An atlas and database of neuropeptide gene expression in the adult zebrafish forebrain

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Author contribution
T. H-K. and Y. Y. designed the study. T. H-K., R. A., Y. Y., N. M., and N. W. conducted experiments. T. H-K. acquired the data, conducted the analysis, and prepared the draft manuscript and figures. H. I. and S. O. designed and constructed the database. Y. Y., N. M., S. O., H. I., R. A., and N. W. edited the manuscript and figures. All the authors have read and approved the final version of the manuscript.

Conflict of interest
The authors report no conflict of interest.

Data availability statement
High-resolution figures and raw data will be uploaded to the CBS Data Sharing Platform. All scan data of glass slides can be viewed and downloaded at the Adult Zebrafish Brain Gene Expression Database (https://ssbd.riken.jp/azebex/).

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Ethics approval statement

All experimental procedures were approved by the Animal Care and Use Committee of RIKEN.

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Abstract

Zebrafish is a useful model organism in neuroscience; however, its gene expression atlas in the adult brain is not well developed. In the present study, we examined the expression of 38 neuropeptides, and glutamatergic neuron marker gene mix (*slc17a6a, slc17a6b, slc17a7a,* and *slc17a7b*) in the adult zebrafish brain using *in situ* hybridization. The results are summarized as an expression atlas in 19 coronal planes of the forebrain. Furthermore, the scan data of all sections

were made publicly available as a database. Based on these data, we performed detailed neuroanatomical analyses of the hypothalamus. By scrutinizing and comparing the expression patterns of neuropeptides, we found that several regions described as one nucleus in the reference zebrafish brain atlas contain two or more subregions with significantly different neuropeptide/neurotransmitter expression profiles, and we proposed them as novel subnuclei. Subsequently, the expression data obtained in this study were compared with those in mice, and a cluster analysis was performed to examine the similarities. As a result, several nuclei in zebrafish and mice were clustered in close vicinity: zebrafish ventral part of the anterior part of the parvocellular preoptic nucleus (PPav)/magnocellular preoptic nucleus (PM) and mouse paraventricular hypothalamic nucleus (Pa), zebrafish posterior part of the parvocellular preoptic nucleus (PPp) and mouse medial preoptic area (MPA), zebrafish dorsal part of the ventral zone of periventricular hypothalamus (Hvd)/anterior tuberal nucleus (ATN) and mouse ventromedial hypothalamic nucleus (VMN). The present expression atlas, database, and anatomical findings will contribute to future neuroscientific research using zebrafish.

Key points

- The expression of 38 neuropeptides and GABAergic/glutamatergic neuronal marker genes in adult zebrafish forebrain was examined and compiled as an atlas.
- All scanned brain section data were published as a database.
- Based on the expression data obtained, multiple subnuclei in the zebrafish hypothalamus were proposed, and comparisons with the mouse hypothalamus were conducted.

Keywords

adult zebrafish brain, gene expression atlas, neuropeptide, gabaergic and glutamatergic neuron marker, subnuclei, comparison with mouse

1. Introduction

Zebrafish (*Danio rerio*) is widely used as a vertebrate animal model in various biology research fields. It has several advantageous properties, such as external fertilization, large clutch size, transparency of eggs and larvae, rapid development, and feasibility for both forward and reverse genetics (Kalueff *et al*., 2014; Stewart *et al*, 2014; Gerlai, 2020). Recently, whole-brain calcium imaging and high-throughput behavioral assays have been successively developed for larval zebrafish, leading to breakthroughs in neuroscience research (Orger and Polavieja, 2017; Vanwalleghem *et al*., 2018; Loring *et al*., 2020; Lee *et al*., 2022). In addition, multiple brain databases have been developed for larval zebrafish, such as the gene expression atlas of 2-, 3-, and 4-days post-fertilization (dpf) larval brains (ViBE-Z, Ronneberger *et al*., 2012), the brain atlas of single neuron tracings and images of transgenes and *in situ* hybridizations in 6-dpf larvae (MapZebrain, Kunst *et al*., 2019), and transgene expression- and immunohistochemistry-based atlas in 6-dpf larvae (Z-Brain Atlas, Randlett *et al*., 2015; Zebrafish Brain Browser 2 (ZBB2), Tabor *et al*., 2019). However, the number of available brain database resources on adult zebrafish is limited so far, which include the reference brain atlas (Wullimann *et al*., 1996), a database of transgene expression patterns in enhancer-trap lines (ZeBrain), magnetic resonance histology-based 3D atlas (Ullmann *et al*., 2010), and 3D atlas of immunohistochemical images of ten marker genes (AZBA, Kenney *et al*., 2021). Thus, no brain atlas maps gene expression patterns on a large scale. As a promising animal model in behavioral neuroscience, adult zebrafish display more diverse and complex behaviors than larval zebrafish, including associative learning, perceptual decision-making, social behavior, and reproductive behavior (Norton and Bally-Cuif, 2010; Oliveira RF, 2013; Yabuki *et al*., 2016; Chou *et a*l., 2016; Stednitz *et al*., 2018, Okamoto *et al*., 2021). Furthermore, as animal models for various neurological and psychiatric diseases, zebrafish have been actively used in the field of translational research in recent years (Kalueff *et al*., 2014; Stewart *et al*., 2014; Fonseka *et al*., 2016; Fontana *et al*., 2021; Kumar *et al*., 2021). A gene expression atlas of adult zebrafish is needed to expand on these trends.

Here, we report an atlas summarizing the expression patterns of 38 neuropeptide genes and GABAergic/glutamatergic neuronal markers in the adult zebrafish forebrain. Neuropeptides are relatively small peptides that are highly conserved from invertebrates to vertebrates (Holmgren and Jensen, 2001; Elphick *et al*., 2018). They function as neuromodulators and play important roles in various physiological phenomena (Hökfelt *et al*., 2000; van den Pol, 2012; Sohn, 2015; van den Burg and Stoop, 2019). As neuropeptides tend to be expressed in specific types or small localized populations of neurons in the brain, they can also be used as markers for groups of neurons of interest and as genetic tools for visualizing and manipulating these neurons. Indeed,

many transgenic zebrafish have been produced using the enhancer/promoter regions of various neuropeptide genes (Liu *et al*., 2003; Levitas-Djerbi *et al*., 2015; Förster *et al*., 2017; Koide *et al*., 2018; Shainer *et al*., 2019; Song *et al*., 2021). By closely examining the expression patterns of neuropeptides and neurotransmitters, we found that some of the hypothalamic nuclei described in the zebrafish reference brain atlas (Wullimann *et al*., 1996) contained multiple subregions with different expression patterns of neuropeptides, which we defined as subnuclei. Furthermore, by comparing the expression patterns of the neuropeptides in the zebrafish hypothalamus with those in the mouse hypothalamus, we identified several nuclei that exhibited similar expression patterns across species.

2 Materials and Methods

2.1 Animals

Zebrafish (*Danio rerio*) were maintained at 28°C under 14-hour light and 10-hour dark cycle and fed with brine shrimp two to three times a day. Adult wild-type zebrafish (RIKEN-Wako) were used in all the experiments. All experimental procedures were approved by the Animal Care and Use Committee of RIKEN.

2.2 cRNA probe synthesis

Coding regions (500–1000 bp) of neuropeptide and neurotransmitter marker genes were PCR-amplified from zebrafish brain cDNA or genomic DNA and cloned into the pGEM-T Easy vector (Promega). Three glutamate decarboxylase genes (*gad1a*, *gad1b*, and *gad2*) were used as marker genes for GABAergic neurons. For the glutamatergic neurons, four vesicular glutamate transporter (*vglut*) genes (*slc17a6a*, *slc17a6b, slc17a7a*, and *slc17a7b*) were used. The genes analyzed and primers used for PCR cloning are listed in Table 1.

The names of the individual genes followed the ZFIN nomenclature, with a few exceptions, for which the correspondences to previous designations are listed in Table 1. One of the *cart* genes was missing from the ZFIN database, and the ZFIN nomenclature did not match the results of the phylogenetic analysis. Therefore, we followed the nomenclature of Cai *et al*. (2015), in which a detailed phylogenetic analysis of *cart* genes was conducted. Zebrafish harbor two galanin genes (*gal* and *gall*); however, the molecular evolutionary positioning of the second galanin-like gene (*gall*, also called *si:rp71-1c10.8*) remains unclear. Hence, we carried out phylogenetic and syntenic analyses of *gall* and revealed that the second galanin-like gene in zebrafish and other teleost species was not a teleost-specific paralogous gene for *gal*, but an orthologous gene for *Galp* in tetrapods (Supplemental Fig. 1). Therefore, it was designated *galp*.

Digoxigenin (DIG)-labeled antisense cRNA probes were synthesized using T7, SP6, or T3 RNA polymerases (Promega) and purified through Micro Bio-Spin Columns with Bio-Gel (BIO-RAD). Plasmids containing *slc17a6a*, *slc17a6b*, and *slc17a7a* genes were kindly provided by Dr. Higashijima (Higashijima *et al*., 2004).

2.3 *In situ* hybridization

Brains of adult male zebrafish (3–12 months old) were sampled during the light period and fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) at 4°C overnight. The brains were then incubated in 50%, 70%, 100% ethanol, and Lemosol (Wako) for dehydration,

230 embedded in paraffin, and sectioned into 10 μm-thick serial coronal sections. The sections were
231 deparaffinized in Lemosol and 100%, 90%, 70%, and 50% ethanol. After washing in PBS, the
232 sections were treated using 1 μg/mL proteinase K (Invitrogen) in PBS at 37°C for 15 min and re-
233 fixed in 4% PFA in PBS for 10 min at room temperature (RT). The sections were acetylated,
234 followed by incubation in pre-hybridization buffer [50% formamide, 5× saline sodium citrate
235 (SSC), 50 μg/mL heparin, 0.1% Tween] at 60°C for 1 h. The sections were then hybridized using
236 DIG-labeled neuropeptide gene probes (0.01 ng/μL for the *oxt* gene, 0.5 ng/μL for the rest of the
237 genes) in hybridization buffer [100 μg/μL RNA from yeast (Roche) added to pre-hybridization
238 buffer] at 60°C overnight. The *gad* mix (a mixture of *gad1a*, *gad1b*, and *gad2* probes; 0.5 ng/μL
239 each) was used as a marker for GABAergic neurons. Moreover, *vglut* mix (a mixture of *slc17a6a*,
240 *slc17a6b, slc17a7a*, and *slc17a7b* probes; 0.5 ng/μL each) was used as a marker for glutamatergic
241 neurons. The sections were then washed in 5× SSC buffer and 0.2× SSC at 60°C. After washing
242 in 0.2× SSC and maleic acid buffer (MAB; 0.1 M maleic acid, 0.15 M NaCl, 0.19 M NaOH), the
243 sections were blocked with 2% blocking reagent (Roche) and 5% normal goat serum in MAB for
244 30 min at RT and incubated with alkaline phosphatase-conjugated anti-DIG antibody (1:10000
245 for *oxt*, 1:1000 for other genes, Sigma-Aldrich) at 4°C overnight. For color development, the
246 sections were washed in MAB and color development buffer (0.1 M Tris-HCl pH 9.5, 0.05 M
247 $MgCl_2$, 0.01 M NaCl) and then incubated with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue
248 tetrazolium (BCIP/NBT) solution at 30°C for 0.5–7 h. The color development was stopped by
249 washing with PBS and water. Some sections (at least one individual fish for each gene) were
250 counterstained using 1% methyl green stain solution (Muto Pure Chemicals) for 3–5 min and
251 washed in water. The sections were then mounted using Aqua-Poly/Mount (Polysciences) or
252 Immu-mount (Thermo Fisher Scientific).
253
254 **2.4 Image acquisition**
255 Whole images of brain sections on glass slides were captured using a NanoZoomer HT slide
256 scanner (Hamamatsu Photonics). The pictures were cut out for each brain plane using NDP.view2
257 (Hamamatsu Photonics) and trimmed using Adobe Photoshop CS6 (Adobe Inc.).
258
259 **2.5 Database construction**
260 The Adult Zebrafish Brain Gene Expression Database (https://ssbd.riken.jp/azebex/) was
261 constructed based on the SSBD (Systems Science of Biological Dynamics) platform (Tohsato *et*
262 *al*., 2016). SSBD:repository was used as the database infrastructure. OMERO (Open Microscopy
263 Environment) system (Allan *et al*., 2012) was used to preview the images.
264
265 **2.6 Phylogenetic and syntenic analyses**

266 Phylogenetic and syntenic analyses were performed to examine homologous relationships
267 between the two galanin-like genes in various animal species. The GenBank accession numbers
268 of the genes used for phylogenetic analysis are summarized in Supplemental Table 1. The amino
269 acid sequences of the galanin-like genes in each species were aligned using ClustalW, and a
270 phylogenetic tree was constructed using the maximum likelihood method. The analysis was
271 performed using Molecular Evolutionary Genetics Analysis (MEGA11) software (version 11)
272 (Tamura *et al*., 2021). For syntenic analysis, genomic information for each species was obtained
273 from the Ensembl or NCBI databases.
274
275 **2.7 Comparison of the hypothalamic nuclei between zebrafish and mouse**
276 To examine the similarity in the hypothalamic nuclei between zebrafish and mice, we compiled a
277 table of gene expression in each nucleus (expressed = 1, not expressed = 0). Comparisons were
278 made for genes that were expressed in both the zebrafish and mouse hypothalamus. Expression
279 profiles of neuropeptide genes in the mouse hypothalamus were obtained from the Allen Brain
280 Atlas (Lein *et al*., 2007) and from previous studies (*Agrp*, Deem *et al*., 2021; *Avp*, Smith *et al*.,
281 1996; Otero-García *et al*., 2015; *Crh*, Alon *et al*., 2009; Keegan *et al*., 1994; *Galp*, Juréus *et al*.,
282 2001; *Oxt*, Otero-García *et al*., 2015; *Pmch*, Croizier *et al*., 2010; Diniz *et al*., 2019; *Npb*, Tanaka
283 *et al*., 2003; *Hcrt*, Peyron *et al*., 1998; *Pomc*, Williams *et al*., 2010; Cowley *et al*., 2001; *Tac1*,
284 Navarro *et al*., 2014; *Tac2*, Duarte *et al*., 2005). The expression of the zebrafish *gad* mix was
285 compared to that of *Gad1* and *Gad2* in mice. Zebrafish *vglut* mix expression was compared with
286 *Vglut2* expression in mice because *Vglut1* was not expressed in the mouse hypothalamus. Because
287 *gad*/*Gad* and *vglut*/*Vglut* are more or less expressed in almost all regions, the expression patterns
288 of *gad* and *vglut* were compared, and those with a higher number of expressing neurons were
289 counted as positive. Both were considered positive when they were equally expressed. When
290 neither was expressed, both were considered negative. The nomenclature of the mouse
291 hypothalamic nuclei follows that of Paxinos and Franklin (2019). Zebrafish often have multiple
292 homologous genes corresponding to a single mouse gene, mainly because of teleost-specific
293 genome duplication (Jaillon *et al*., 2004, Kassahn *et al*., 2009). Teleost-specific paralogous genes
294 expressions, that have been shown to have arisen from teleost-specific genome duplication, were
295 added together (*ccka*/*cckb*, Dupré and Tostivint, 2014; Kurokawa *et al*., 2003; *crha*/*crhb*, Grone
296 and Maruska, 2015; *penka*/*penkb*, Bojnik *et al*., 2011; *pomca*/*pomcb*, Forlano and Cone, 2007;
297 *tac3a*/*tac3b*, Zhou *et al*., 2012; *vipa*/*vipb*, Nam *et al*., 2009). The *cart* genes were expressed in
298 both zebrafish and mice but were not included in the comparison analysis because none of the
299 *cart* genes found in the present zebrafish genome data were considered homologous to the mouse
300 *Cart* gene (Cai *et al*., 2015). Furthermore, zebrafish and mouse hypothalamic nuclei and
301 neuropeptide/neurotransmitter genes were clustered using Ward's method based on Euclidean

distance using the hclust function in R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing).

3. Results

3.1 Neuropeptide expressions in the adult zebrafish forebrain

Expression patterns of 38 neuropeptide genes, *agrp*, *asip2b*, *avp*, *calca*, *cart2a*, *cart2b*, *cart3a*, *cart3b*, *cart4*, *cart5*, *ccka*, *cckb*, *crha*, *crhb*, *gal*, *galp*, *gnrh2*, *gnrh3*, *grp*, *kiss1*, *kiss2*, *npb*, *npvf*, *npy*, *hcrt*, *oxt*, *pdyn*, *penka*, *penkb*, *pmch*, *pmchl*, *pomca*, *pomcb*, *tac1*, *tac3a*, *tac3b*, *vipa*, *vipb*, glutamatergic neuron marker gene mix (*slc17a6a*, *slc17a6b*, *slc17a7a*, and *slc17a7b*), and GABAergic neuron marker gene mix (*gad1a*, *gad1b*, and *gad2*) were examined by *in situ* hybridization throughout the adult zebrafish forebrain (telencephalon and diencephalon). Fig. 1 shows a schematic of the zebrafish brain and the location of each plane. Figs. 2–20 show schematics of the coronal planes and microscopic images of the genes that are expressed in each plane. Schematics were based on the zebrafish brain atlas (Wullimann *et al*., 1996) with several modifications. The division of nuclei in the dorsal telencephalic area was modified according to Ganz *et al.* (2014) and Yáñez *et al*. (2022) (Figs. 3–10). The intermediate nucleus of the ventral telencephalon (Vi) was added, as described by Biechl *et al*. (2017) (Figs. 9, 10). Subregions in the dorsal habenula (Had), medial Had (Hadm), and lateral Had (Hadl) were added according to Agetsuma *et al*. (2010) (Figs. 11–13). The nucleus lateralis tuberis (NLT) in the hypothalamus was added as in recent papers in zebrafish (Akash *et al*., 2014) or other teleost species (Karoubi *et al*., 2016) (Figs. 14, 15). The intercalated nucleus (IC) was added, as described by Mueller and Guo (2009). The intermediate nucleus (IN) was added, and the positions of the paraventricular organ (PVO) and posterior tuberal nucleus (PTN) were altered according to Rink and Wullimann (2001) (Figs. 15, 16). The neuropeptide expression profiles in each nucleus are summarized in Tables 2–6. All scanned data of the whole glass slides are available in the Adult Zebrafish Brain Gene Expression Database (https://ssbd.riken.jp/azebex). The database allows users to view whole-brain scan data online. Raw data in .ndpi format can also be downloaded from the database and viewed using NDP.view2, which is free software. The database also provides images of sections in the hindbrain and expression data in female zebrafish.

3.1.1 Telencephalon

3.1.1.1 Olfactory bulb

In the zebrafish olfactory bulb, the glomerular layer (GL) and the external cellular layer (ECL) contain two types of projection neurons, mitral cells and ruffed cells, and two types of interneurons, periglomerular cells and short-axon cells. The internal cellular layer (ICL) mainly contains GABAergic interneurons and granule cells (Olivares and Schmachtenberg, 2019). The distribution of GABAergic and glutamatergic neurons was consistent with that of these distinct cell types (Figs. 2 and 3, Table 2). *crhb* was strongly expressed in a small number of large-

diameter cells in ECL/GL, which presumably are a subset of mitral cells, as was reported in mammals (Bassett *et al*., 1992) (Figs. 2, 3, Table 2). *penkb* was expressed in small-diameter cells surrounding glomeruli in GL/ECL and ICL, which are likely periglomerular cells and granule cells, respectively, as is the case in mammals (Matsutani *et al*., 1989) (Figs. 2, 3, Table 2). *npy*-positive cells in GL/ECL might be short-axon cells, as described in mammals (Scott *et al*., 1987). *cart3b*, *cckb*, and *tac1* were expressed intensely and exclusively in ICL cells, which are likely granule cells. *gnrh3* was expressed in terminal nerve neurons, with a very small number of cells located in the ventral-most region of the olfactory bulb (Figs. 2 and 3, Table 2).

3.1.1.2 Dorsal telencephalon

The dorsal telencephalic area (D) contains nine nuclei, including several newly defined nuclei (Ganz *et al*., 2014; Yáñez *et al*., 2022) (Figs. 3–10, Table 2). All nuclei showed distinct neuropeptide expression patterns, some of which validated the new definition of nuclei (Figs. 3–10, Table 2). For example, *cart3b* and *tac3b* were selectively expressed in the dorsal part of the lateral zone of D (Dld), but not in the ventral part of the lateral zone of D (Dlv) (Figs. 3 and 4, Table 2). *tac1* was strongly expressed in the posterior part of the medial zone of D (Dmp) but not in the medial zone of D (Dm) (Figs. 4–10, Table 2). Dmp was also characterized by the absence of *cckb* expression (Fig. 8–10, Table 2). The dorsal telencephalon was characterized by high *vglut* expression and low *gad* expression (Figs. 3–10). The dorsal telencephalon expressed fewer neuropeptides than the ventral telencephalon; however, *cckb* and *cart3b* were commonly expressed in most of the dorsal telencephalic areas, except that *cckb* was absent in Dmp, whereas *cart3b* was absent in Dlv and the posterior zone of D (Dp) (Figs. 3–10, Table 2).

3.1.1.3 Ventral telencephalon

The ventral telencephalic area (V) contains nine nuclei (Figs. 3–10, Table 3). In contrast to the abundant presence of glutamatergic neurons in the dorsal telencephalon, most neurons in the ventral telencephalic area were GABAergic inhibitory neurons (Figs. 3–10, Table 3). The dorsal nucleus of V (Vd) and supracommissural nucleus of V (Vs) are anteroposteriorly adjacent structures in the dorsomedial part of the ventral telencephalon and show exactly the same pattern of neuropeptide expression, implying similar functional features of these nuclei (Figs. 3–7. Table 3). The postcommissural nucleus of V (Vp) also expressed a similar repertoire of neuropeptides as Vd and Vs but additionally expressed *cart5* and *npb* (Figs. 8, 9, Table 3). The intermediate nucleus of V (Vi) is located in the most posterodorsomedial part of the ventral telencephalon, and its presence has recently been proposed in zebrafish (Biechl *et al*., 2017). Vi shared the expression of many neuropeptides with Vd, Vs, and Vp but was characterized by the lack of *crhb* and *npy* (Figs. 9 and 10, Table 3). The ventral part of the entopeduncular nucleus (ENv) and dorsal part of

the entopeduncular nucleus (ENd) differed greatly in the neurotransmitter profiles: ENd was mostly occupied by GABAergic neurons (Figs. 5-8), while ENv was composed predominantly of glutamatergic neurons (Figs. 8–10). In addition, *crhb* and *npy* were selectively and strongly expressed in ENd, but not in ENv (Figs. 5–10, Table 3).

