sections at the lateral level, where hippocampal CA3 and dentate gyrus (DG) are not visible, show Lypd1 (a), Nnat (b), and Cdh8 (c) expression in the PoR and nearby regions at P4. The PoR displays moderate Nnat and Cdh8 expression in its layers 2–3 and 5. Note the strong expression of Nnat in the dorsal PaS (indicated by star) and layers 2 and 5–6 of the MEC (b), and that of Cdh8 in layer 3 of the MEC (c). (d–f) Three sections at the medial level, where the dorsal MEC inserted between the dorsal and ventral PaS, demonstrate Lypd1 (d), Nnat (e), and Cdh8 (f) expression in the PoR and nearby regions at P4. Note the differential gene expression patterns in the MEC, PaS, PoR, and PrS. (g–i) Three lateral sections display Lypd1 (d), Nnat (e), and Cdh8 (f) expression in the PoR and nearby regions at P14. Note the strong gene expression in the PoR and slightly weaker expression in the dorsal PaS. PS for prosubiculum; Sub for subiculum. Scale bars: 330 µm in panel (a) for panels (a–f); 350 µm in panel (g) for panels (g–i).
FIGURE 20  Comparison of Id2 and Grp expression in the prostriata (Pro) of the marmosets and mice. Panels (a–d) and (e–h) are four sequential coronal sections from the marmoset at P0. (a–d) Id2 expression in the Pro, area 29 (A29), area 30 (A30), area 23 (A23), V1, and V2 of the marmoset. Note the weak and strong Id2 expression in layers 2–3 of A29 (a, b) and the Pro (b, c), respectively. The arrows with A29 in panels (a) and (b) point to layers 2–3 of A29, which has faint Id2 expression. The superficial layers of A30, A23, and V1 also display strong Id2 expression. Note the existence of layer 4 in A23 (with faint Id2 expression; pointed by arrows). (e–h) Grp expression in the Pro, A29, A30, A23, V1, and V2 of the marmoset. Strong Grp expression in layers 2–3 of A29 (e, f) and relatively stronger expression in layer 5 of the Pro (f, g) compared to adjoining regions are observed. Layer 5 of the V1 shows the weakest Grp expression (g, h). The arrows with A29 in panels (e) and (f) point to layers 2–3 of A29, which show strong Grp expression. (i–l) Id2 (i, j) and Grp (k, l) expression in the Pro, PrSd, V1, A29, and A30 in sagittal sections from the mice at P4. Strong Id2 expression is observed in layers 2–3 of A29, A30 (i), and the Pro (j) and in layers 2 and 5 of the PrSd (j). Grp is strongly expressed in layers 2–3 of A29 (k) and layer 5 of the Pro (l). The inset in panel (i) shows Id2 expression in A29 and A30 of a mouse at P28. At this age, weak and strong Id2 expression exists in layers 2–3 of A29 and A30, respectively, and this pattern is similar to that found in the marmoset (a, b). It should be mentioned that strong Id2 expression also exists in layers 2–3 of A29 and A30 at P14. cc, corpus callosum; CF, calcarine fissure. Scale bars: 1 mm in panel (a) for panel (a–h); 200 µm in panel (j) for panels (j) and (l); 420 µm in panel (i) for panels (i, k) and the inset in panel (i).
rodent RS (RSg, RSag, and RSagl) display differential gene expression (Hu et al., 2020; Lu et al., 2020). Since the RSg and RSag correspond to areas 29 and 30, respectively, it is possible that the RSagl or part of it corresponds to A23 in terms of spatial topography of these three subdivisions (see Figure 20a). However, A23 in nonhuman primates has a thin but clear layer 4 (Ding et al., 2003; Figures 20b and 20c), whereas the RSagl in rodents has almost no layer 4 (Hu et al., 2020; Lu et al., 2020). Similarly, the Pro-p of the prostriata in the macaque monkeys has a very thin layer 4, while both subdivisions of the rodent prostriata do not appear to have a layer 4 (see Figure 10). Therefore, multimodal data including connectional and comparative ones are needed to establish the correspondence of A23 between these two species and the relationship between the prostriata and A23.