3.1.2 Diencephalon

3.1.2.1 Preoptic area

Four nuclei were observed in the preoptic area, all of which expressed numerous neuropeptides (Figs. 7–12, Table 4). All nuclei commonly expressed *ccka*, *crhb*, *npy*, *pdyn*, *penka*, *penkb*, *tac1*, and *tac3a* (Figs. 7–12, Table 4). The anterior part of the parvocellular preoptic nucleus (PPa) expressed 21 neuropeptides among the 38 tested, with the largest number in the preoptic area (Figs. 7–9. Table 4). The dorsal and ventral parts of the PPa showed different neuropeptide expression patterns (see Section 3.2.1). The posterior part of the parvocellular preoptic nucleus (PPp) was characterized by densely packed GABAergic neurons, and its neuropeptide-expressing cells were sparser than those in other preoptic nuclei (Figs. 10–12). The magnocellular preoptic nucleus (PM) contained *avp*-, *oxt*-, *crhb*-, or *penka*-expressing magnocellular cells, as well as parvocellular cells positive for *asip2b*, *cart5*, *ccka*, *gal*, *galp*, *npy*, *pdyn*, *penkb*, *tac1*, and *tac3a* (Figs. 10 and 11). In addition, cells with very weak expressions of *crha* and *npb* were also observed (Figs. 10 and 11).

3.1.2.2 Hypothalamus

The hypothalamus contains nine nuclei (Figs. 12–20, Table 4). NLT, which is a thin cell layer at the outer rim of the hypothalamus, expressed *gal*, *npy*, *pmch*, *pmchl*, and *pomca* (Figs. 12, 13, Table 4). The ventral zone of the periventricular hypothalamus (Hv), lateral hypothalamic nucleus (LH), dorsal zone of the periventricular hypothalamus (Hd), and caudal zone of the periventricular hypothalamus (Hc) commonly expressed *cart3b*, *cart5*, *ccka*, *cckb*, *crhb*, *kiss2*, *npb*, *npy*, *pdyn*, *penka*, *penkb*, *pomcb*, *tac3a*, and *vipa* (Figs. 12–20, Table 4). Furthermore, Hv expressed 23 neuropeptides, many of which were localized ventrally (see 3.2.2) (Figs. 12–16). Neuropeptide expression in Hd and Hc was also not uniform, with distinct subregions within the nucleus that displayed different neuropeptide expression profiles (see 3.2.3 and 3.2.4) (Figs. 14–18). The IN is adjacent to the PTN and is distinguishable from it by the predominance of GABAergic neurons, while PTN neurons are glutamatergic (Figs. 15, 16). The anterior tuberal nucleus (ATN) differed significantly from other hypothalamic nuclei in that it expressed only two neuropeptides, *tac1* and *tac3a*, and contained only glutamatergic neurons (Figs. 13–16), which is a feature similar to the mammalian ventromedial hypothalamic nucleus (VMN) (see 3.3). Furthermore, the central (CIL) and diffuse (DIL) nucleus of the inferior lobe differed from other hypothalamic nuclei in the

413 predominance of glutamatergic neurons and low neuropeptide expression (Figs. 14–20). A large
414 part of the inferior lobe, including the CIL and DIL, has recently been shown to be of
415 mesencephalic origin, and it has been debated whether these nuclei should be included or
416 excluded from the hypothalamus (Bloch *et al*., 2019). Our results also suggest that these nuclei
417 differ significantly from other hypothalamic nuclei in terms of neuropeptide expression profiles.
418
419 3.1.2.3 Prethalamus
420 Prethalamus contains four nuclei (Figs. 10–13, Table 5). The rostrolateral nucleus (R) did not
421 express any of the neuropeptides examined (Fig. 11). The intermediate thalamic nucleus (I),
422 ventromedial thalamic nucleus (VM), and ventrolateral thalamic nucleus (VL) are located adjacent
423 to each other; however, their neuropeptide expression patterns differed. The three nuclei
424 commonly expressed *cart3b* and *penka* (Figs. 10–13). VM expressed seven neuropeptides, among
425 which *npb*, *pdyn*, *vipa*, and *vipb* were present only in the VM among the prethalamic nuclei (Figs.
426 1–13). The VL had a higher percentage of glutamatergic neurons than the VM, and *cckb*
427 expression was specific to the VL in the prethalamus (Figs. 11–13). *npy* and *penkb* were expressed
428 only in I among the prethalamic nuclei (Fig. 11).
429
430 3.1.2.4 Posterior tuberculum
431 The periventricular nucleus of the posterior tuberculum (TPp), PVO, and PTN in the posterior
432 tuberculum, which are known to contain many dopaminergic neurons (Rink and Wullimann,
433 2001), also showed expression of multiple neuropeptides (Figs. 13–18, Table 5). Of note is the
434 specific expression of *calca* only in the TPp of the forebrain (Figs. 14 and 15). Furthermore, *hcrt*
435 and *npvf* were selectively localized to the PVO in the forebrain (Figs. 14 and 15).
436
437 3.1.2.5 Thalamus
438 The zebrafish thalamus has seven nuclei (Figs. 10–14, Table 5). The habenula is a conserved
439 structure among all vertebrates. In teleost, it is divided into the dorsal (Had) and ventral (Hav)
440 habenular nuclei, which differ in neuronal connectivity and function (Okamoto *et al*., 2012).
441 Neuropeptide expression also differed between Had and Hav, with *kiss1* being the only
442 neuropeptide expressed in Hav (Figs. 11–13). Had can be further divided into Hadm and Hadl
443 (Agetsuma *et al*., 2010). *pdyn* and *tac1* were expressed in Hadl, whereas *tac3a* was expressed in
444 Hadm (Figs. 11 and 12). The dorsal posterior thalamic nucleus (DP) was the only nucleus other
445 than the hypothalamus expressing *asip2b* (Fig. 16). IC was recently defined based on its high
446 expression in *gad* (Mueller and Guo, 2009). Strong expression of *npy* and *penkb* also
447 distinguished this nucleus from other adjacent nuclei (Figs. 14,15).
448

3.1.2.6 Preglomerular complex

Neuropeptide expression was low in the nuclei of the preglomerular complex. The subglomerular nucleus (SG), tertiary gustatory nucleus (TGN), anterior preglomerular nucleus (PGa), caudal preglomerular nucleus (PGc), and corpus mamillare (CM) did not express any of the neuropeptides examined (Figs. 13–20, Table 6). In contrast, a few neuropeptides were expressed in the lateral (PGl) and medial (PGm) preglomerular nuclei. In addition, the torus lateralis (TLa) and posterior thalamic nucleus (P) only expressed *tac1* (Figs. 15–17).

3.1.2.7 Pretectum

Neuropeptide expression was low in the nuclei of the pretectum (Figs. 10–14, Table 6). The dorsal accessory optic nucleus (DAO), magnocellular superficial pretectal nucleus (PSm), posterior pretectal nucleus (PO), and ventral accessory optic nucleus (VAO) did not express the examined neuropeptides. The remaining nuclei expressed neuropeptides with distinct expression patterns.

3.1.3 Individual differences

For each gene, we examined the brains of 3–5 male zebrafish and found that, in most cases, the expression patterns were consistent across individuals (although not compared quantitatively). Therefore, only representative pictures from one fish for each gene are shown in Figs. 2–20. In contrast, several genes showed marked individual differences in their expression patterns, as summarized in Supplemental Fig. 2. *asip2b* in PPa was observed in only two of the four fish examined. *asip2b* in DP was also observed in only two of the four fish examined. As *asip2b* (also referred to as *agrp2*) has been reported to play a role in background adaptation (Zhang *et al*., 2010) and stress axis regulation (Shainer *et al*., 2019), these individual differences might be related to these factors. *agrp* was expressed in Hv in all four fish examined; however, the signal densities were highly variable. As for *pomca*, in four fish examined, one showed expression in both NLT and Hv, while the other three showed expression only in Hv. As *agrp* and *pomca* expression is known to be regulated by feeding conditions (*agrp*, Jeong *et al*., 2018; *pomca*, Löhr *et al*., 2018), these individual differences may reflect different feeding statuses.

3.2 Novel subnuclei revealed by neuropeptide expressions in the hypothalamus

Neuropeptides are prominently expressed in the hypothalamus and are, therefore, considered to play crucial roles in various hypothalamic functions. Our scrutinized observation of the expression patterns of neuropeptides in the hypothalamus revealed that within some of the hypothalamic nuclei defined by the zebrafish brain atlas (Wullimann *et al*., 1996), there were distinct subnuclei that were not annotated, but displayed distinct neuropeptide expression patterns.

3.2.1 Subnclei in the PPa

The anterior part of the parvocellular preoptic nucleus (PPa) is a relatively large region covering all of the anterior preoptic area (Fig. 21 A). Within the PPa, there is a dense mass of neurons in the dorsal part of the PPa (PPad), segregated from the ventral part of the PPa (PPav) by a thin blank area with few neurons (Fig. 21, see the counterstain). The expressions of four neuropeptides (*cart5*, *ccka*, *gnrh3*, and *vipa*) were restricted to the PPav (Figs. 7-9), as represented by *ccka* and *vipa* in Fig. 21. Although other neuropeptides present in PPa were expressed in both PPad and PPav, their expression patterns were not uniform: some genes such as *gal* and *pdyn* were expressed predominantly in PPav, while others such as *cart3b* and *tac3a* were expressed more prominently in PPad (Fig. 21). In addition, glutamatergic neurons were mostly restricted to PPav, whereas GABAergic neurons were present in both PPad and PPav (Fig. 21). Thus, based on the expression patterns of neuropeptides/neurotransmitters, we proposed two subnuclei in PPa: PPad and PPav.

3.2.2 Subnuclei in Hv

The ventral zone of the periventricular hypothalamus (Hv) is a layer of neurons surrounding the diencephalic ventricle (DiV) (Fig. 22). Most of the neuropeptides present in the Hv (*agrp*, *asip2b*, *cart3b*, *cart3a*, *ccka*, *crha*, *crhb*, *gnrh3*, *npb*, *penka*, *penkb*, *pomcb*, and *vipa*) were expressed exclusively in the ventral part of Hv (Hvv) (Figs. 12-16), as represented by *agrp* and *ccka* in Fig. 22. In contrast, four genes (*pdyn*, *tac1*, *tac3a*, and *tac3b*) were expressed in the dorsal part of Hv (Hvd) (Figs. 12-16) as represented by *pdyn* and *tac3a* in Fig. 22. The boundary between Hvd and Hvv was clearly defined by *pdyn* expression (Fig. 22 B). The genes expressed in Hvd and Hvv were mutually exclusive, with only one exception: *cckb*, which was highly expressed in Hvv and slightly expressed in the anterior part of Hvd (Fig. 22). Hvd and Hvv also differed in neurotransmitter expression patterns: Hvd contained mostly glutamatergic neurons, whereas Hvv harbored mostly GABAergic neurons (Fig. 22). Thus, we propose that Hv can be divided into two subnuclei, Hvd and Hvv.

3.2.3 Subnuclei in Hd

The dorsal zone of the periventricular hypothalamus (Hd) is the nucleus that surrounds the lateral recess of the DiV (LR). At the anterior end (Fig. 14; plane 13 in Fig. 23), Hd was located medially, adjacent to Hv. More posteriorly (Fig. 15; plane 14 in Fig. 23), Hd extends laterally and continues caudally through the lateral part of the hypothalamus. The expression of neuropeptide genes differed along the mediolateral and dorsoventral axes of Hd (Fig. 23). Genes such as *cart5*, *gal*, *kiss2*, *npy*, *pdyn,* and *tac3a* were expressed only in the medial part of Hd (Hdm) (Figs. 14 and 15), as represented by *npy* in Fig. 23. Within the lateral part of the Hd, the neuropeptide expression patterns were different between the dorsal and ventral parts. *cart3b* and *npb* were only expressed

521 in the dorsolateral part of Hd (Hddl) (Figs. 15–20), as represented by *cart3b* in Fig. 23. In contrast,
522 *ccka*, *cckb,* and *pomcb* were only expressed in the ventrolateral part of Hd (Hdvl) (Figs. 15–20),
523 as represented by *ccka* in Fig. 23. The boundary between Hddl and Hdvl is clearly delineated by
524 *ccka* expression. Five genes (*galp*, *penka*, *penkb*, *tac1*, and *vipa*) were expressed in all the Hd
525 subnuclei (Figs. 14–20), as shown by *penka* and *vipa* in Fig. 23. Both Hddl and Hdvl were *gad*-
526 positive but not *vglut*-positive (Fig. 23), whereas Hdm harbored both *gad*-positive and *vglut*-
527 positive neurons. Altogether, we propose that Hd be divided into three subnuclei: Hdm, Hddl, and
528 Hdvl.
529
530 **3.2.4 Subnuclei in Hc**
531 The caudal zone of the periventricular hypothalamus (Hc) refers to the hypothalamic area caudal
532 to the Hv and LH (Fig. 24). The anterior part of Hc is not recessed, while the posterior part
533 surrounds the posterior recess (PR) (Fig. 24). The expression of neuropeptides in the anterior and
534 posterior parts of Hc was distinct. In the anterior part of Hc, seven genes (*cart5*, *kiss2*, *npy*, *penkb*,
535 *pmchl*, *pomcb*, and *tac3a*) were expressed only in the dorsal part (the anterodorsal part of Hc,
536 Hcad) (Figs. 17, 18), as shown by *pomcb* and *tac3a* in Fig. 24. Underneath Hcad, there was a
537 triangular dense cell population (the anterointermediate part of Hc, Hcai). *npb* and *cckb* were
538 expressed only in Hcai (Figs. 17 and 18), as represented by *npb* in Fig. 24. *agrp* and *tac1* were
539 expressed in the ventral cell population (the anteroventral part of Hc, Hcav).
540 The posterior part of the Hc was divided into three regions. The dense neuronal population
541 surrounding the PR was divided into two parts: the subregion dorsal to the PR
542 (posterointermediate part of the Hc, Hcpi) and the subregion ventral to the PR (posteroventral
543 part of the Hc, Hcpv). Dorsal to the Hcpi is another subregion with a lower cell density (the
544 posterodorsal part of Hc, Hcpd). These three subregions showed different neuropeptide
545 expression patterns. Nine genes were expressed only in Hcpd (*cart5*, *gal*, *kiss2*, *npy*, *pdyn*, *pomcb*,
546 *tac1*, *tac3a*, and *vipa*) (Fig. 19), as represented by *pomcb*, *tac3a,* and *pdyn* in Fig. 24. Three genes
547 were expressed in Hcpd and Hcpv (*cart3b*, *cckb*, and *penkb*) (Fig. 19), as represented by *cckb* and
548 *penkb* in Fig. 24. Four genes (*ccka*, *crhb*, *npb*, and *penka*) were expressed in all three regions (Fig.
549 19), as shown by *penka* and *npb* in Fig. 24. Genes expressed in both Hcpi and Hcpv were not
550 uniformly expressed; all neuropeptides, except *penka*, were more abundantly expressed in Hcpv.
551 *vglut* was expressed in Hcpd and in some cells in Hcpv, but not in Hcpi. *gad* was expressed in all
552 three subregions. Thus, based on the differential expression patterns of neuropeptide genes, we
553 propose the delineation of six subnuclei in the Hc: Hcad, Hcai, Hcav, Hcpd, Hcpi, and Hcpv.
554
555 **3.3 Comparison of neuropeptide expressions in the hypothalamus between zebrafish and**
556 **mouse**

Using the data obtained in this study, we next compared the neuropeptide expression profiles in the hypothalamic nuclei of zebrafish and mice (Fig. 25). Hypothalamic nuclei were hierarchically clustered based on the expression matrix. Several hypothalamic nuclei in zebrafish were highly clustered with those in mice and showed high similarity in neuropeptide expression. These include the zebrafish Hvd/ATN with mouse ventromedial hypothalamic nucleus (VMN), zebrafish PPav/PM with mouse paraventricular hypothalamic nucleus (Pa), and zebrafish PPp with mouse medial preoptic area (MPA) (Fig. 25). Both zebrafish Hvd and ATN had only one gene that differed from the mouse VMN in either the presence (*Cck*) or absence (*Pdyn*) of expression, respectively. The expression profiles of zebrafish PM and mouse Pa were mostly identical, except that *Galp* and *Vip* were expressed only in zebrafish PM. The zebrafish PPav also showed similar expression patterns to the mouse Pa, but *Vip* and *Pomc* were only expressed in the PPav. In addition, zebrafish PPav contained high numbers of both GABAergic and glutamatergic neurons, whereas mouse Pa predominantly contained glutamatergic neurons. The zebrafish PPp and mouse MPA were also remarkably similar, except that *Vip* and *Gal* were expressed only in mouse MPA.

4. Discussion

4.1 Mapping of neuropeptide and neurotransmitter marker gene expressions

The expression of 38 neuropeptide genes and GABAergic/glutamatergic neuron marker genes was mapped throughout the adult zebrafish forebrain. This study presents the first large-scale atlas and database of gene expression in the adult zebrafish brain. Although expression of some of the neuropeptide genes was previously analyzed by *in situ* hybridization in the adult zebrafish brain in separate papers (*agrp*, Jeong *et al*., 2018; *avp*, Carreño Gutiérrez *et al*., 2019; *cart2b*, Akash *et al*., 2014; *cart3a*, Akash *et al*., 2014; *cart3b*, Akash *et al*., 2014; *cart5* Akash *et al*., 2014; *crha*, Grone, and Maruska, 2015; *crhb*, Alderman and Bernier, 2007; *gnrh2*, Steven *et al*., 2003; *gnrh3*, Steven *et al*., 2003; *kiss1*, Servili *et al*., 2011; *kiss2*, Servili *et al*., 2011; *hcrt*, Appelbaum *et al*., 2009; *npy*, Jeong *et al*., 2018; *npvf*, Marvel *et al*., 2018; *oxt*, Carreño Gutiérrez *et al*., 2019; *pmch*, Berman *et al*., 2009; *pmchl*, Berman *et al*., 2009; *tac1*, Ogawa *et al*., 2012; *tac3a*, Biran *et al*., 2012; Ogawa *et al*., 2012; *tac3b*, Ogawa *et al*., 2012; *gad1b*, Mueller *et al*., 2009), it has been difficult to perform detailed comparisons due to different sampling and staining conditions. The gene expression atlas is a fundamental resource in neuroscience research and will be useful for a variety of studies, including searching for marker genes for groups of neurons of interest, detailed anatomical analysis of each brain area, and investigation of homologous relationships with other species. The latter two points were further analyzed in the present study.

4.2 Subnuclei in the hypothalamus

The hypothalamus plays an important role as a central hub that controls the autonomic nervous system, endocrine system, and behavior. However, in teleost species, the anatomical relevance of the hypothalamic nuclei to these functions remains unclear. In addition, the definition of nuclei in the zebrafish hypothalamus is relatively simple compared with that in mammals. The zebrafish hypothalamus is divided into ten nuclei in the commonly used brain atlas (Wullimann *et al*., 1996), whereas the mouse hypothalamus is divided into over 25 nuclei in the brain atlas (Paxinos and Franklin, 2019). In this study, we attempted to define "subnuclei" in the zebrafish hypothalamus based on differential neuropeptide and neurotransmitter expression profiles to further increase the resolution of the current atlas. The information on these subnuclei defined in the present study will provide useful insights for future research on the neuroanatomy and functions of the teleost hypothalamus.

4.2.1 The anterior part of the parvocellular preoptic nucleus (PPa)

The neuropeptide expression patterns revealed that PPa could be clearly divided into two subnuclei: PPad and PPav. Previous studies have shown that the expression of genes other than

607 neuropeptides is also different between PPad and PPav, although differences in preparation make
608 it difficult to compare the exact location of expression. Estrogen receptor 1 (*esr1*) and androgen
609 receptor (*ar*) are mostly expressed in PPav (*esr1*, Diotel *et al*., 2011; *ar*, Gorelick *et al*., 2008).
610 Cholinergic neurons are predominantly localized to PPad (Clemente *et al.*, 2004), whereas
611 dopaminergic neurons are confined to PPav (Yamamoto *et al*., 2011). Orthopedia a (*otpa*), a
612 transcription factor important for the development of neurosecretory neurons, is also found only
613 in PPav (Eugenin von Bernhardi *et al*., 2022). Recently, PPa has been shown to be critical in
614 zebrafish social behavior (Nunes *et al*., 2021; Tallafus *et al*., 2022). PPad and PPav may play
615 different roles in social information processing.
616
617 **4.2.2 The ventral zone of the periventricular hypothalamus (Hv)**
618 Based on the expression patterns of neurotransmitters and neuropeptides, we proposed two
619 subnuclei in Hv: Hvd and Hvv. The Hv in the teleost (also referred to as the NLTv, ventral part of
620 the lateral tuberal nucleus) is considered homologous to the mammalian arcuate nucleus (Arc),
621 based on the shared expression of feeding-related neuropeptide genes such as *agrp*, *pomc*, *npy*,
622 and *cart* (Forlano and Cone, 2007; Soengas *et al*., 2018). All of these feeding-related
623 neuropeptides are expressed exclusively in the Hvv, but not in the Hvd, suggesting a role for Hvv
624 in the control of feeding. However, as Hvv also expresses many other neuropeptide genes, such
625 as *ccka*/*b*, *crha*/*b*, *npb*, *penka*/*b*, and *vipa*, its function may not be confined to feeding. The
626 function of Hvd is not yet clear, but its neuropeptide and neurotransmitter expression patterns
627 resemble those of the mammalian ventromedial hypothalamus (VMN), as discussed below.
628
629 **4.2.3 The dorsal zone of the periventricular hypothalamus (Hd)**
630 Hd can be divided into three subnuclei, Hdm, Hddl, and Hdvl. Recently, it has been reported that
631 Hd is activated by feeding behavior (Wee *et al*., 2018) or food-related cues (Muto *et al*., 2017;
632 Wakisaka *et al*., 2017). However, the subnuclei in the Hd that are activated and the types of
633 neurons that are involved in feeding remain unknown. The neuropeptide expression atlas of the
634 present study will help to clarify these important questions in the near future.
635
636 **4.2.4 The caudal zone of the periventricular hypothalamus (Hc)**
637 Our analysis revealed that Hc could be divided into six subnuclei: Hcad, Hcai, Hcav, Hcpd, Hcpi,
638 and Hcpv. The posterior region of the Hc has been reported to harbor cerebrospinal fluid (CSF)-
639 contacting cells that produce dopamine and/or serotonin, which are absent in mammals (Xavier
640 *et al*., 2017). Within the posterior part of the Hc, the expression patterns of neuropeptides varied
641 greatly among the three subnuclei, suggesting that CSF-contacting cells in the posterior Hc are
642 not uniform. In addition, the colocalization of two dopamine-synthesizing enzymes, tyrosine

643 hydroxylase (TH) 1 and TH 2, is confined to Hcpv (Yamamoto *et al*., 2011). Functional
644 differences in the Hc subnuclei have also been suggested in a previous study with larval zebrafish,
645 in which the Hcpi and Hcpv responded differently to the visual cues of conspecifics (Tunbak *et*
646 *al*., 2020). These regions might play distinct roles in the social reward system.
647
648 **4.3 Comparison of hypothalamic neuropeptide expression patterns between zebrafish and**
649 **mouse**
650 To date, few studies have compared the structure of the teleost hypothalamus with that of other
651 vertebrate lineages, and homologous relationships are not yet clear. In the present study, a
652 comprehensive and detailed examination of the expression profiles of a large number of
653 neuropeptides and neurotransmitters in the same preparation enabled us to conduct the most
654 extensive comparison of the hypothalamic nuclei between zebrafish and mice to date and to reveal
655 several similarities across species.
656
657 **4.3.1 Zebrafish magnocellular preoptic nucleus (PM), ventral part of the anterior part of**
658 **the parvocellular preoptic nucleus (PPav), and mouse paraventricular hypothalamic**
659 **nucleus (Pa)**
660 It has been considered that the teleost PM and mammalian Pa are homologous because they share
661 many features, such as the existence of avp- and oxt-expressing magnocellular neurons (Goodson
662 and Bass, 2001; Olivereau and Olivereau, 1988), innervation by *agrp* and *pomc* neurons (Forlano
663 and Cone, 2007), and the expression of other marker genes such as *nurr1* (Kapsimali *et al*., 2001),
664 *ccka*, *nts*, *penka*, *penkb*, *vipa*, *sst1.1*, *crhb*, and *otpa* (Herget *et al*., 2014). Consistently, the
665 zebrafish PM was clustered closest to the mouse Pa in our analysis. Fifteen of the 17 neuropeptide
666 genes examined showed identical expression status between PM and Pa, suggesting highly similar
667 functionality. Zebrafish PPav also clustered close to the PM. In the brain atlases of some fish
668 species, a portion of the PM is considered to be parvocellular, referred to as the parvocellular part
669 of the PM (PMp), and it is located anteriorly adjacent to the magnocellular part of the PM (PMm)
670 (medaka, Anken and Bourrat, 1998; goldfish, Braford and Northcutt, 1983). In the zebrafish
671 reference atlas (Wullimann *et al*., 1996) it was considered that the PMp was not evident; however,
672 the PPav may correspond to the PMp in other fish species and is closely related to the PM, and
673 therefore, highly similar to Pa in mice.
674
675 **4.3.2 Zebrafish posterior part of the parvocellular preoptic nucleus (PPp) and mouse medial**
676 **preoptic area (MPA)**
677 Zebrafish PPp clustered close to the mouse MPA. The preoptic area as a whole is thought to be
678 conserved between teleosts and mammals (Goodson, 2005; O'Connell and Hofmann, 2011);

679 however, the homology of specific nuclei or regions within the preoptic area has not been
680 discussed. The fact that the neuropeptide expression profile of zebrafish PPp was particularly
681 similar to that of mouse MPA may indicate functional similarities between these nuclei. In
682 mammals, MPA harbors estrogen receptor (*Esr1*)-expressing neurons, which play important roles
683 in sexual and parental behaviors (Tsuneoka and Funato, 2021). Although there is no available data
684 on the presence or absence of *esr1* in zebrafish PPp, *esr1* expression in the PPp has been reported
685 in several fish species, such as Atlantic croaker, sea bass, and medaka (Hawkins *et al*., 2005;
686 Muriach *et al*., 2008; Zempo *et al*., 2013). In contrast, *esr1* is also expressed in the PPa and PM
687 in many teleost species. Furthermore, galanin, which is excessively expressed in mammalian MPA
688 (Wu *et al*., 2014; Moffitt *et al*., 2018), was not observed in zebrafish PPp but was abundantly
689 expressed in PPa. Collectively, it may be more accurate to consider that many of the cell
690 populations corresponding to mammalian MPA are located in the PPp in zebrafish, but some are
691 also distributed in other regions of the preoptic area.
692
693 **4.3.3 Zebrafish dorsal subnucleus of the ventral zone of periventricular hypothalamus**
694 **(Hvd), anterior tuberal nucleus (ATN), and mouse ventromedial hypothalamic nucleus**
695 **(VMN)**
696 Zebrafish Hvd and ATN clustered close to the mouse VMN. The similarity between the ATN and
697 VMN has been proposed previously, based on the location, expression of *esr1*, and connections
698 with the preoptic area (Forlano *et al*., 2005; Goodson, 2005; Forlano and Bass, 2011; O'Connell
699 and Hofmann, 2011). Hvd is located medially adjacent to the ATN, but the homologous
700 relationship between Hvd and VMN has not been reported so far. Our data showed that *pdyn*,
701 which is present predominantly in the dorsomedial part of the mouse VMN (Allen Brain Atlas),
702 is strongly expressed in zebrafish Hvd but not in ATN, indicating that Hvd and ATN might
703 represent homologs of different subnuclei in the VMN. Other genes that are highly expressed in
704 the mouse VMN include *Adcyap* and *Bdnf* (Khodai *et al*., 2021). *adcyap1b* is expressed in both
705 the Hvd and ATN in teleosts (Nakamachi *et al*., 2018), and *bndf* is predominantly expressed in
706 the Hvd(Cacialli *et al*., 2016).
707 Mammalian VMN controls a variety of behavioral and physiological traits such as sexual
708 behavior, aggression, fear response, and energy homeostasis (Silva *et al*., 2013; Hashikawa *et al*.,
709 2017; Khodai *et al*., 2021). In teleost fishes, it has been reported that the Hv and ATN in males
710 are activated by the courtship of competing males (midshipman fish, Petersen *et al*., 2013), and
711 during the change from subordinate to dominant (cichlid fish, Maruska *et al*., 2013), suggesting
712 a role for Hv and ATN in social information processing. Further studies are required to elucidate
713 the degree to which mammalian VMN and fish Hvd/ATN functions are conserved.
714

5. Conclusion

We constructed an expression atlas and database of 38 neuropeptide genes and GABAergic/glutamatergic neuron marker genes in the forebrain of adult zebrafish. By utilizing these data, we proposed subnuclei in the hypothalamic nuclei and compared them with mouse hypothalamic nuclei. This atlas and database will become a useful resource that will contribute to the future development of neuroscience research using zebrafish.

References

1. Agetsuma, M., Aizawa, H., Aoki, T., Nakayama, R., Takahoko, M., Goto, M., Sassa, T., Amo, R., Shiraki, T., Kawakami, K., Hosoya, T., Higashijima, S., & Okamoto, H. (2010). The habenula is crucial for experience-dependent modification of fear responses in zebrafish. *Nature neuroscience*, 13, 1354–1356.
2. Alderman, S. L., & Bernier, N. J. (2007). Localization of corticotropin-releasing factor, urotensin I, and CRF-binding protein gene expression in the brain of the zebrafish, Danio rerio. *The Journal of comparative neurology*, 502, 783–793.
3. Allan, C., Burel, J. M., Moore, J., Blackburn, C., Linkert, M., Loynton, S., Macdonald, D., Moore, W. J., Neves, C., Patterson, A., Porter, M., Tarkowska, A., Loranger, B., Avondo, J., Lagerstedt, I., Lianas, L., Leo, S., Hands, K., Hay, R. T., Patwardhan, A., Best, C., Kleywegt, G. J., Zanetti, G., Swedlow, J. R. (2012). OMERO: flexible, model-driven data management for experimental biology. *Nature methods*, 9, 245–253.
4. Allen Mouse Brain Atlas. 2004 Allen Institute for Brain Science. Available from: https://mouse.brain-map.org/
5. Alon, T., Zhou, L., Pérez, C. A., Garfield, A. S., Friedman, J. M., & Heisler, L. K. (2009). Transgenic mice expressing green fluorescent protein under the control of the corticotropin-releasing hormone promoter. *Endocrinology*, 150, 5626–5632.
6. Anken, R., & Bourrat, F. (1998). Brain atlas of the medakafish: *Oryzias latipes*. Editions Quae
7. Appelbaum, L., Wang, G. X., Maro, G. S., Mori, R., Tovin, A., Marin, W., Yokogawa, T., Kawakami, K., Smith, S. J., Gothilf, Y., Mignot, E., & Mourrain, P. (2009). Sleep-wake regulation and hypocretin-melatonin interaction in zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 21942–21947.
8. Bassett, J. L., Shipley, M. T., & Foote, S. L. (1992). Localization of corticotropin-releasing factor-like immunoreactivity in monkey olfactory bulb and secondary olfactory areas. *The Journal of comparative neurology*, 316, 348–362.
9. Berman, J. R., Skariah, G., Maro, G. S., Mignot, E., & Mourrain, P. (2009). Characterization of two melanin-concentrating hormone genes in zebrafish reveals evolutionary and physiological links with the mammalian MCH system. The Journal of comparative neurology, 517, 695–710.
10. Biechl, D., Tietje, K., Ryu, S., Grothe, B., Gerlach, G., & Wullimann, M. F. (2017). Identification of accessory olfactory system and medial amygdala in the zebrafish. *Scientific reports*, 7, 44295.
11. Biran, J., Palevitch, O., Ben-Dor, S., & Levavi-Sivan, B. (2012). Neurokinin Bs and neurokinin B receptors in zebrafish-potential role in controlling fish reproduction.

Proceedings of the National Academy of Sciences of the United States of America, 109, 10269–10274.

12. Bloch, S., Thomas, M., Colin, I., Galant, S., Machado, E., Affaticati, P., Jenett, A., & Yamamoto, K. (2019). Mesencephalic origin of the inferior lobe in zebrafish. *BMC biology*, 17, 22.
13. Bojnik, E., Boynik, E., Corbani, M., Babos, F., Magyar, A., Borsodi, A., & Benyhe, S. (2011). Phylogenetic diversity and functional efficacy of the C-terminally expressed heptapeptide unit in the opioid precursor polypeptide proenkephalin A. *Neuroscience*, 178, 56–67.
14. Braford MR Jr, Northcutt RG. 1983. Organization of the diencephalon and pretectum of the ray-finned fishes. In: Fish Neurobiology (Northcutt RG, Davis RE, eds). vol 2. University of Michigan Press. p117-163.
15. Cacialli, P., Gueguen, M. M., Coumailleau, P., D'Angelo, L., Kah, O., Lucini, C., & Pellegrini, E. (2016). BDNF Expression in Larval and Adult Zebrafish Brain: Distribution and Cell Identification. *PloS one*, 11, e0158057.
16. Cai, G., Mo, C., Huang, L., Li, J., & Wang, Y. (2015). Characterization of the Two CART Genes (CART1 and CART2) in Chickens (Gallus gallus). *PloS one*, 10, e0127107.
17. Carreño Gutiérrez, H., Colanesi, S., Cooper, B., Reichmann, F., Young, A. M. J., Kelsh, R. N., & Norton, W. H. J. (2019). Endothelin neurotransmitter signalling controls zebrafish social behaviour. *Scientific reports*, 9, 3040.
18. Chou, M. Y., Amo, R., Kinoshita, M., Cherng, B. W., Shimazaki, H., Agetsuma, M., Shiraki, T., Aoki, T., Takahoko, M., Yamazaki, M., Higashijima, S., & Okamoto, H. (2016). Social conflict resolution regulated by two dorsal habenular subregions in zebrafish. *Science (New York, N.Y.)*, 352, 87–90.
19. Clarkson, J., d'Anglemont de Tassigny, X., Colledge, W. H., Caraty, A., & Herbison, A. E. (2009). Distribution of kisspeptin neurones in the adult female mouse brain. *Journal of neuroendocrinology*, 21, 673–682.
20. Clemente, D., Porteros, A., Weruaga, E., Alonso, J. R., Arenzana, F. J., Aijón, J., & Arévalo, R. (2004). Cholinergic elements in the zebrafish central nervous system: Histochemical and immunohistochemical analysis. *The Journal of comparative neurology*, 474, 75–107.
21. Cowley, M. A., Smart, J. L., Rubinstein, M., Cerdán, M. G., Diano, S., Horvath, T. L., Cone, R. D., & Low, M. J. (2001). Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*, 411, 480–484.
22. Croizier, S., Franchi-Bernard, G., Colard, C., Poncet, F., La Roche, A., & Risold, P. Y. (2010). A comparative analysis shows morphofunctional differences between the rat and mouse melanin-concentrating hormone systems. *PloS one*, 5, e15471.
23. Deem, J. D., Faber, C. L., & Morton, G. J. (2022). AgRP neurons: Regulators of feeding,

energy expenditure, and behavior. *The FEBS journal*, 289, 2362–2381.

24. Diniz, G. B., Battagello, D. S., Cherubini, P. M., Reyes-Mendoza, J. D., Luna-Illades, C., Klein, M. O., Motta-Teixeira, L. C., Sita, L. V., Miranda-Anaya, M., Morales, T., & Bittencourt, J. C. (2019). Melanin-concentrating hormone peptidergic system: Comparative morphology between muroid species. *The Journal of comparative neurology*, 527, 2973–3001.
25. Diotel, N., Do Rego, J. L., Anglade, I., Vaillant, C., Pellegrini, E., Vaudry, H., & Kah, O. (2011). The brain of teleost fish, a source, and a target of sexual steroids. *Frontiers in neuroscience*, 5, 137.
26. Duarte, C. R., Schütz, B., & Zimmer, A. (2006). Incongruent pattern of neurokinin B expression in rat and mouse brains. *Cell and tissue research*, 323, 43–51.
27. Dupré, D., & Tostivint, H. (2014). Evolution of the gastrin-cholecystokinin gene family revealed by synteny analysis. *General and comparative endocrinology*, 195, 164–173.
28. Elphick, M. R., Mirabeau, O., & Larhammar, D. (2018). Evolution of neuropeptide signalling systems. *The Journal of experimental biology*, 221, jeb151092.
29. Eugenin von Bernhardi, J., Biechl, D., Miek, L., Herget, U., Ryu, S., & Wullimann, M. F. (2022). A versatile transcription factor: Multiple roles of orthopedia a (otpa) beyond its restricted localization in dopaminergic systems of developing and adult zebrafish (Danio rerio) brains. *The Journal of comparative neurology*, 530, 2537–2561.
30. Fonseka, T. M., Wen, X. Y., Foster, J. A., & Kennedy, S. H. (2016). Zebrafish models of major depressive disorders. *Journal of neuroscience research*, 94, 3–14.
31. Fontana BD, Gibbon AJ, Cleal M, et al. Moderate early life stress improves adult zebrafish (*Danio rerio*) working memory but does not affect social and anxiety-like responses. Dev Psychobiol. 2021;63(1):54-64.
32. Forlano, P. M., & Cone, R. D. (2007). Conserved neurochemical pathways involved in hypothalamic control of energy homeostasis. *The Journal of comparative neurology*, 505, 235–248.
33. Förster, D., Arnold-Ammer, I., Laurell, E., Barker, A. J., Fernandes, A. M., Finger-Baier, K., Filosa, A., Helmbrecht, T. O., Kölsch, Y., Kühn, E., Robles, E., Slanchev, K., Thiele, T. R., Baier, H., & Kubo, F. (2017). Genetic targeting and anatomical registration of neuronal populations in the zebrafish brain with a new set of BAC transgenic tools. *Scientific reports*, 7, 5230.
34. Ganz, J., Kroehne, V., Freudenreich, D., Machate, A., Geffarth, M., Braasch, I., Kaslin, J., & Brand, M. (2014). Subdivisions of the adult zebrafish pallium based on molecular marker analysis. *F1000Research*, 3, 308.
35. Gerlai R. (2020). Evolutionary conservation, translational relevance and cognitive function:

The future of zebrafish in behavioral neuroscience. *Neuroscience and biobehavioral reviews*, 116, 426–435.

36. Goodson, J. L., & Bass, A. H. (2001). Social behavior functions and related anatomical characteristics of vasotocin/vasopressin systems in vertebrates. Brain research. *Brain research reviews*, 35, 246–265.
37. Goodson J. L. (2005). The vertebrate social behavior network: evolutionary themes and variations. *Hormones and behavior*, 48, 11–22.
38. Gorelick, D. A., Watson, W., & Halpern, M. E. (2008). Androgen receptor gene expression in the developing and adult zebrafish brain. *Developmental dynamics : an official publication of the American Association of Anatomists*, 237, 2987–2995.
39. Gospodarska, E., Kozak, L. P., & Jaroslawska, J. (2019). Isolation and identification of endogenous RFamide-related peptides 1 and 3 in the mouse hypothalamus. *Journal of neuroendocrinology*, 31, e12668.
40. Grone, B. P., & Maruska, K. P. (2015). Divergent evolution of two corticotropin-releasing hormone (CRH) genes in teleost fishes. *Frontiers in neuroscience*, 9, 365.
41. Hashikawa, Y., Hashikawa, K., Falkner, A. L., & Lin, D. (2017). Ventromedial Hypothalamus and the Generation of Aggression. *Frontiers in systems neuroscience*, 11, 94.
42. Hawkins, M. B., Godwin, J., Crews, D., & Thomas, P. (2005). The distributions of the duplicate oestrogen receptors ER-beta a and ER-beta b in the forebrain of the Atlantic croaker (Micropogonias undulatus): evidence for subfunctionalization after gene duplication. *Proceedings. Biological sciences*, 272, 633–641.
43. Herget, U., Wolf, A., Wullimann, M. F., & Ryu, S. (2014). Molecular neuroanatomy and chemoarchitecture of the neurosecretory preoptic-hypothalamic area in zebrafish larvae. *The Journal of comparative neurology*, 522, 1542–1564.
44. Higashijima, S., Mandel, G., & Fetcho, J. R. (2004). Distribution of prospective glutamatergic, glycinergic, and GABAergic neurons in embryonic and larval zebrafish. *The Journal of comparative neurology*, 480, 1–18.
45. Hökfelt, T., Broberger, C., Xu, Z. Q., Sergeyev, V., Ubink, R., & Diez, M. (2000). Neuropeptides--an overview. *Neuropharmacology*, 39, 1337–1356.
46. Holmgren, S., & Jensen, J. (2001). Evolution of vertebrate neuropeptides. *Brain research bulletin*, 55, 723–735.
47. Jaillon, O., Aury, J. M., Brunet, F., Petit, J. L., Stange-Thomann, N., Mauceli, E., Bouneau, L., Fischer, C., Ozouf-Costaz, C., Bernot, A., Nicaud, S., Jaffe, D., Fisher, S., Lutfalla, G., Dossat, C., Segurens, B., Dasilva, C., Salanoubat, M., Levy, M., Boudet, N., … Roest Crollius, H. (2004). Genome duplication in the teleost fish Tetraodon nigroviridis reveals the early vertebrate proto-karyotype. *Nature*, 431, 946–957.

48. Jeong, I., Kim, E., Kim, S., Kim, H. K., Lee, D. W., Seong, J. Y., & Park, H. C. (2018). mRNA expression and metabolic regulation of npy and agrp1/2 in the zebrafish brain. *Neuroscience letters*, 668, 73–79.
49. Juréus, A., Cunningham, M. J., Li, D., Johnson, L. L., Krasnow, S. M., Teklemichael, D. N., Clifton, D. K., & Steiner, R. A. (2001). Distribution and regulation of galanin-like peptide (GALP) in the hypothalamus of the mouse. *Endocrinology*, 142, 5140–5144.
50. Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in pharmacological sciences*, 35, 63–75.
51. Kapsimali, M., Bourrat, F., & Vernier, P. (2001). Distribution of the orphan nuclear receptor Nurr1 in medaka (Oryzias latipes): cues to the definition of homologous cell groups in the vertebrate brain. *The Journal of comparative neurology*, 431, 276–292.
52. Kassahn, K. S., Dang, V. T., Wilkins, S. J., Perkins, A. C., & Ragan, M. A. (2009). Evolution of gene function and regulatory control after whole-genome duplication: comparative analyses in vertebrates. *Genome research*, 19, 1404–1418.
53. Karoubi, N., Segev, R., & Wullimann, M. F. (2016). The Brain of the Archerfish Toxotes chatareus: A Nissl-Based Neuroanatomical Atlas and Catecholaminergic/Cholinergic Systems. *Frontiers in neuroanatomy*, 10, 106.
54. Keegan, C. E., Herman, J. P., Karolyi, I. J., O'Shea, K. S., Camper, S. A., & Seasholtz, A. F. (1994). Differential expression of corticotropin-releasing hormone in developing mouse embryos and adult brain. *Endocrinology*, 134, 2547–2555.
55. Kenney, J. W., Steadman, P. E., Young, O., Shi, M. T., Polanco, M., Dubaishi, S., Covert, K., Mueller, T., & Frankland, P. W. (2021). A 3D adult zebrafish brain atlas (AZBA) for the digital age. *eLife*, 10, e69988.
56. Khodai T, Luckman SM. Ventromedial nucleus of the hypothalamus neurons under the magnifying glass. Endocrinology. 2021;162(10):bqab141.
57. Kim, J. H., Kromm, G. H., Barnhill, O. K., Sperber, J., Heuer, L. B., Loomis, S., Newman, M. C., Han, K., Gulamali, F. F., Legan, T. B., Jensen, K. E., Funderburk, S. C., Krashes, M. J., & Carter, M. E. (2022). A discrete parasubthalamic nucleus subpopulation plays a critical role in appetite suppression. *eLife*, 11, e75470.
58. Koide, T., Yabuki, Y., & Yoshihara, Y. (2018). Terminal Nerve GnRH3 Neurons Mediate Slow Avoidance of Carbon Dioxide in Larval Zebrafish. *Cell reports*, 22, 1115–1123.
59. Kumar, V., Singh, C., & Singh, A. (2021). Zebrafish an experimental model of Huntington's disease: molecular aspects, therapeutic targets and current challenges. *Molecular biology reports*, 48, 8181–8194.
60. Kunst, M., Laurell, E., Mokayes, N., Kramer, A., Kubo, F., Fernandes, A. M., Förster, D., Dal Maschio, M., & Baier, H. (2019). A Cellular-Resolution Atlas of the Larval Zebrafish

Brain. *Neuron*, 103, 21–38.e5.