AUTHOR CONTRIBUTIONS
SLD conceptualized the study, performed supervision, and wrote the manuscript. SLD, SQC, CHC, XJX, and SYZ performed data investigation and analysis. All authors have read and approved the submitted manuscript.

ACKNOWLEDGMENT
The authors would like to thank Allen Institute for providing publicly accessible mouse brain in situ hybridization data set.

FUNDING INFORMATION
This work is partially supported by National Natural Science Foundation of China (#31771327).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
All data sets presented in this study are included in the article. These data are available online (http://www.brain-map.org) and from the corresponding author upon reasonable request.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/cne.25346.

ORCID
Song-Lin Ding https://orcid.org/0000-0002-7072-5272

REFERENCES
Allman, J. M., & Kaas, J. H. (1971). Representation of the visual field in striate and adjoining cortex of the owl monkey (Aotus trivirgatus). Brain Research, 35, 89–106. https://doi.org/10.1016/0006-8993(71)90596-8
Barbas, H. (1993). Organization of cortical afferent input to orbitofrontal areas in the rhesus monkey. Neuroscience, 56, 841–864. https://doi.org/10.1016/0306-4522(93)90132-Y
Blackstad, T. W. (1956). Commisural connections of the hippocampal region in the rat, with special reference to their mode of termination. Journal of Comparative Neurology, 105, 417–537. https://doi.org/10.1002/cne.901050305
Bocchera, C. N., Sargolini, F., Thoresen, V. H., Solstad, T., Witter, M. P., Moser, E. I., & Moser, M. B. (2010). Grid cells in pre- and parasubiculum. Nature Neuroscience, 13, 987–994. https://doi.org/10.1038/nn.2408
Falcieri, A., Schroeder, C. E., Hackett, T. A., Lakatos, P., Nascimento-Silva, S., Ulbert, I., Karmos, G., & Smiley, J. F. (2010). Projection from visual areas...
hippocampal formation projections. *Journal of Comparative Neurology*, 302, 515–528. https://doi.org/10.1002/cne.903020308

van Groen, T., & Wyss, J. M. (1990c). The postsubicular cortex in the rat: Characterization of the fourth region of the subicular cortex and its connections. *Brain Research*, 529, 165–177. https://doi.org/10.1016/0006-8993(90)90824-U

van Groen, T., & Wyss, J. M. (1992). Projections from the laterodorsal nucleus of the thalamus to the limbic and visual cortices in the rat. *Journal of Comparative Neurology*, 324, 427–448. https://doi.org/10.1002/cne.903240310

Vaz Ferreira, A. (1951). The cortical areas of the albino rat studied by silver impregnation. *Journal of Comparative Neurology*, 95, 177–243. https://doi.org/10.1002/cne.900950202

Yoder, R. M., & Taube, J. S. (2011). Projections to the anterodorsal thalamus and lateral mammillary nuclei arise from different cell populations within the postsubiculum: Implications for the control of head direction cells. *Hippocampus*, 21, 1062–1073. https://doi.org/10.1002/hipo.20820

Yu, H.-H., Chaplin, T. A., Davies, A. J., Verma, R., & Rosa, M. G. P. (2012). A specialized area in limbic cortex for fast analysis of peripheral vision. *Current Biology*, 22, 1351–1357. https://doi.org/10.1016/j.cub.2012.05.029

---

**How to cite this article:** Chen, S.-Q., Chen, C.-H., Xiang, X.-J., Zhang, S.-Y., & Ding, S.-L. (2022). Chemoarchitecture of area prostriata in adult and developing mice: Comparison with presubiculum and parasubiculum. *Journal of Comparative Neurology*, 530, 2486–2517. https://doi.org/10.1002/cne.25346