61. Kurokawa, T., Suzuki, T., & Hashimoto, H. (2003). Identification of gastrin and multiple cholecystokinin genes in teleost. *Peptides*, 24, 227–235.
62. Lee, D. A., Oikonomou, G., & Prober, D. A. (2022). Large-scale Analysis of Sleep in Zebrafish. *Bio-protocol*, 12, e4313.
63. Lein, E. S., Hawrylycz, M. J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A. F., Boguski, M. S., Brockway, K. S., Byrnes, E. J., Chen, L., Chen, L., Chen, T. M., Chin, M. C., Chong, J., Crook, B. E., Czaplinska, A., Dang, C. N., Datta, S., Dee, N. R., … Jones, A. R. (2007). Genome-wide atlas of gene expression in the adult mouse brain. *Nature*, 445, 168–176.
64. Levitas-Djerbi, T., Yelin-Bekerman, L., Lerer-Goldshtein, T., & Appelbaum, L. (2015). Hypothalamic leptin-neurotensin-hypocretin neuronal networks in zebrafish. *The Journal of comparative neurology*, 523, 831–848.
65. Liu, N. A., Huang, H., Yang, Z., Herzog, W., Hammerschmidt, M., Lin, S., & Melmed, S. (2003). Pituitary corticotroph ontogeny and regulation in transgenic zebrafish. *Molecular endocrinology (Baltimore, Md.)*, 17, 959–966.
66. Löhr, H., Hess, S., Pereira, M. M. A., Reinoß, P., Leibold, S., Schenkel, C., Wunderlich, C. M., Kloppenburg, P., Brüning, J. C., & Hammerschmidt, M. (2018). Diet-Induced Growth Is Regulated via Acquired Leptin Resistance and Engages a Pomc-Somatostatin-Growth Hormone Circuit. *Cell reports*, 23, 1728–1741.
67. Loring, M. D., Thomson, E. E., & Naumann, E. A. (2020). Whole-brain interactions underlying zebrafish behavior. *Current opinion in neurobiology*, 65, 88–99.
68. MapZebrain (Max-Planck Zebrafish Brain Atlas), Available from: https://fishatlas.neuro.mpg.de/lines
69. Maruska, K. P., Zhang, A., Neboori, A., & Fernald, R. D. (2013). Social opportunity causes rapid transcriptional changes in the social behaviour network of the brain in an African cichlid fish. *Journal of neuroendocrinology*, 25, 145–157.
70. Marvel, M., Spicer, O. S., Wong, T. T., Zmora, N., & Zohar, Y. (2018). Knockout of the Gnrh genes in zebrafish: effects on reproduction and potential compensation by reproductive and feeding-related neuropeptides. *Biology of reproduction*, 99, 565–577.
71. Matsutani, S., Senba, E., & Tohyama, M. (1989). Distribution of neuropeptidelike immunoreactivities in the guinea pig olfactory bulb. The Journal of comparative neurology, 280(4), 577–586.
72. Menuet, A., Pellegrini, E., Anglade, I., Blaise, O., Laudet, V., Kah, O., & Pakdel, F. (2002). Molecular characterization of three estrogen receptor forms in zebrafish: binding characteristics, transactivation properties, and tissue distributions. *Biology of reproduction*,

66, 1881–1892.

73. Mitra, S. W., Hoskin, E., Yudkovitz, J., Pear, L., Wilkinson, H. A., Hayashi, S., Pfaff, D. W., Ogawa, S., Rohrer, S. P., Schaeffer, J. M., McEwen, B. S., & Alves, S. E. (2003). Immunolocalization of estrogen receptor β in the mouse brain: comparison with estrogen receptor α. *Endocrinology*, 144, 2055–2067.
74. Moffitt, J. R., Bambah-Mukku, D., Eichhorn, S. W., Vaughn, E., Shekhar, K., Perez, J. D., Rubinstein, N. D., Hao, J., Regev, A., Dulac, C., & Zhuang, X. (2018). Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. *Science (New York, N.Y.)*, 362(6416), eaau5324.
75. Mueller, T., & Guo, S. (2009). The distribution of GAD67-mRNA in the adult zebrafish (teleost) forebrain reveals a prosomeric pattern and suggests previously unidentified homologies to tetrapods. *The Journal of comparative neurology*, 516, 553–568.
76. Muriach, B., Cerdá-Reverter, J. M., Gómez, A., Zanuy, S., & Carrillo, M. (2008). Molecular characterization and central distribution of the estradiol receptor alpha (ER α) in the sea bass (Dicentrarchus labrax). *Journal of chemical neuroanatomy*, 35, 33–48.
77. Muto, A., Lal, P., Ailani, D., Abe, G., Itoh, M., & Kawakami, K. (2017). Activation of the hypothalamic feeding centre upon visual prey detection. *Nature communications*, 8, 15029.
78. Mathew, R. T., & Chang, C. F. (2022). Expression and Transcript Localization of *star*, *sf-1,* and *dax-1* in the Early Brain of the Orange-Spotted Grouper *Epinephelus coioides*. *International journal of molecular sciences*, 23, 2614.
79. Nakamachi, T., Kamata, E., Tanigawa, A., Konno, N., Shioda, S., & Matsuda, K. (2018). Distribution of pituitary adenylate cyclase-activating polypeptide 2 in zebrafish brain. *Peptides*, 103, 40–47.
80. Nam, B. H., Kim, Y. O., Kong, H. J., Kim, W. J., Lee, S. J., & Choi, T. J. (2009). Identification and characterization of the prepro-vasoactive intestinal peptide gene from the teleost Paralichthys olivaceus. *Veterinary immunology and immunopathology*, 127, 249–258.
81. Navarro, V. M., Bosch, M. A., León, S., Simavli, S., True, C., Pinilla, L., Carroll, R. S., Seminara, S. B., Tena-Sempere, M., Rønnekleiv, O. K., & Kaiser, U. B. (2015). The integrated hypothalamic tachykinin-kisspeptin system as a central coordinator for reproduction. *Endocrinology*, 156, 627–637.
82. Norton, W., & Bally-Cuif, L. (2010). Adult zebrafish as a model organism for behavioural genetics. *BMC neuroscience*, 11, 90.
83. Nunes, A. R., Gliksberg, M., Varela, S. A. M., Teles, M., Wircer, E., Blechman, J., Petri, G., Levkowitz, G., & Oliveira, R. F. (2021). Developmental Effects of Oxytocin Neurons on Social Affiliation and Processing of Social Information. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 41, 8742–8760.

84. O'Connell, L. A., & Hofmann, H. A. (2011). The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *The Journal of comparative neurology*, 519, 3599–3639.
85. Ogawa, S., Ramadasan, P. N., Goschorska, M., Anantharajah, A., Ng, K. W., & Parhar, I. S. (2012). Cloning and expression of tachykinins and their association with kisspeptins in the brains of zebrafish. *The Journal of comparative neurology*, 520, 2991–3012.
86. Okamoto, H., Agetsuma, M., & Aizawa, H. (2012). Genetic dissection of the zebrafish habenula, a possible switching board for selection of behavioral strategy to cope with fear and anxiety. *Developmental neurobiology*, 72, 386–394.
87. Okamoto, H., Cherng, B. W., Nakajo, H., Chou, M. Y., & Kinoshita, M. (2021). Habenula as the experience-dependent controlling switchboard of behavior and attention in social conflict and learning. *Current opinion in neurobiology*, 68, 36–43.
88. Oliveira R. F. (2013). Mind the fish: zebrafish as a model in cognitive social neuroscience. *Frontiers in neural circuits*, 7, 131.
89. Olivereau, M., & Olivereau, J. (1988). Localization of CRF-like immunoreactivity in the brain and pituitary of teleost fish. *Peptides*, 9(1), 13–21.
90. Olivares, J., & Schmachtenberg, O. (2019). An update on anatomy and function of the teleost olfactory system. *PeerJ*, 7, e7808.
91. Orger, M. B., & de Polavieja, G. G. (2017). Zebrafish Behavior: Opportunities and Challenges. *Annual review of neuroscience*, 40, 125–147.
92. Otero-García, M., Agustín-Pavón, C., Lanuza, E., & Martínez-García, F. (2016). Distribution of oxytocin and co-localization with arginine vasopressin in the brain of mice. *Brain structure & function*, 221, 3445–3473.
93. Paxinos G, Franklin K. Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates Academic Press, 2019
94. Petersen, C. L., Timothy, M., Kim, D. S., Bhandiwad, A. A., Mohr, R. A., Sisneros, J. A., & Forlano, P. M. (2013). Exposure to advertisement calls of reproductive competitors activates vocal-acoustic and catecholaminergic neurons in the plainfin midshipman fish, Porichthys notatus. *PloS one*, 8, e70474.
95. Peyron, C., Tighe, D. K., van den Pol, A. N., de Lecea, L., Heller, H. C., Sutcliffe, J. G., & Kilduff, T. S. (1998). Neurons containing hypocretin (orexin) project to multiple neuronal systems. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 18, 9996–10015.
96. Randlett, O., Wee, C. L., Naumann, E. A., Nnaemeka, O., Schoppik, D., Fitzgerald, J. E., Portugues, R., Lacoste, A. M., Riegler, C., Engert, F., & Schier, A. F. (2015). Whole-brain activity mapping onto a zebrafish brain atlas. *Nature methods*, 12(11), 1039‑1046.

97. Rink, E., & Wullimann, M. F. (2001). The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). *Brain research*, 889, 316–330.
98. Ronneberger, O., Liu, K., Rath, M., Rueβ, D., Mueller, T., Skibbe, H., Drayer, B., Schmidt, T., Filippi, A., Nitschke, R., Brox, T., Burkhardt, H., & Driever, W. (2012). ViBE-Z: a framework for 3D virtual colocalization analysis in zebrafish larval brains. *Nature methods*, 9, 735–742.
99. Scott, J. W., McDonald, J. K., & Pemberton, J. L. (1987). Short axon cells of the rat olfactory bulb display NADPH-diaphorase activity, neuropeptide Y-like immunoreactivity, and somatostatin-like immunoreactivity. *The Journal of comparative neurology*, 260, 378–391.
100. Servili, A., Le Page, Y., Leprince, J., Caraty, A., Escobar, S., Parhar, I. S., Seong, J. Y., Vaudry, H., & Kah, O. (2011). Organization of two independent kisspeptin systems derived from evolutionary-ancient kiss genes in the brain of zebrafish. *Endocrinology*, 152, 1527–1540.
101. Shah, T., Dunning, J. L., & Contet, C. (2022). At the heart of the interoception network: Influence of the parasubthalamic nucleus on autonomic functions and motivated behaviors. *Neuropharmacology*, 204, 108906.
102. Shainer, I., Michel, M., Marquart, G. D., Bhandiwad, A. A., Zmora, N., Ben-Moshe Livne, Z., Zohar, Y., Hazak, A., Mazon, Y., Förster, D., Hollander-Cohen, L., Cone, R. D., Burgess, H. A., & Gothilf, Y. (2019). Agouti-Related Protein 2 Is a New Player in the Teleost Stress Response System. *Current biology* : CB, 29, 2009–2019.e7.
103. Silva, B. A., Mattucci, C., Krzywkowski, P., Murana, E., Illarionova, A., Grinevich, V., Canteras, N. S., Ragozzino, D., & Gross, C. T. (2013). Independent hypothalamic circuits for social and predator fear. *Nature neuroscience*, 16(12), 1731–1733.
104. Smith, M., & Carter, D. A. (1996). In situ hybridization analysis of vasopressin mRNA expression in the mouse hypothalamus: diurnal variation in the suprachiasmatic nucleus. *Journal of chemical neuroanatomy*, 12, 105–112.
105. Soengas, J. L., Cerdá-Reverter, J. M., & Delgado, M. J. (2018). Central regulation of food intake in fish: an evolutionary perspective. *Journal of molecular endocrinology*, 60, R171–R199.
106. Sohn J. W. (2015). Network of hypothalamic neurons that control appetite. *BMB reports*, 48, 229–233.
107. Song, Y., Golling, G., Thacker, T. L., & Cone, R. D. (2003). Agouti-related protein (AGRP) is conserved and regulated by metabolic state in the zebrafish, Danio rerio. *Endocrine*, 22, 257–265.
108. Song, Y., Peng, W., Luo, J., Zhu, Z., & Hu, W. (2021). Organization of the gonadotropin-inhibitory hormone (Lpxrfa) system in the brain of zebrafish (Danio rerio). *General and*

comparative endocrinology, 304, 113722.

109. Stednitz, S. J., McDermott, E. M., Ncube, D., Tallafuss, A., Eisen, J. S., & Washbourne, P. (2018). Forebrain Control of Behaviorally Driven Social Orienting in Zebrafish. *Current biology* : CB, 28, 2445–2451.e3.
110. Steven, C., Lehnen, N., Kight, K., Ijiri, S., Klenke, U., Harris, W. A., & Zohar, Y. (2003). Molecular characterization of the GnRH system in zebrafish (Danio rerio): cloning of chicken GnRH-II, adult brain expression patterns and pituitary content of salmon GnRH and chicken GnRH-II. *General and comparative endocrinology*, 133, 27–37.
111. Stewart, A. M., Braubach, O., Spitsbergen, J., Gerlai, R., & Kalueff, A. V. (2014). Zebrafish models for translational neuroscience research: from tank to bedside. *Trends in neurosciences*, 37, 264–278.
112. Tabor, K. M., Marquart, G. D., Hurt, C., Smith, T. S., Geoca, A. K., Bhandiwad, A. A., Subedi, A., Sinclair, J. L., Rose, H. M., Polys, N. F., & Burgess, H. A. (2019). Brain-wide cellular resolution imaging of Cre transgenic zebrafish lines for functional circuit-mapping. *eLife*, 8, e42687.
113. Tallafuss, A., Stednitz, S. J., Voeun, M., Levichev, A., Larsch, J., Eisen, J., & Washbourne, P. (2022). Egr1 Is Necessary for Forebrain Dopaminergic Signaling during Social Behavior. *eNeuro*, 9, ENEURO.0035-22.2022.
114. Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular biology and evolution*, 38, 3022–3027.
115. Tanaka, H., Yoshida, T., Miyamoto, N., Motoike, T., Kurosu, H., Shibata, K., Yamanaka, A., Williams, S. C., Richardson, J. A., Tsujino, N., Garry, M. G., Lerner, M. R., King, D. S., O'Dowd, B. F., Sakurai, T., & Yanagisawa, M. (2003). Characterization of a family of endogenous neuropeptide ligands for the G protein-coupled receptors GPR7 and GPR8. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 6251–6256.
116. Tohsato, Y., Ho, K. H., Kyoda, K., & Onami, S. (2016). SSBD: a database of quantitative data of spatiotemporal dynamics of biological phenomena. *Bioinformatics (Oxford, England)*, 32, 3471–3479.
117. Tsuneoka, Y., & Funato, H. (2021). Cellular Composition of the Preoptic Area Regulating Sleep, Parental, and Sexual Behavior. *Frontiers in neuroscience*, 15, 649159.
118. Tunbak, H., Vazquez-Prada, M., Ryan, T. M., Kampff, A. R., & Dreosti, E. (2020). Whole-brain mapping of socially isolated zebrafish reveals that lonely fish are not loners. *eLife*, 9, e55863.
119. Ullmann, J. F., Cowin, G., Kurniawan, N. D., & Collin, S. P. (2010). A three-dimensional digital atlas of the zebrafish brain. *NeuroImage*, 51, 76–82.

120. van den Burg, E. H., & Stoop, R. (2019). Neuropeptide signalling in the central nucleus of the amygdala. *Cell and tissue research*, 375(1), 93–101.
121. van den Pol A. N. (2012). Neuropeptide transmission in brain circuits. *Neuron*, 76, 98–115.
122. Vanwalleghem, G. C., Ahrens, M. B., & Scott, E. K. (2018). Integrative whole-brain neuroscience in larval zebrafish. *Current opinion in neurobiology*, 50, 136–145.
123. Vizziano-Cantonnet, D., Anglade, I., Pellegrini, E., Gueguen, M. M., Fostier, A., Guiguen, Y., & Kah, O. (2011). Sexual dimorphism in the brain aromatase expression and activity, and in the central expression of other steroidogenic enzymes during the period of sex differentiation in monosex rainbow trout populations. *General and comparative endocrinology*, 170, 346–355.
124. Wakisaka, N., Miyasaka, N., Koide, T., Masuda, M., Hiraki-Kajiyama, T., & Yoshihara, Y. (2017). An Adenosine Receptor for Olfaction in Fish. *Current biology*, 27, 1437–1447.e4.
125. Wee, C. L., Song, E. Y., Johnson, R. E., Ailani, D., Randlett, O., Kim, J. Y., Nikitchenko, M., Bahl, A., Yang, C. T., Ahrens, M. B., Kawakami, K., Engert, F., & Kunes, S. (2019). A bidirectional network for appetite control in larval zebrafish. *eLife*, 8, e43775.
126. Weger, M., Weger, B. D., Diotel, N., Rastegar, S., Hirota, T., Kay, S. A., Strähle, U., & Dickmeis, T. (2013). Real-time in vivo monitoring of circadian E-box enhancer activity: a robust and sensitive zebrafish reporter line for developmental, chemical and neural biology of the circadian clock. *Developmental biology*, 380, 259–273.
127. Williams, K. W., Margatho, L. O., Lee, C. E., Choi, M., Lee, S., Scott, M. M., Elias, C. F., & Elmquist, J. K. (2010). Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *The Journal of neuroscience*, 30, 2472–2479.
128. Wu, Z., Autry, A. E., Bergan, J. F., Watabe-Uchida, M., & Dulac, C. G. (2014). Galanin neurons in the medial preoptic area govern parental behaviour. *Nature*, 509, 325–330.
129. Wullimann, Mario F., Barbara Rupp, and Heinrich Reichert. Neuroanatomy of the zebrafish brain: a topological atlas. Birkhäuser, 1996.
130. Xavier, A. L., Fontaine, R., Bloch, S., Affaticati, P., Jenett, A., Demarque, M., Vernier, P., & Yamamoto, K. (2017). Comparative analysis of monoaminergic cerebrospinal fluid-contacting cells in Osteichthyes (bony vertebrates). *The Journal of comparative neurology*, 525, 2265–2283.
131. Yabuki, Y., Koide, T., Miyasaka, N., Wakisaka, N., Masuda, M., Ohkura, M., Nakai, J., Tsuge, K., Tsuchiya, S., Sugimoto, Y., & Yoshihara, Y. (2016). Olfactory receptor for prostaglandin F2α mediates male fish courtship behavior. *Nature neuroscience*, 19, 897–904.
132. Yamamoto, K., Ruuskanen, J. O., Wullimann, M. F., & Vernier, P. (2011). Differential expression of dopaminergic cell markers in the adult zebrafish forebrain. *The Journal of comparative neurology*, 519, 576–598.

133. Yáñez, J., Folgueira, M., Lamas, I., & Anadón, R. (2022). The organization of the zebrafish pallium from a hodological perspective. *The Journal of comparative neurology*, 530, 1164–1194.
134. Z-Brain Atlas, Available from: https://engertlab.fas.harvard.edu/Z-Brain/home/
135. ZeBrain, Available from: https://zebrain.nig.ac.jp/zebrain/page.do?p=welcome
136. Zebrafish Brain Browser 2, Available from: http://zbbrowser.com
137. Zempo, B., Kanda, S., Okubo, K., Akazome, Y., & Oka, Y. (2013). Anatomical distribution of sex steroid hormone receptors in the brain of female medaka. *The Journal of comparative neurology*, 521, 1760–1780.
138. Zhang, C., Song, Y., Thompson, D. A., Madonna, M. A., Millhauser, G. L., Toro, S., Varga, Z., Westerfield, M., Gamse, J., Chen, W., & Cone, R. D. (2010). Pineal-specific agouti protein regulates teleost background adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 20164–20171.
139. Zhou, W., Li, S., Liu, Y., Qi, X., Chen, H., Cheng, C. H., Liu, X., Zhang, Y., & Lin, H. (2012). The evolution of tachykinin/tachykinin receptor (TAC/TACR) in vertebrates and molecular identification of the TAC3/TACR3 system in zebrafish (Danio rerio). *Molecular and cellular endocrinology*, 361, 202–212.

Tables

Table 1. Genes examined in this study and primers used for *in situ* hybridization (ISH) probe synthesis.

Table 2. The summary of gene expressions in the olfactory bulb and dorsal telencephalon.

Table 3. The summary of gene expressions in the ventral telencephalon.

Table 4. The summary of gene expressions in the preoptic area and hypothalamus.

Table 5. The summary of gene expressions in the prethalamus, posterior tuberculum, and thalamus.

Table 6. The summary of gene expressions in the preglomerular complex and pretectum.

Supplemental Table 1. The database of *gal* and *galp* genes in various species was used for phylogenetic tree analysis.

Figure Legends

Figure 1. Schematic of the zebrafish brain viewed from the lateral side. The anterior is to the left, and the dorsal is to the top. Lines 1–19 indicate the locations of each plane in Figs. 2–20.

Figure 2. Schematic of plane 1 and photographs of ISH results of genes expressed in plane 1. Scale bar = 100 μm.

Figure 3. Schematic of plane 2 and photographs of ISH results of genes expressed in plane 2. Scale bar = 200 μm.

Figure 4. Schematic of plane 3 and photographs of ISH results of genes expressed in plane 3. Scale bar = 200 μm.

Figure 5. Schematic of plane 4 and photographs of ISH results of genes expressed in plane 4. Scale bar = 200 μm.

Figure 6. Schematic of plane 5 and photographs of ISH results of genes expressed in plane 5. Scale bar = 200 μm.

Figure 7. Schematic of plane 6 and photographs of ISH results of genes expressed in plane 6. Scale bar = 200 μm.

Figure 8. Schematic of plane 7 and photographs of ISH results of genes expressed in plane 7. Scale bar = 200 μm.

Figure 9. Schematic of plane 8 and photographs of ISH results of genes expressed in plane 8. Scale bar = 200 μm.

Figure 10. Schematic of plane 9 and photographs of ISH results of genes expressed in plane 9. Scale bar = 200 μm

Figure 11. Schematic of plane 10 and photographs of ISH results of genes expressed in plane 10. Scale bar = 200 μm.

Figure 12. Schematic of plane 11 and photographs of ISH results of genes expressed in plane 11. Scale bar = 200 μm.

Figure 13. Schematic of plane 12 and photographs of ISH results of genes expressed in plane 12. Scale bar = 200 μm.

Figure 14. Schematic of plane 13 and photographs of ISH results of genes expressed in plane 13. Scale bar = 200 μm.

Figure 15. Schematic of plane 14 and photographs of ISH results of genes expressed in plane 14. Scale bar = 200 μm.

Figure 16. Schematic of plane 15 and photographs of ISH results of genes expressed in plane 15. Scale bar = 200 μm.

Figure 17. Schematic of plane 16 and photographs of ISH results of genes expressed in plane 16. Scale bar = 200 μm.

Figure 18. Schematic of plane 17 and photographs of ISH results of genes expressed in plane 17. Scale bar = 200 μm.

Figure 19. Schematic of plane 18 and photographs of ISH results of genes expressed in plane 18. Scale bar = 200 μm.

Figure 20. Schematic of plane 19 and photographs of ISH results of genes expressed in plane 19. Scale bar = 200 μm.

Figure 21. Subnuclei in the anterior part of the parvocellular preoptic nucleus (PPa). (A) The PPa (grey) in the original zebrafish brain atlas. (B) The PPa can be divided into two subnuclei: the ventral part of PPa (PPav, pink) and dorsal part of PPa (PPad, purple). Neuropeptides such as *ccka* and *vipa* and *vglut* mix are exclusively expressed in the PPav. *gal* and *pdyn* are expressed in both the PPad and PPav, but predominantly in the PPav. *cart3b* and *tac3a* are also expressed in the PPav and PPad, but predominantly in the PPad. *gad* mix is expressed in both the PPad and PPav. Scale bar = 100 μm.

Figure 22. Subnuclei in the ventral zone of the periventricular hypothalamus (Hv). (A) The Hv (grey) in the original zebrafish brain atlas. (B) The Hv can be divided into two subnuclei: the ventral part of Hv (Hvv, purple) and the dorsal part of Hv (Hvd, pink). Neuropeptides such as *pdyn* and *tac3a* are expressed only in the Hvd, whereas *agrp* and *ccka* are expressed only in the Hvv. *cckb* is expressed in both the Hvd and Hvv. *gad* mix is predominantly expressed in the Hvv and the *vglut* mix is more densely expressed in the Hvd. Scale bar = 100 μm.

Figure 23. Subnuclei in the dorsal zone of the periventricular hypothalamus (Hd). (A) The Hd (grey) in the original zebrafish brain atlas. (B) The Hd can be divided into three subnuclei: the medial (Hdm, pink), dorsolateral (Hddl, purple), and ventrolateral (Hdvl, yellow) part of Hd. Some neuropeptides are expressed solely in the Hdm, Hddl, or Hdvl (represented by *npy*, *cart*, and *ccka*, respectively). Neuropeptides such as *penka* and *vipa* are expressed in all three subnuclei. *gad* mix is also expressed in all subnuclei. *vglut* mix is absent in all subnuclei. Scale bar = 100 μm.

Figure 24. Subnuclei in the caudal zone of the periventricular hypothalamus (Hc). (A) The anterior part of Hc (grey) in the original zebrafish brain atlas. (B) The anterior part of Hc can be divided into three subnuclei: the anterodorsal (Hcad, yellow), anterointermediate (Hcai, blue), anteroventral (Hcav, pink) part of Hc. Neuropeptides such as *pomcb* and *tac3a* are solely expressed in the Hcad. Neuropeptides such as *npb* and *cckb* are expressed exclusively in the Hcai. *agrp* and *tac1* are only expressed in the Hcav. Neuropeptides such as *cckb*, *pdyn*, and *penka* are expressed across multiple subnuclei. *gad* mix and *vglut* mix are expressed in all three subnuclei. (C) Posterior part of Hc (grey) in original zebrafish brain atlas. (D) The posterior part of Hc can be divided into three subnuclei: the posterodorsal (Hcpd, green), posterointermediate (Hcpi, light green), posteroventral (Hcpv, purple) part of Hc. Neuropeptides such as *pomcb*, *tac3a*, and *pdyn* are solely expressed in the Hcpd. *cckb*, *penkb*, and *vglut* mix are expressed in the Hcpd and Hcpv. *penka*, *npb*, and *gad* mix are expressed in all three subnuclei. Scale bar = 100 μm.

Figure 25. Comparison between zebrafish and mouse hypothalamic nuclei based on their neuropeptide expression patterns. Expression of neuropeptides (green) and neurotransmitter markers (purple) in the hypothalamic nuclei of zebrafish (light yellow) and mice (light blue) were extracted from the present ISH results (zebrafish), the Allen Mouse Brain Atlas, and previous papers (mouse) and summarized in a table (expressed = 1, not expressed = 0). Ward's method hierarchically clustered zebrafish and mouse nuclei and expressed genes.

1262 **Supplemental Figure 1.** Phylogenetic tree and synteny analyses of *gal* and *galp* gene families in
1263 vertebrates. A: Phylogenetic tree of GAL and GALP amino acid sequences. The accession
1264 numbers of the sequences used in this analysis are listed in Supplementary Table 1. Sequences
1265 are aligned using ClustalW, and a phylogenetic tree is constructed by the maximum likelihood
1266 method using the Molecular Evolutionary Genetics Analysis (MEGA11) software. Numbers at the
1267 branch nodes represent the bootstrap confidence level (500 replicates). The scale bar indicates the
1268 number of substitutions per site. B: Schematic showing the genes in the genomic area around *gal*
1269 and *galp* genes. The arrowhead represents the orientation of the gene. Orthologous genes among
1270 vertebrate species are indicated in the same color.
1271
1272 **Supplemental Figure 2.** Summary of individual differences in neuropeptide expression.
1273 Photographs of four individual fish are shown for each gene for which individual differences in
1274 expression are observed. Some sections are subjected to methyl green nuclear staining. Scale bar
1275 = 100 μm.

Gene	Peptide	Synonim	Forward primer	Reverse primer	NCBI gene ID	mRNA GenBank accession no.
agrp	agouti-related neuropeptide		AGGATCTGGGCAAAGCTGTCCACCTGCAGAGAAGAG	GCAATAATCAGAGTTTATTGAATGCATATATACAAA	100149042	NM_001328012.1
asip2b	agouti-signaling protein 2b	agrp2	CTTCATAAAAACTCCCCTTTATCAGGTCGG	AAGCTTGCAGCTATGTGCTGTCATTATGGC	796595	NM_001271291.1
avp	arginine vasopressin	arginin vasotocin	AGTGTATGTCGTGTGGCCCCGGGGGTGTGGGCCGC	CTGAATCGCAGCAGATGCCCTCAGCGGCGCAGCG	352922	NM_178293.2
calca	calcitonin related polypeptide alpha	cgrp	GGTCACCAGTCCACACGTCCACAGTTCCTCTTTG	CTTGCTCAAATTCACAATTGTTACAATTTGTAATTC	436744	NM_001002471.1
cart2a	cocaine- and amphetamine-regulated transcript 2a		ATGTTTAGCAGACGGATTGTCTTTTTCGCTGCTGCTTCTGCCC	TTAAAGGCATTTTAAAATAAAAAAATGGCATGATGTTCCTTGTGG		OQ363808
cart2b	cocaine- and amphetamine-regulated transcript 2b	cart1	CCACATGAATTTTGCTAGCTGGGATATGACGC	ATTTGATGATGGTAAACAACACACTGGAGC	562883	XM_021473557.1
cart3a	cocaine- and amphetamine-regulated transcript 3a	cart3	TTGCGCTGTGCTGCTCTCCTGCATACAGGC	ATCTTCAAACGCATCTGATAGTATCATAGC	557301	NM_001327818.1
cart3b	cocaine- and amphetamine-regulated transcript 3b	cart2	TCCAGTCTGAGGATGCGCATGGCAGTG	GCGGACAGTCGCACATCTTGCCGATTC	550232	NM_001017570.2
cart4	cocaine- and amphetamine-regulated transcript 4	cartl	ATGACGAGCTCTGAGATGCTGATGACAGGACTCCTCGTAGCGGG	TCAGATGCATTTTAAGAGAAATGAGTTACAGTTTGATCCTCTGCC	100329270	NM_001386407.1
cart5	cocaine- and amphetamine-regulated transcript 5	cart4	GCACACAAGAGCTGGGAAACGCTTAG	ACAACAGAGTCAGTAGTAGGTCACC	799423	NM_001082932.1
ccka	cholecystokinin a		GTGTTTCTCCAGCTCTCTCTGCGTCTCTGC	GTGACTCTGTACAGATGATCGGCTAATCGG	100007763	NM_001386383.1
cckb	cholecystokinin b		AGGCTCCGTTCGCCGGAACTCCTCGATGAACAGCC	TAGGGTTTAAGGCCAAAGTTGAATGCTGACCTG	100000095	XM_002665615.6
crha	corticotropin-releasing hormone a		AGATTACCGTGAATTCCCCTATGAATCTGCACGTGG	TATTTCACTCAGTTTGGGTTTAAATAACCC	796284	XM_009298729.3
crhb	corticotropin-releasing hormone b		CAGATTCTCCTCGCCACTTTTTGACATGAAGCTC	GAAAAATATAAATATAAGACATTCTCTTGC	492507	NM_001007379.1
gal	galanin		GAATCCACAGGAGCATCAGAGGACCAGCAGCAG	GAATGCCATTTATGAGTTTTATTTTTGAATCC	100006361	XM_005162992.4
galp	galanin-like peptide	gall, si:rp71-1c10.8	GCAAACCTGTTATTTGAAGGTTACAGGCGG	CCATGATTCAGTAAAGGGGAGAGATGGAGG	100538216	NM_001327976.1
gnrh2	gonadotoropin-releasing hormone 2		GCTGGATGCCCTCATTCGTGATTTCCAAAAGAG	CAAAATACCAAAATAAAGCCTTTATTGTAGGAAC	353222	NM_181439.4
gnrh3	gonadotoropin-releasing hormone 3		ATCTTGAACAAACACAGCAGTTTTAGCATGGAGTG	GTTCCTTCAGTGAAGTGTATTTATTAAATCCATAA	360141	NM_182887.2
grp	gastrin-releasing peptide		GGCTGAGGACTTGTTACGGGCTTTAAGAATGCAG	TCAAATGATATGCAAAACAGTCATTTTTTATAAACC	559978	NM_001161350.1
hcrt	hypocretin	orexin	AGAAGCTCCAGGTGCTCGTCTTCATGGCGCTGC	TAGCGACAAGTGTCATCGTTTTTCATAACTGTCC	613239	NM_001077392.2
kiss1	kisspeptide 1		TATGAAGCGAACACAAGCTCCATACCTGC	AACATGAAGGCAAATACCTCAGAGAGGACC	100134982	NM_001113489.1
kiss2	kisspeptide 2		ATATAAGCTCTACAATTCAGCAATATGAATACC	TAGGCTACAATAACTCAATAAAACAAAGTGCGC	100216472	NM_001142585.1
npb	neuropeptide b		ACAACAGAGAACACTATCACCCGAGCGCGC	TGTCAACAGCACGTCTGGCTGCCTCGGCGG	563766	XM_009296109.3
npvf	neuropeptide VF	gnih	TCAGAGAATGCTCAAGAGATTCCCCGCAGTCTGGAG	CCTTTCTTTTGGAAATGATGGTGATTCAGGTGTGCG	100002108	NM_001082949.1
npy	neuropeptide y		CCCACAGAGCAAGAATTCCAATCAAGATCTC	TCAGGGGCAAAAGACAGCAAACAAAGACGAC	30281	NM_131074.2
oxt	oxytocin	isotocin	ACGCGTCCGCAACTCTTCTTCTTCTTCTTCTCC	TCTGCACTAATGTACAGTCAAGCTAATCATCAG	352920	NM_178291.2
pdyn	pro-dynorphin		GACTTGTACTTTAGAGTGTGAAGGAGCCGTTCCGC	GAGTTCTAGTTGAGTTATCAATGATACAACAGCC	414850	XM_009297105.1
penka	pro-enkephalin a		GGCCTGCACATTAGAATGTGAAGGGTCTGTGGAC	ATTCTGTACAGCTAATAGCTCAAAATGTTGCCTCG	368228	NM_200083.2
penkb	pro-enkephalin b		GAATGTGTGTTGGAGTGTGAGGAGCAACCGAACGC	CTGTACAACAAATGACAAGATTCACACTGGTACCC	360136	NM_182883.1
pmch	pro-melanin-concentrating hormone	pmch2	GTGCAAAGCTAGGTTCTGCAACCATCACAGCCG	TCATACATTTCTGCTCGTCATGTTTATATTTGG	100003610	NM_001202542.1
pmchl	pro-melanin-concentrating hormone, like	pmch1	GACAGGAAACAGCAGCCAAACCGCTAAAGC	AGAACAGAACAGAACATGAACAGACCTGTG	100216485	NM_001162488.1
pomca	pro-opiomelanocortin a		GGAATGCATACAATTATGCAGGTCTGAACTTACAG	TAGGAACCTCTCATCCAGAAGTTTGCTGGG	353221	NM_181438.3
pomcb	pro-opiomelanocortin b		GCAGTGTCTCAGAAAGTGCAGATCGGACCAAGAATC	TTAATCTGTGAACTGCTGTCCATTGCCGATGGCG	100034412	XM_005170169.4
tac1	tachykinin 1		GAGTCGTATGAATGGGGAACAGTACAGATCTACGAC	GTTCCACCAATAAAGAAAATATCmAATATCAAGC	555634	NM_001256391.1
tac3a	tachykinin 3a		ATGTACCGTGGACTTGTGTTACTGTTCTTGGTTTTGG	CTATAACCCACGACGAAACCTCAGTTTGCATTTG	100320280	XM_005162221.4
tac3b	tachykinin 3b		CTGAACAGCATCCTGTTAACACCAGCTCATACGAG	TCATACACCACAGCAAAACCTCAGTCTTCCATCC	569642	NM_001256390.1
vipa	vasoactive intestinal peptide a		GCCGCTCTGAGGTCAGTCCGAACGCAAGAAATCAAG	CTTTGAATGGCAAAAATAAAGATTTCCAATGCCCG	795653	NM_001114553.3
vipb	vasoactive intestinal peptide b		GTCAGCGGACAGAGATCGTATACCAACCAC	CTGTGTCATTCACACCAGAGTCGCAATCAC	100000558	XM_021479523.1
gad1a	glutamate decarboxylase 1a		TCTGGCGGCGTTCCGGATCCCAACTC	TGGTAACCGCCGGATTGGAGACCACC	100329827	XM_002663304.5
gad1b	glutamate decarboxylase 1b	gad67	ATGGCGTCTTCTGCACCTTCTTCCTCGGCTGGTG	TTACAGATCCTGACCGAGCCGCTCGATCTCATCG	378441	NM_194419.1
gad2	glutamate decarboxylase 2	gad65	CGGCTGGAAACGGCAGTCAAAGCCCG	GGCTGGTAGCTCACCATGGTCGTGCC	550403	NM_001017708.2
slc17a6a	solute carrier family 17 member 6a	VGLUT2.2, vglut2b	Higashijima et al., 2004	Higashijima et al., 2004		
slc17a6b	solute carrier family 17 member 6b	VGLUT2.1, vglut2a	Higashijima et al., 2004	Higashijima et al., 2004		
slc17a7a	solute carrier family 17 member 7a	VGLUT1, vglut1a	Higashijima et al., 2004	Higashijima et al., 2004		
slc17a7b	solute carrier family 17 member 7b	vglut1b	ATTCCCACTGCGGCACGAATGCACTTCGGC	GCTGTGGAAGTCATTCGTTCATCCCTCTGCCC	100331980	XM_009297642.3

Table 1. Genes examined in this study and primers used for in situ hybridization (ISH) probe synthesis.

	Olfactory bulb			Dorsal telencephalon								
	ICL	ECL/GL	Terminal nerve neruon	Dma	Dld	Dlv	Dm	Dd	Dc	Dp	Dl	Dmp
agrp	-	-	-	-	-	-	-	-	-	-	-	-
asip2b	-	-	-	-	-	-	-	-	-	-	-	-
avp	-	-	-	-	-	-	-	-	-	-	-	-
calca	-	-	-	-	-	-	-	-	-	-	-	-
cart2a	-	-	-	-	-	-	-	-	-	-	-	-
cart2b	-	-	-	-	-	-	-	-	-	-	-	-
cart3a	+	+	-	-	-	-	-	-	+	-	-	-
cart3b	+	-	-	+	+	-	+	+	+	-	+	+
cart4	-	-	-	-	-	-	-	-	-	-	-	-
cart5	-	-	-	-	-	-	+	-	-	-	+	-
ccka	-	-	-	-	-	-	-	-	-	-	-	-
cckb	+	-	-	+	+	+	+	+	+	+	+	-
crha	-	-	-	-	-	-	-	-	-	-	-	-
crhb	+	+	-	-	-	-	+	+	+	+	+	-
gal	-	-	-	-	-	-	-	-	-	-	-	-
galp	-	-	-	-	-	-	-	-	-	-	-	-
gnrh2	-	-	-	-	-	-	-	-	-	-	-	-
gnrh3	-	-	+	-	-	-	-	-	-	-	-	-
grp	-	-	-	-	-	-	-	-	-	-	-	-
hcrt	-	-	-	-	-	-	-	-	-	-	-	-
kiss1	-	-	-	-	-	-	-	-	-	-	-	-
kiss2	-	-	-	-	-	-	-	-	-	-	-	-
npb	-	-	-	-	-	-	-	-	-	-	-	-
npvf	-	-	-	-	-	-	-	-	-	-	-	-
npy	-	+	-	-	-	-	-	-	+	-	-	-
oxt	-	-	-	-	-	-	-	-	-	-	-	-
pdyn	-	-	-	-	-	-	-	-	-	-	-	-
penka	+	-	-	-	-	-	+	-	-	-	+	+
penkb	+	+	-	-	-	-	-	-	-	-	-	-
pmch	-	-	-	-	-	-	-	-	-	-	-	-
pmchl	-	-	-	-	-	-	-	-	-	-	-	-
pomca	-	-	-	-	-	-	-	-	-	-	-	-
pomcb	-	-	-	-	-	-	-	-	-	-	-	-
tac1	+	-	-	-	-	-	-	-	-	+	+	+
tac3a	-	-	-	-	-	-	-	-	-	-	-	-
tac3b	-	+	-	-	+	-	-	-	-	-	-	-
vipa	-	-	-	-	-	-	+	-	-	-	+	-
vipb	-	-	-	-	-	-	-	-	-	-	-	-
gad mix	+	+	-	+	+	+	+	+	+	+	+	+
vglut mix	+	+	-	+	+	+	+	+	+	+	+	+

Table 2. The summary of gene expressions in the olfactory bulb and dorsal telencephalon.

	Ventral telencephalon								
	Vl	Vc	Vv	Vd	Vs	Vp	Vi	ENd	ENv
agrp	-	-	-	-	-	-	-	-	-
asip2b	-	-	-	-	-	-	-	-	-
avp	-	-	-	-	-	-	-	-	-
calca	-	-	-	-	-	-	-	-	-
cart2a	-	-	-	-	-	-	-	-	-
cart2b	-	-	-	-	-	-	-	-	-
cart3a	-	-	+	+	+	+	+	+	+
cart3b	-	-	+	+	+	+	+	+	-
cart4	-	-	-	-	-	-	-	-	-
cart5	-	-	+	-	-	+	-	-	+
ccka	-	-	-	-	-	-	-	-	-
cckb	-	+	-	+	+	+	+	+	-
crha	-	-	-	-	-	-	-	-	-
crhb	-	-	+	+	+	+	-	+	-
gal	-	-	-	-	-	-	-	-	-
galp	-	-	-	-	-	-	-	-	-
gnrh2	-	-	-	-	-	-	-	-	-
gnrh3	-	-	-	-	-	-	-	-	-
grp	-	-	-	-	-	-	-	-	-
hcrt	-	-	-	-	-	-	-	-	-
kiss1	-	-	-	-	-	-	-	-	-
kiss2	-	-	-	-	-	-	-	-	-
npb	+	-	+	-	-	+	-	-	-
npvf	-	-	-	-	-	-	-	-	-
npy	-	-	+	+	+	+	-	+	-
oxt	-	-	-	-	-	-	-	-	-
pdyn	-	-	+	+	+	+	+	-	-
penka	+	-	+	+	+	+	+	-	+
penkb	-	+	+	+	+	+	+	-	-
pmch	-	-	-	-	-	-	-	-	-
pmchl	-	-	+	-	-	-	-	-	-
pomca	-	-	-	-	-	-	-	-	-
pomcb	-	-	+	-	-	-	-	-	-
tac1	-	+	+	+	+	+	+	-	-
tac3a	-	-	+	+	+	+	+	-	-
tac3b	-	-	-	-	-	-	-	-	-
vipa	-	-	+	-	-	-	-	-	-
vipb	-	-	-	-	-	-	-	-	-
gad mix	+	+	+	+	+	+	+	+	+
vglut mix	+	-	+	+	+	+	+	-	+

Table 3. The summary of gene expressions in the ventral telencephalon.

	Preoptic area				Hypothalamus								
	PPa	PPp	SC	PM	NLT	Hv	LH	Hd	Hc	IN	ATN	DIL	CIL
agrp	-	-	-	-	-	+	-	-	+	-	-	-	-
asip2b	+	-	-	+	-	+	-	-	+	-	-	-	-
avp	+	+	-	+	-	+	-	-	-	-	-	-	-
calca	-	-	-	-	-	-	-	-	-	-	-	-	-
cart2a	-	-	-	-	-	-	-	-	-	-	-	-	-
cart2b	-	-	-	-	-	-	-	-	-	-	-	-	-
cart3a	+	+	-	-	-	+	-	-	+	-	-	-	-
cart3b	+	+	+	-	-	+	+	+	+	+	-	-	-
cart4	+	-	-	-	-	-	-	-	-	-	-	-	-
cart5	+	+	+	-	-	+	+	+	+	-	-	-	-
ccka	+	+	+	+	-	+	+	+	+	+	-	+	+
cckb	+	+	-	-	-	+	+	+	+	-	-	-	-
crha	-	-	-	+	-	+	-	-	-	-	-	-	-
crhb	+	+	+	+	-	+	+	+	+	+	-	-	-
gal	+	-	+	+	+	-	+	+	+	-	-	-	-
galp	-	-	-	+	-	-	+	+	-	-	-	-	-
gnrh2	-	-	-	-	-	-	-	-	-	-	-	-	-
gnrh3	+	-	-	-	-	+	-	-	-	-	-	-	-
grp	-	-	-	-	-	-	-	-	-	-	-	-	-
hcrt	-	-	-	-	-	-	-	-	-	-	-	-	-
kiss1	-	-	-	-	-	-	-	-	-	-	-	-	-
kiss2	-	-	-	-	-	+	+	+	+	-	-	-	-
npb	+	-	+	+	-	+	+	+	+	-	-	-	-
npvf	-	-	-	-	-	-	-	-	-	-	-	-	-
npy	+	+	+	+	+	+	+	+	+	-	-	+	-
oxt	+	+	-	+	-	-	-	-	-	-	-	-	-
pdyn	+	+	+	+	-	+	+	+	+	+	-	+	+
penka	+	+	+	+	-	+	+	+	+	+	-	-	-
penkb	+	+	+	+	-	+	+	+	+	+	-	-	-
pmch	-	-	-	-	+	-	+	-	-	-	-	-	-
pmchl	-	-	-	-	+	-	+	-	+	+	-	-	-
pomca	-	-	-	-	+	+	+	-	+	-	-	-	-
pomcb	+	-	-	-	-	+	+	+	+	-	-	+	-
tac1	+	+	+	+	-	+	-	+	+	-	+	-	-
tac3a	+	+	+	+	-	+	+	+	+	+	+	-	-
tac3b	-	-	-	-	-	+	-	-	-	-	-	-	-
vipa	+	-	-	+	-	+	+	+	+	+	-	+	-
vipb	-	-	-	-	-	-	-	-	-	-	-	-	-
gad mix	+	+	+	+	-	+	+	+	+	+	-	+	+
vglut mix	+	+	+	+	-	+	+	+	+	-	+	+	+

Table 4. The summary of gene expressions in the preoptic area and hypothalamus.

	Prethalamus				Posterior tuberculum			Thalamus					
	R	VM	VL	I	TPp	PVO	PTN	Had	Hav	A	DP	IC	CP
agrp	-	-	-	-	-	-	-	-	-	-	-	-	-
asip2b	-	-	-	-	-	-	-	-	-	-	+	-	-
avp	-	-	-	-	-	-	-	-	-	-	-	-	-
calca	-	-	-	-	+	-	-	-	-	-	-	-	-
cart2a	-	-	-	-	-	-	-	-	-	-	-	-	-
cart2b	-	-	-	-	-	-	-	-	-	-	-	-	-
cart3a	-	-	-	-	+	-	+	-	-	+	+	-	-
cart3b	-	+	+	+	+	-	+	-	-	-	+	+	-
cart4	-	-	-	-	-	-	-	-	-	-	-	-	-
cart5	-	-	-	-	-	-	-	-	-	-	-	-	-
ccka	-	-	-	-	+	+	+	-	-	-	-	+	+
cckb	-	-	+	-	+	+	+	+	-	+	+	-	+
crha	-	-	-	-	-	-	-	-	-	-	-	-	-
crhb	-	+	+	-	+	+	+	-	-	-	+	+	-
gal	-	-	-	-	-	-	-	-	-	-	-	-	-
galp	-	-	-	-	+	-	-	-	-	-	-	-	-
gnrh2	-	-	-	-	-	-	-	-	-	-	-	-	-
gnrh3	-	-	-	-	-	-	-	-	-	-	-	-	-
grp	-	-	-	-	-	-	-	-	-	-	-	-	-
hcrt	-	-	-	-	-	+	-	-	-	-	-	-	-
kiss1	-	-	-	-	-	-	-	-	+	-	-	-	-
kiss2	-	-	-	-	+	-	-	-	-	-	-	-	-
npb	-	+	-	-	+	-	+	-	-	-	-	+	-
npvf	-	-	-	-	-	+	-	-	-	-	-	-	-
npy	-	-	-	+	+	-	+	-	-	+	-	+	-
oxt	-	-	-	-	-	-	-	-	-	-	-	-	-
pdyn	-	+	-	-	-	+	+	+	-	-	-	+	-
penka	-	+	+	+	+	+	+	-	-	-	-	+	+
penkb	-	-	-	+	+	+	+	-	-	+	-	+	-
pmch	-	-	-	-	-	-	-	-	-	-	-	-	-
pmchl	-	-	-	-	-	+	-	-	-	-	-	-	-
pomca	-	-	-	-	-	+	-	-	-	-	-	-	-
pomcb	-	-	-	-	-	-	+	-	-	-	-	-	-
tac1	-	-	-	-	+	-	-	+	-	-	-	-	+
tac3a	-	-	-	-	+	+	+	+	-	-	-	-	-
tac3b	-	-	-	-	+	-	-	-	-	-	-	-	+
vipa	-	+	-	-	+	-	+	-	-	-	-	-	-
vipb	-	+	-	-	-	-	-	-	-	-	-	-	-
gad mix	+	+	+	+	+	+	+	-	-	+	-	+	-
vglut mix	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 5. The summary of gene expressions in the prethalamus, posterior tuberculum, and thalamus.

	Preglomerular complex									Pretectum									
	TLa	P	PGl	PGm	SG	TGN	PGa	PGc	CM	CPN	PSp	APN	DAO	PSm	PO	VAO	PPv	PPd	PCN
agrp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
asip2b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
avp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
calca	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cart2a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cart2b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cart3a	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
cart3b	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
cart4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cart5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ccka	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
cckb	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-
crha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
crhb	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+
gal	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
galp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
gnrh2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
gnrh3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
grp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hcrt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
kiss1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
kiss2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
npb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
npvf	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
npy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
oxt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pdyn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
penka	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
penkb	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
pmch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pmchl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pomca	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pomcb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
tac1	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
tac3a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
tac3b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
vipa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
vipb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
gad mix	-	+	+	+	-	+	+	-	-	+	-	+	+	-	+	+	+	+	+
vglut mix	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+

Table 6. The summary of gene expressions in the preglomerular complex and pretectum.

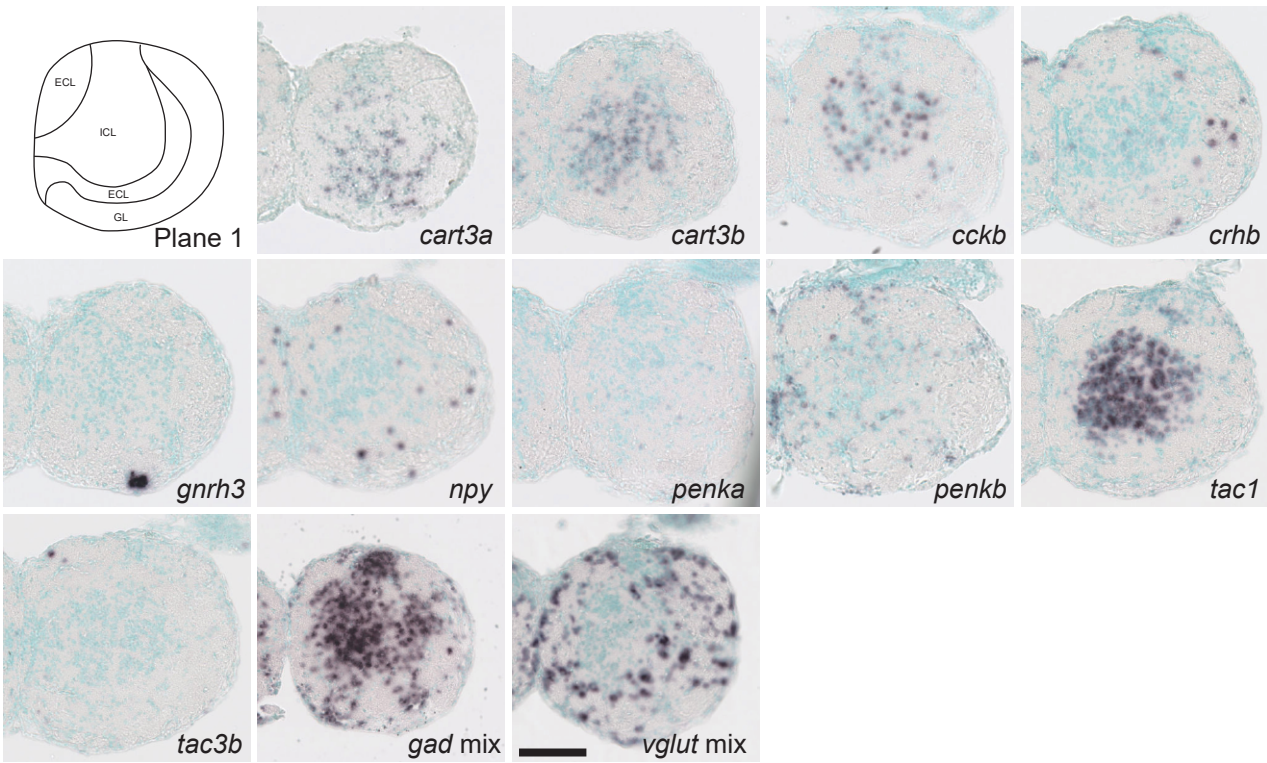


Figure 2

A schematic of plane 1 and photographs of ISH results of genes expressed in plane 1. Scale bar = 100 μm.

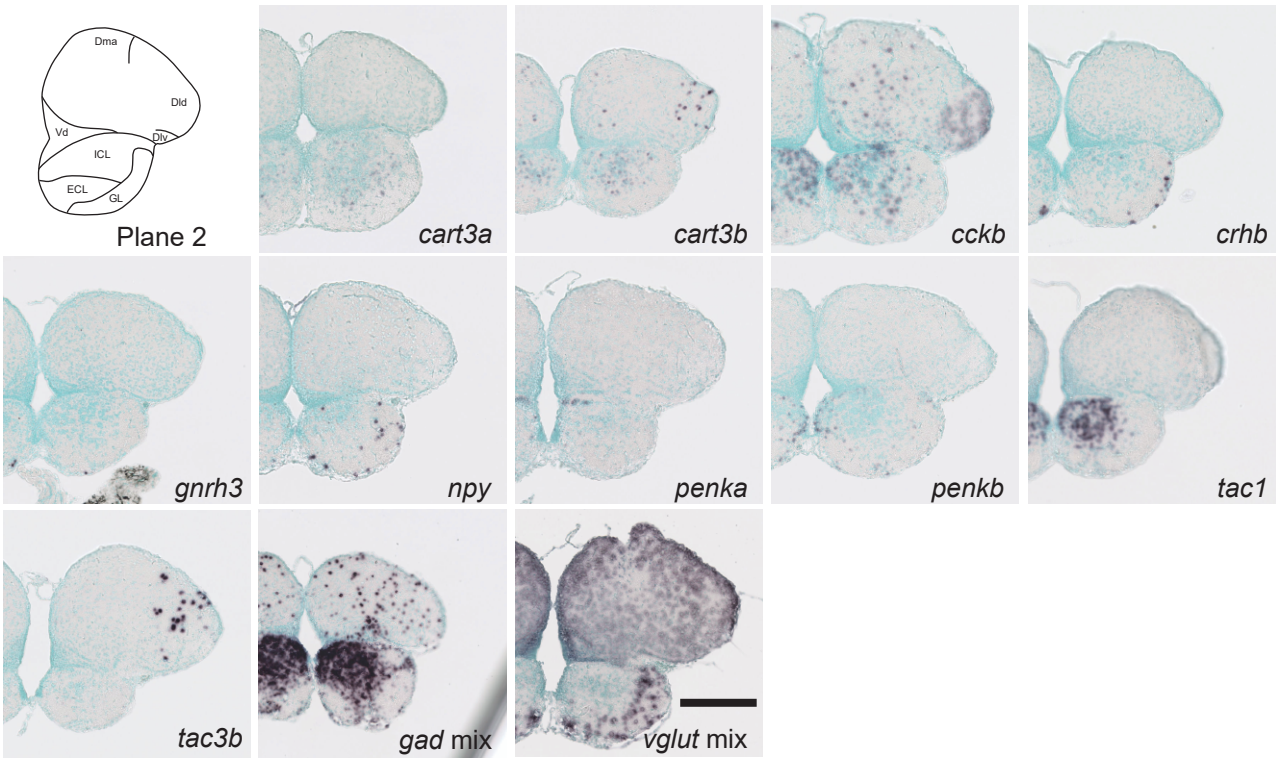


Figure 3

A schematic of plane 2 and microscopic images of genes expressed in the plane 2. Scale bar = 200 μm.

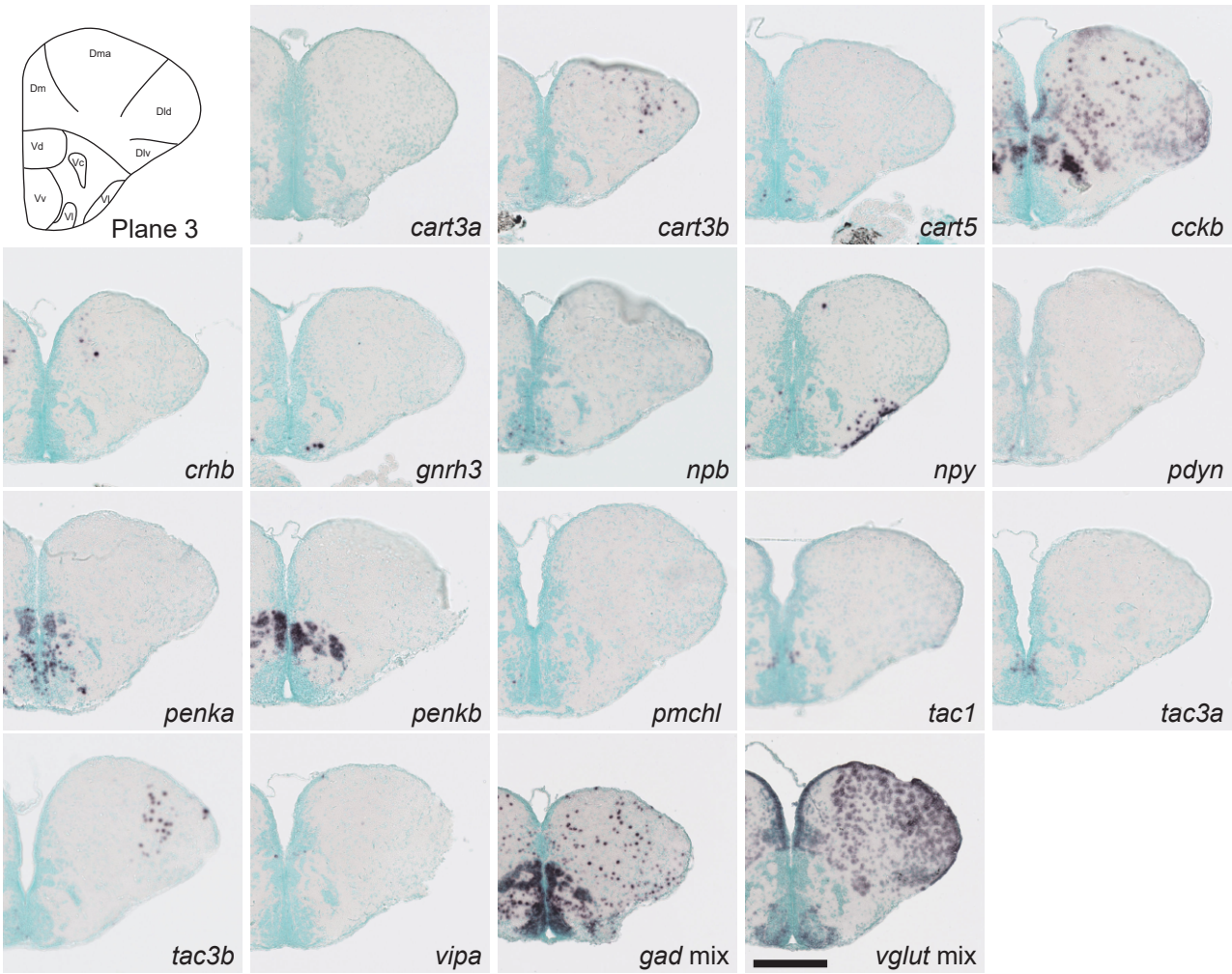


Figure 4

A schematic of plane 3 and microscopic images of genes expressed in the plane 3. Scale bar = 200 μm.

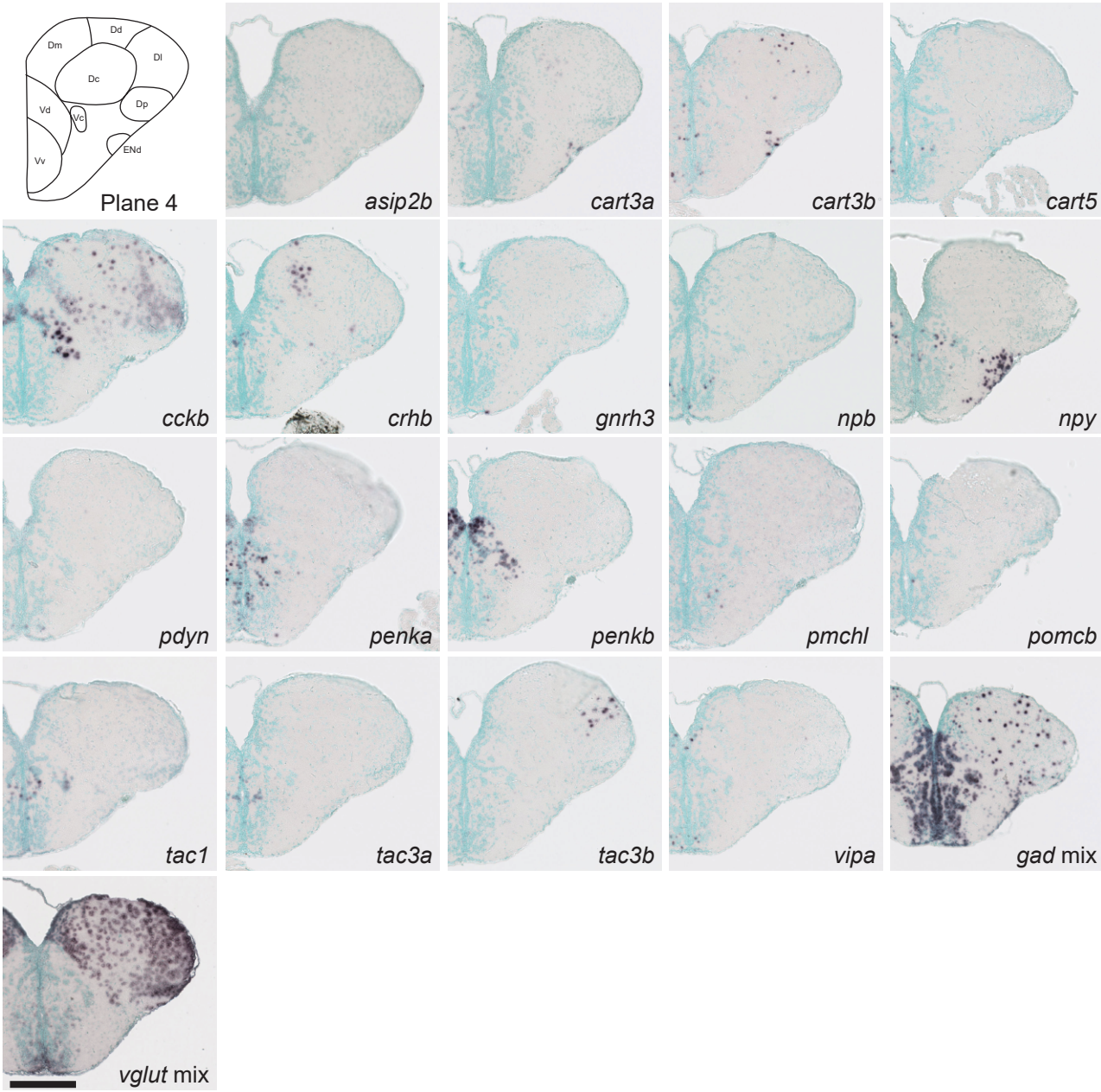


Figure 5

A schematic of plane 4 and microscopic images of genes expressed in the plane 4. Scale bar = 200 μm.

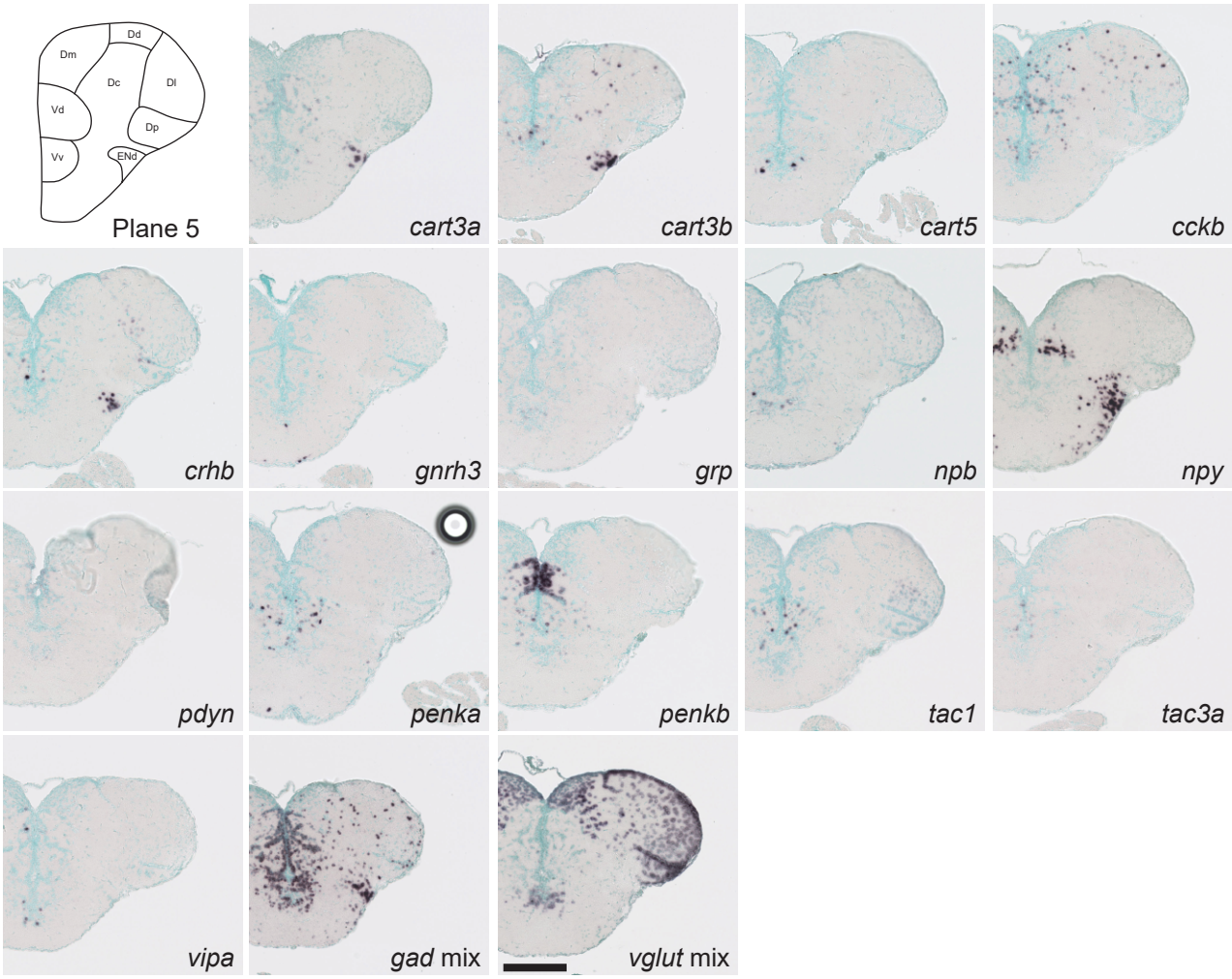


Figure 6

A schematic of plane 5 and microscopic images of genes expressed in the plane 5. Scale bar = 200 μm.

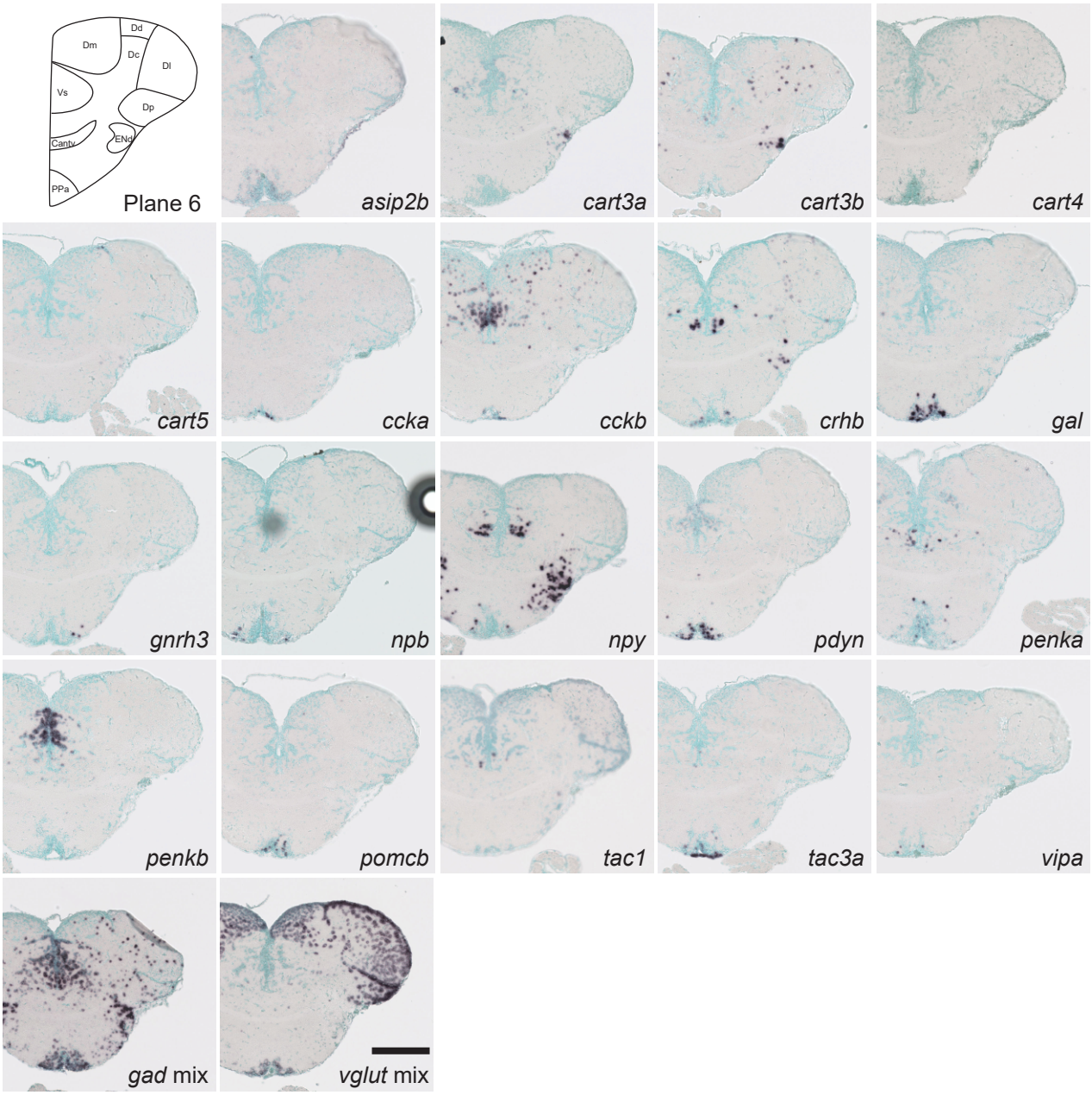


Figure 7

A schematic of plane 6 and microscopic images of genes expressed in the plane 6. Scale bar = 200 μm.

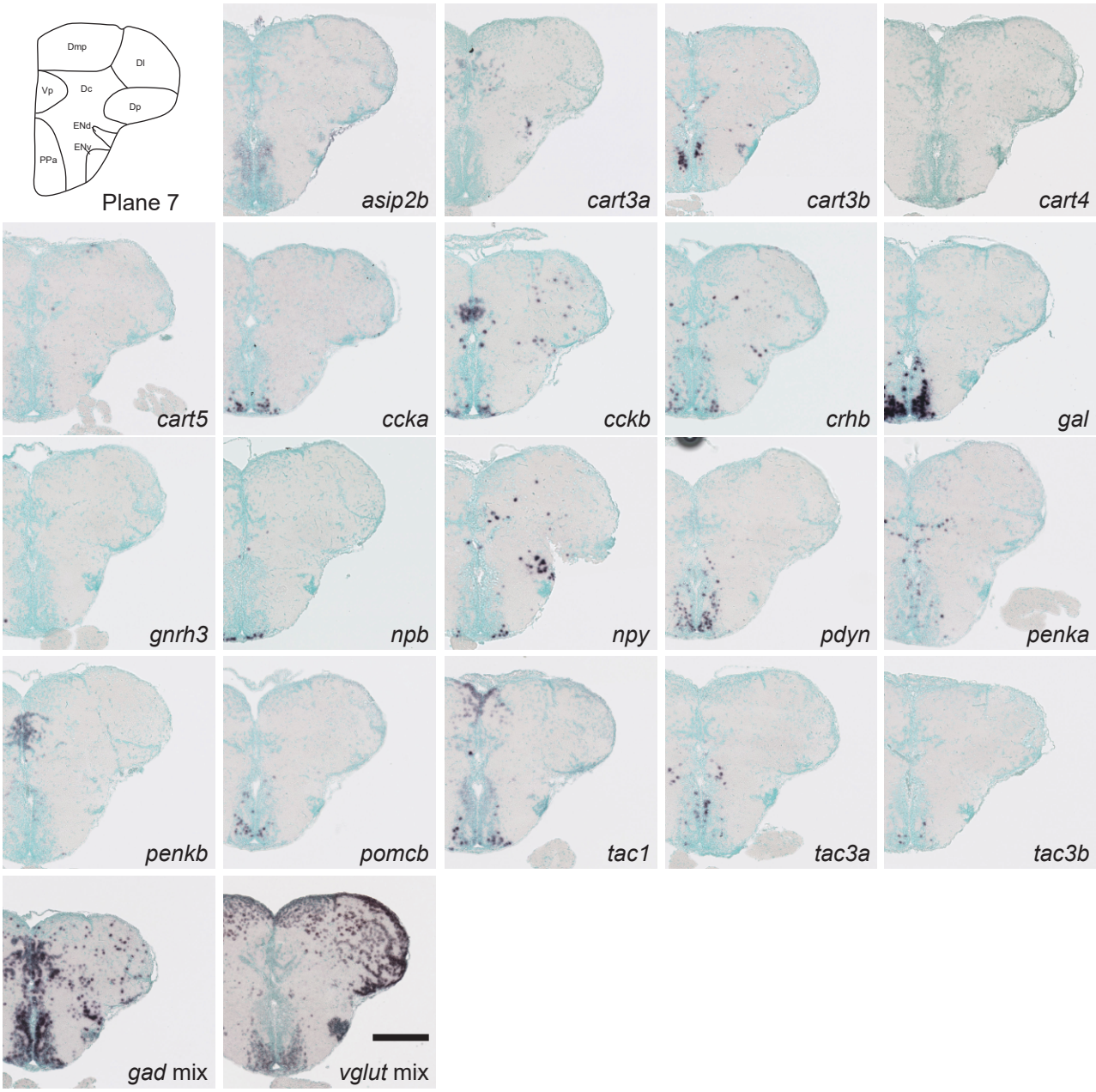


Figure 8

A schematic of plane 7 and microscopic images of genes expressed in the plane 7. Scale bar = 200 μm.

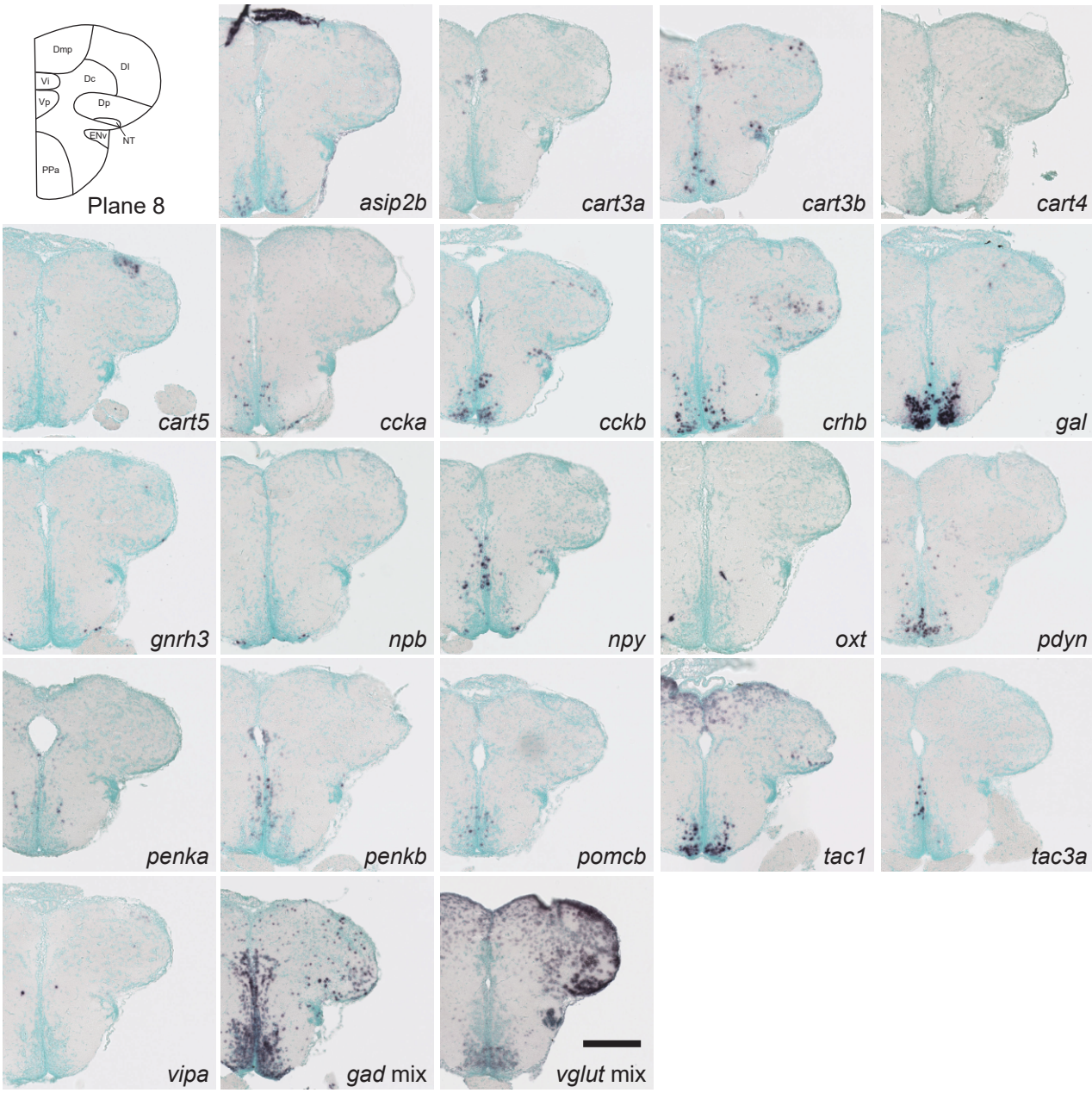


Figure 9

A schematic of plane 8 and microscopic images of genes expressed in the plane 8. Scale bar = 200 μm.

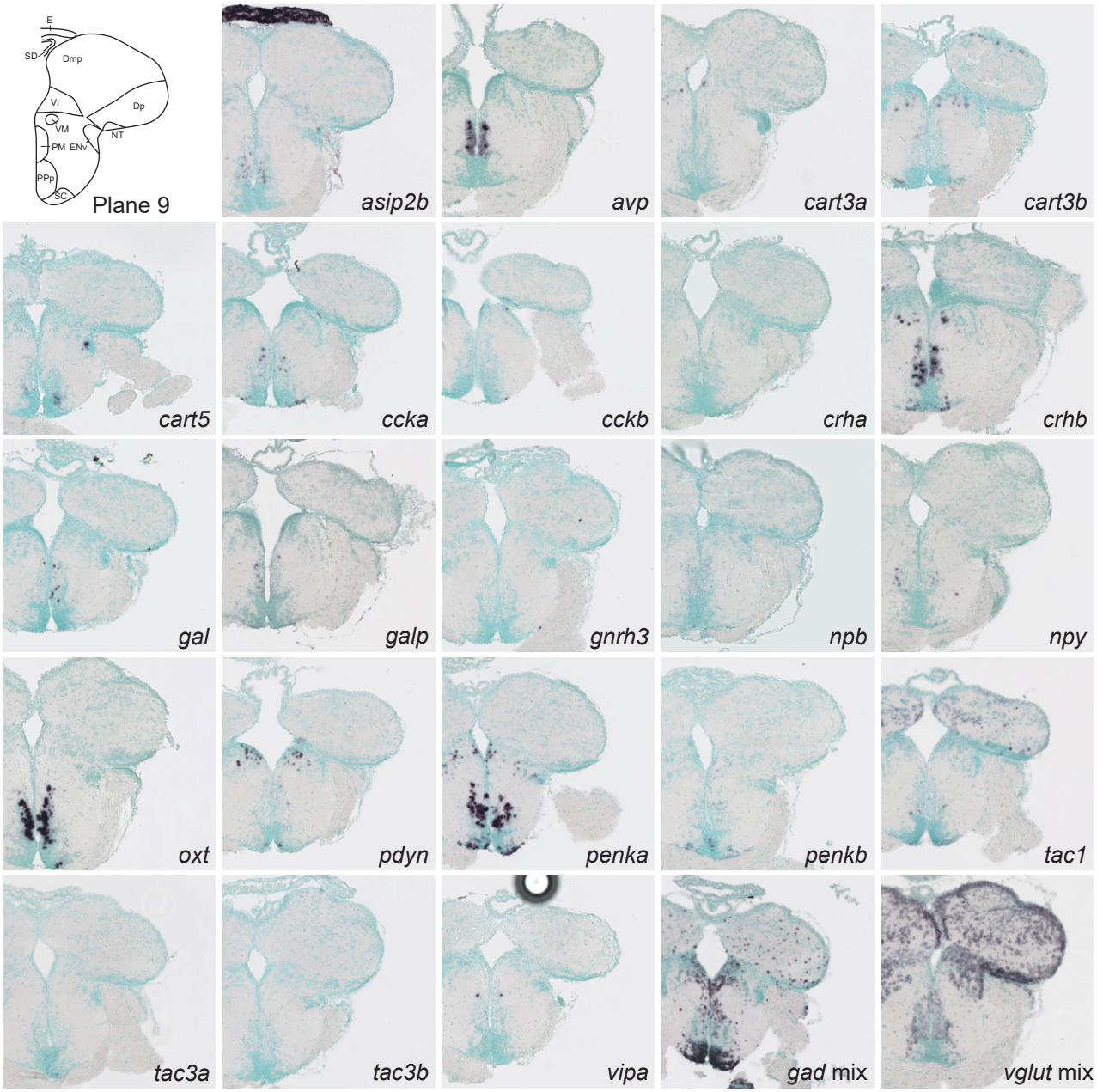


Figure 10

A schematic of plane 9 and microscopic images of genes expressed in the plane 9. Scale bar = 200 μm.

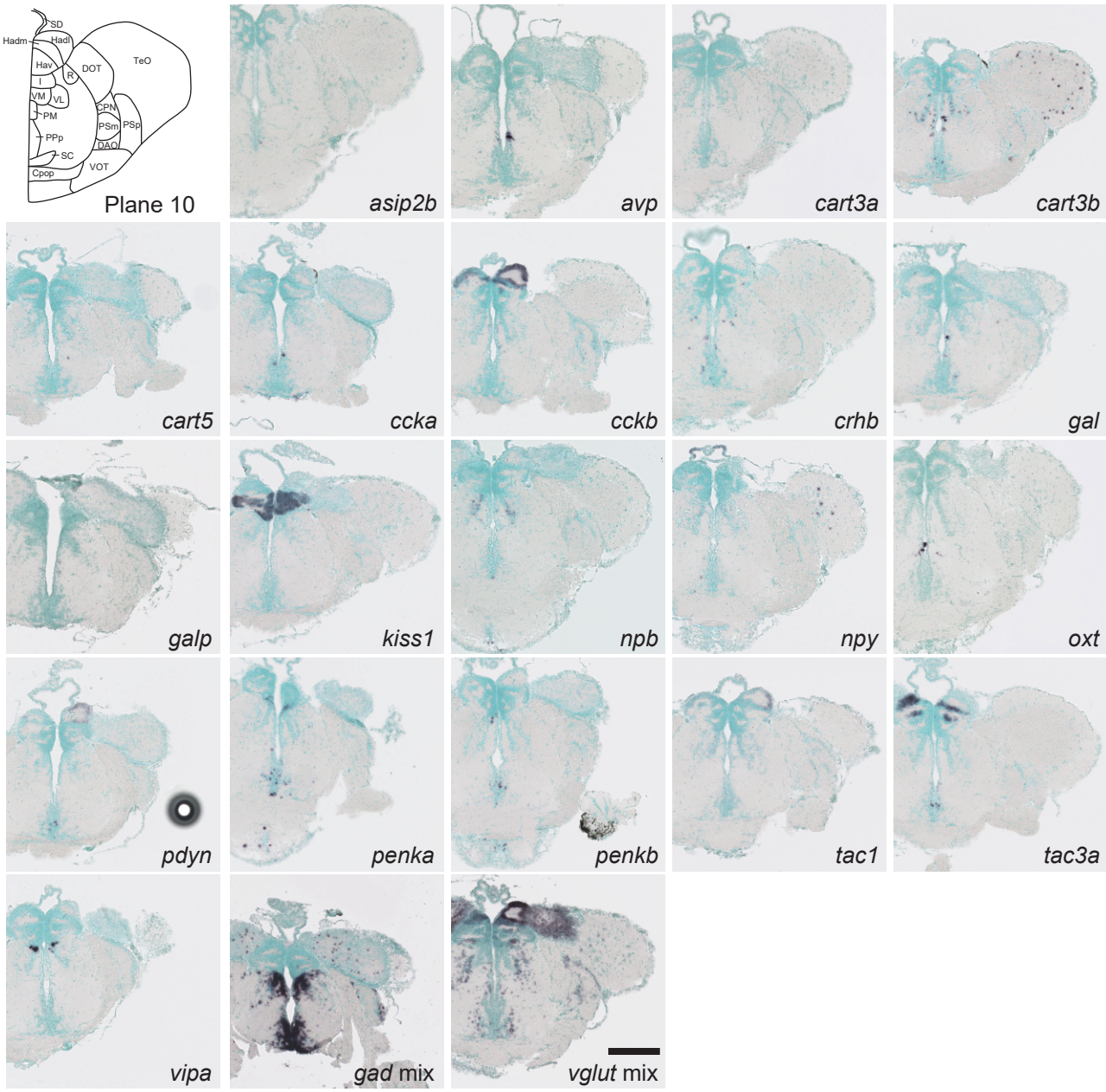


Figure 11

A schematic of plane 10 and microscopic images of genes expressed in the plane 10. Scale bar = 200 μm.

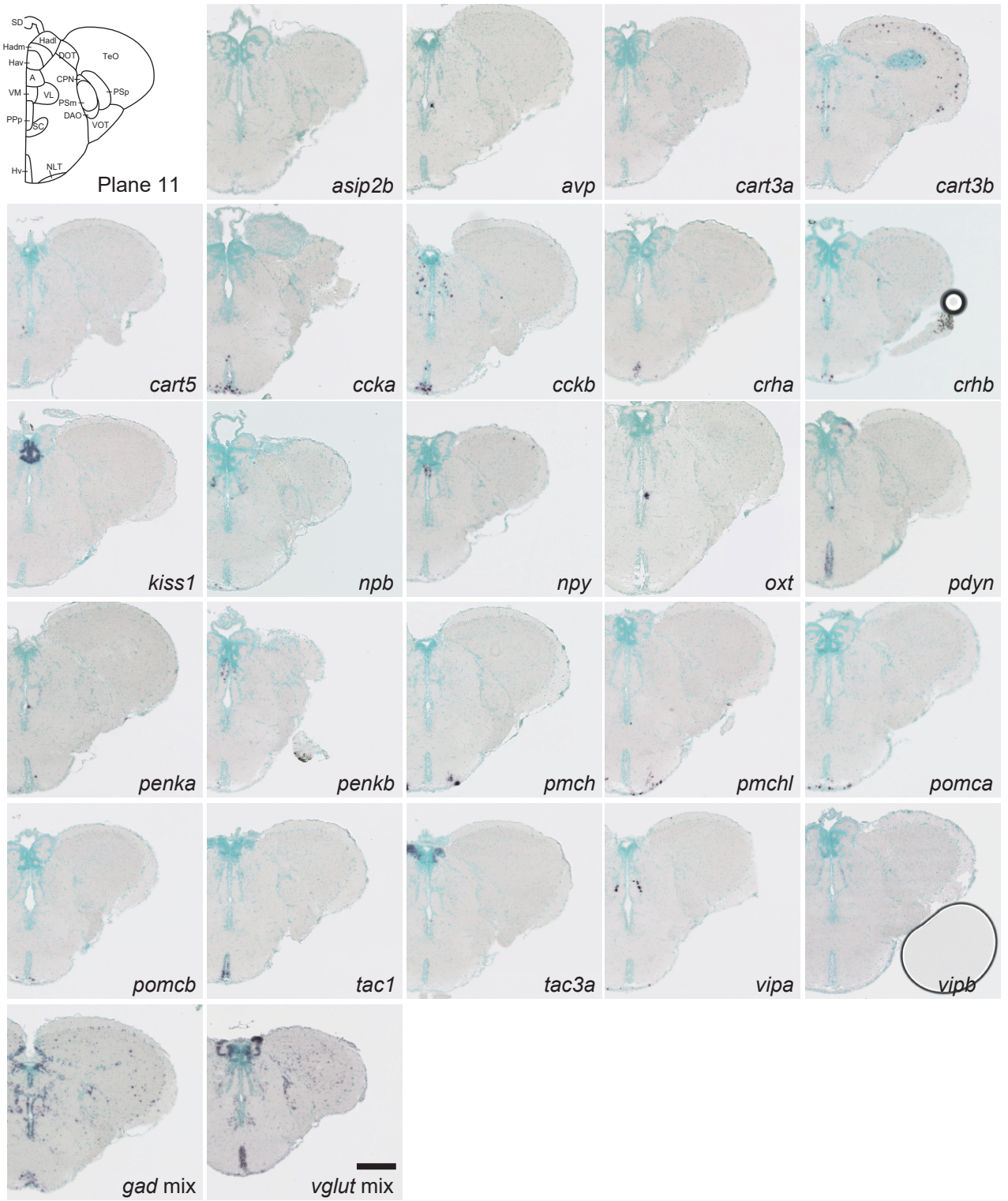


Figure 12

A schematic of plane 11 and microscopic images of genes expressed in the plane 11. Scale bar = 200 μm.

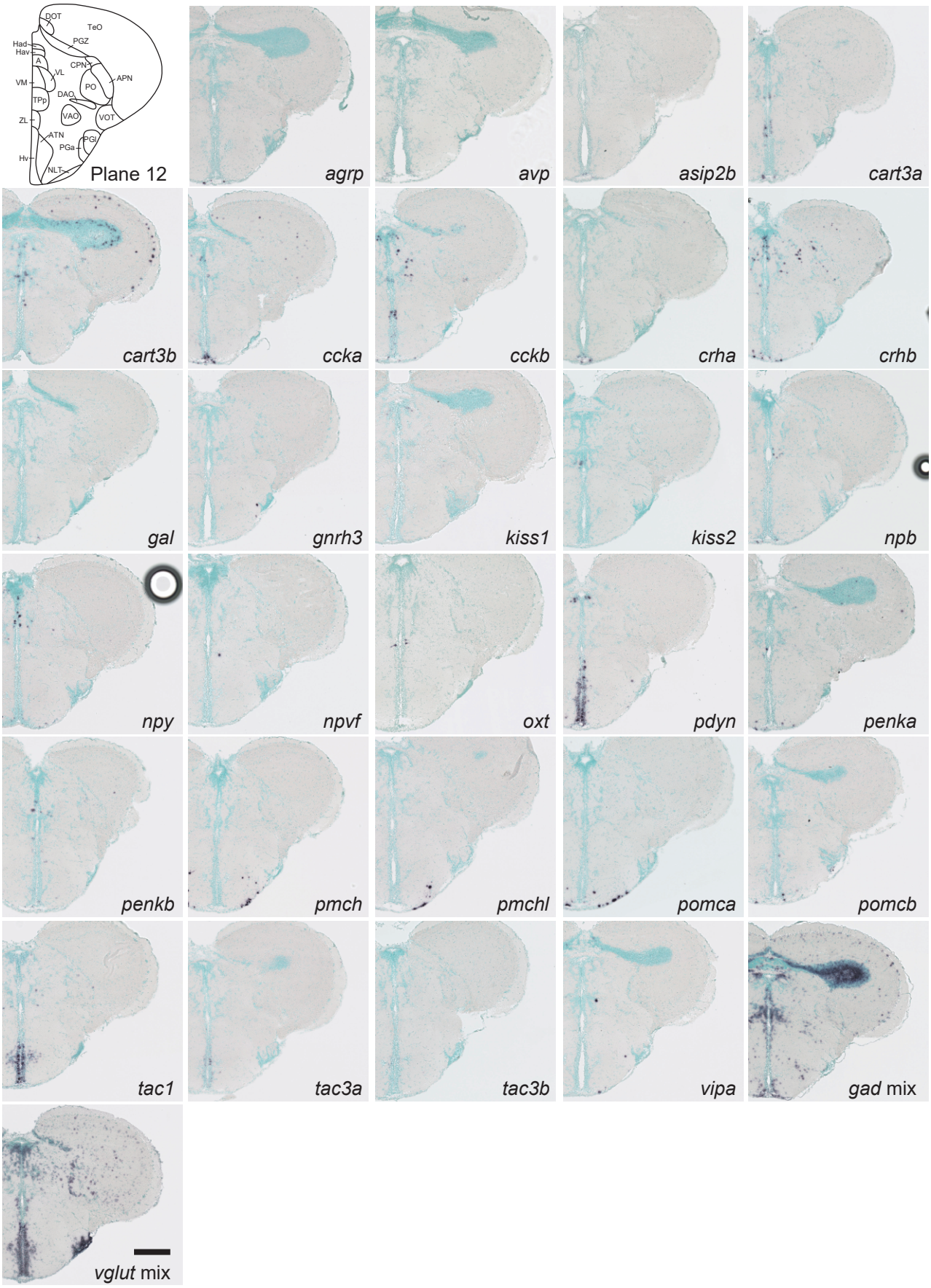


Figure 13

A schematic of plane 12 and microscopic images of genes expressed in the plane 12. Scale bar = 200 µm.

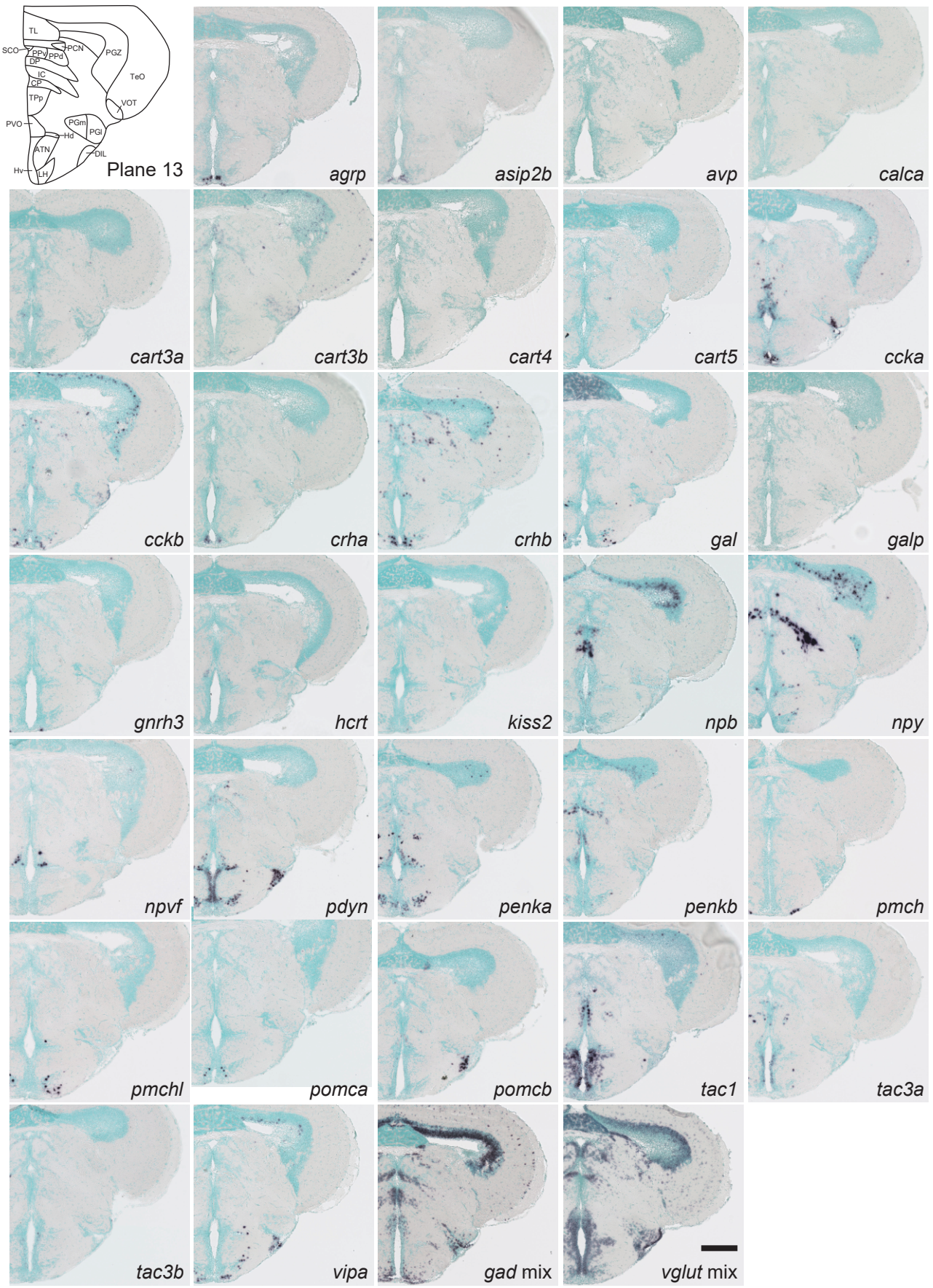


Figure 14

A schematic of plane 13 and microscopic images of genes expressed in the plane 13. Scale bar = 200 μm.

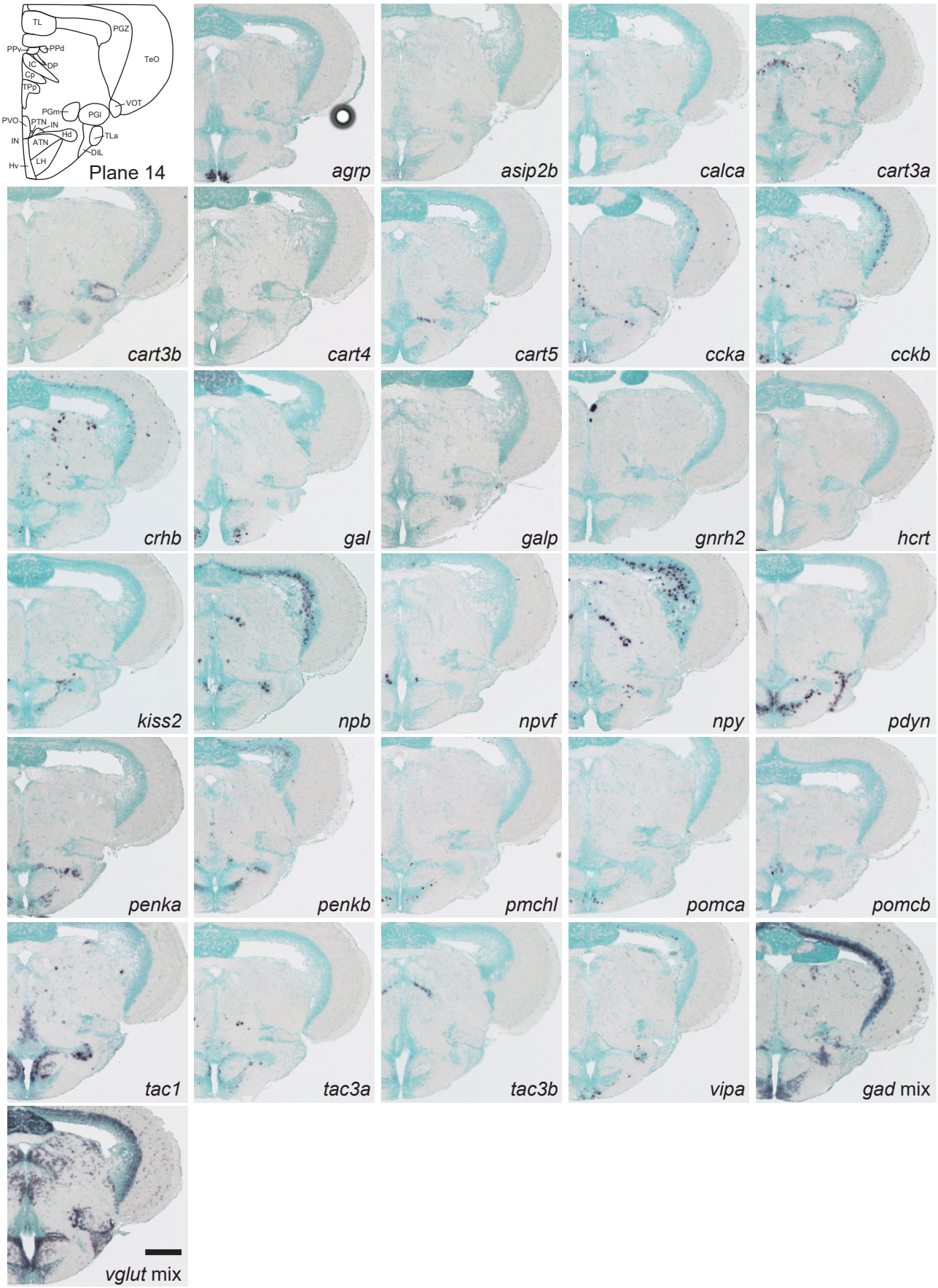


Figure 15

A schematic of plane 14 and microscopic images of genes expressed in the plane 14. Scale bar = 200 μm.

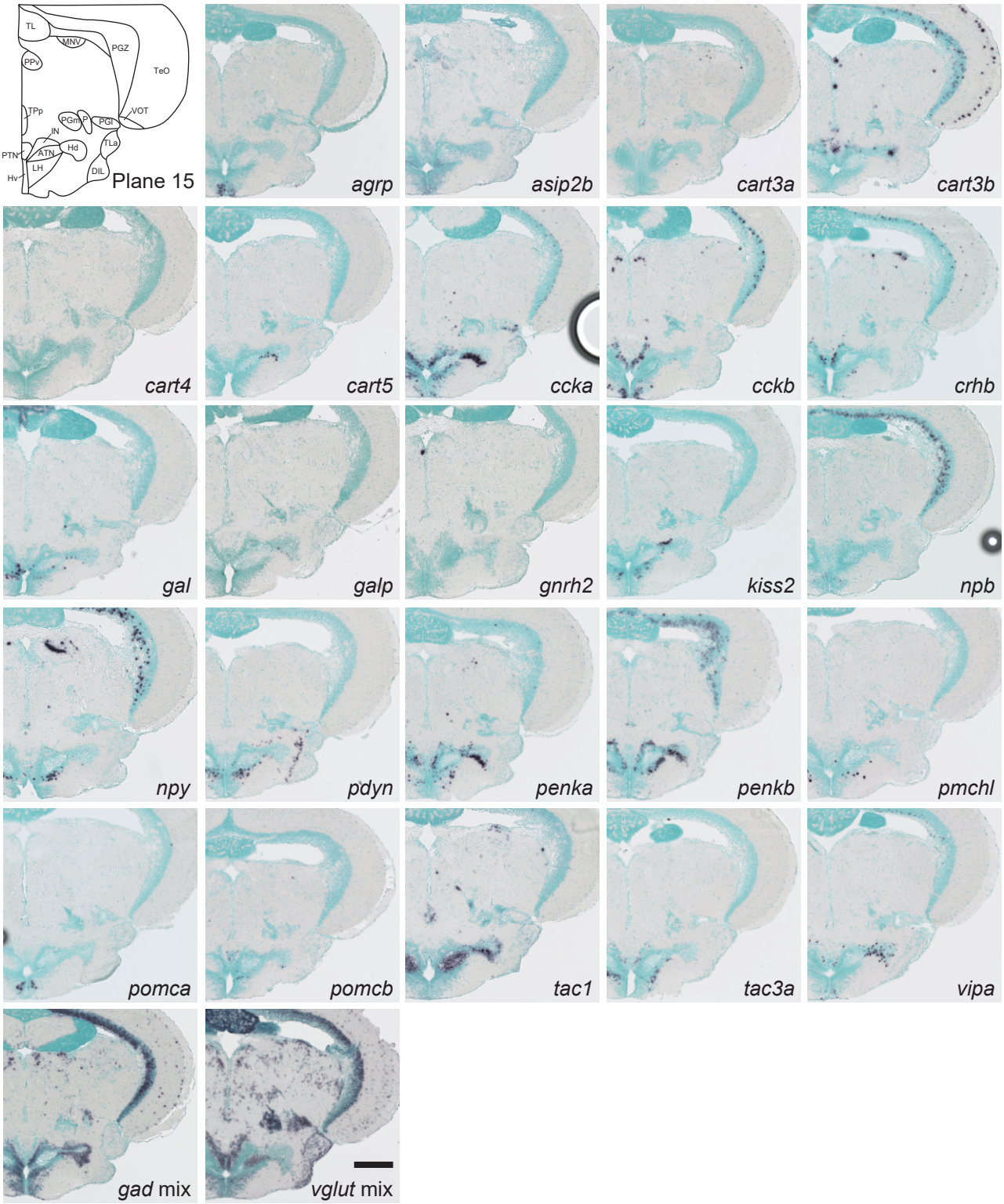


Figure 16

A schematic of plane 15 and microscopic images of genes expressed in the plane 15. Scale bar = 200 μm.

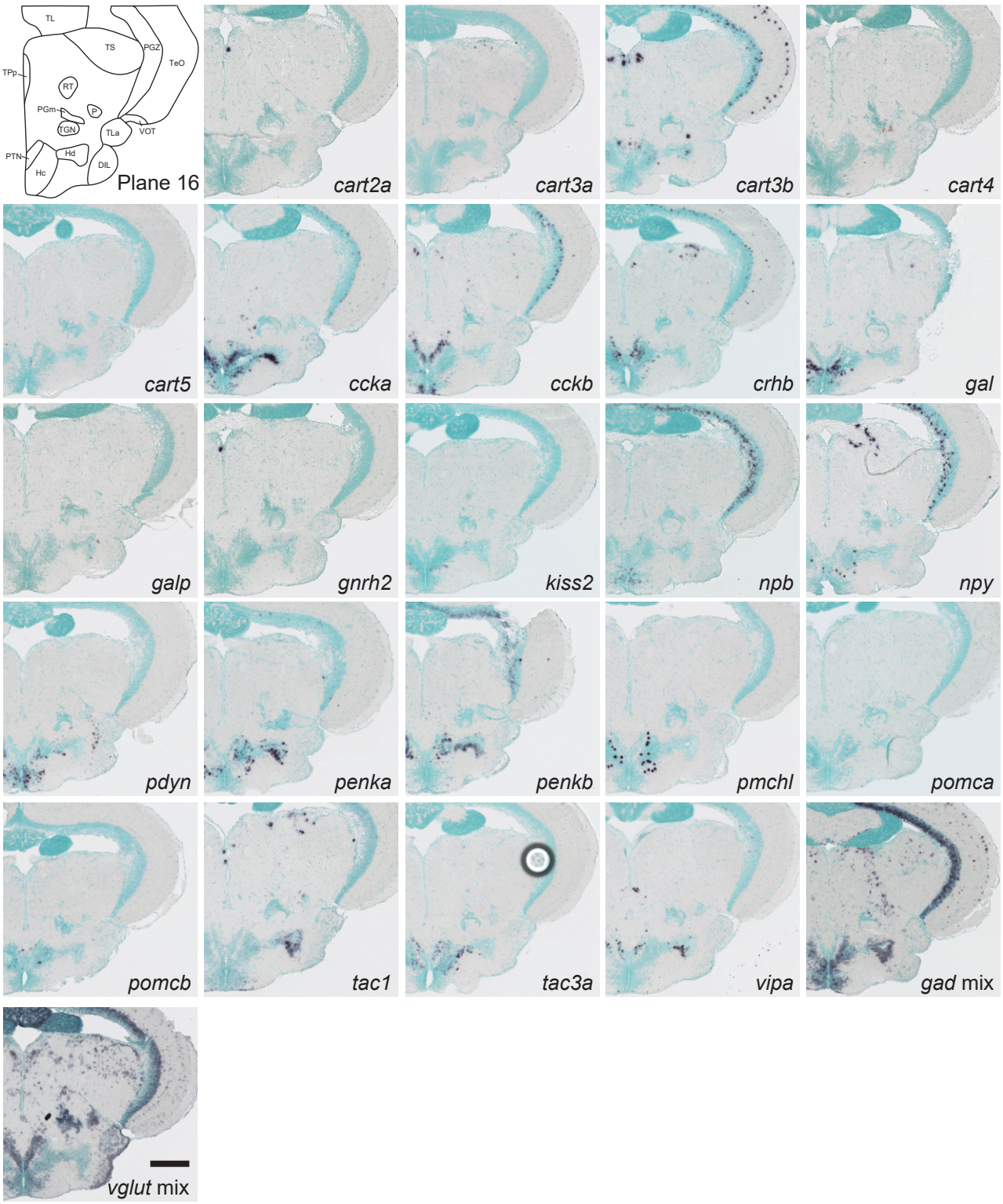


Figure 17

A schematic of plane 16 and microscopic images of genes expressed in the plane 16. Scale bar = 200 μm.

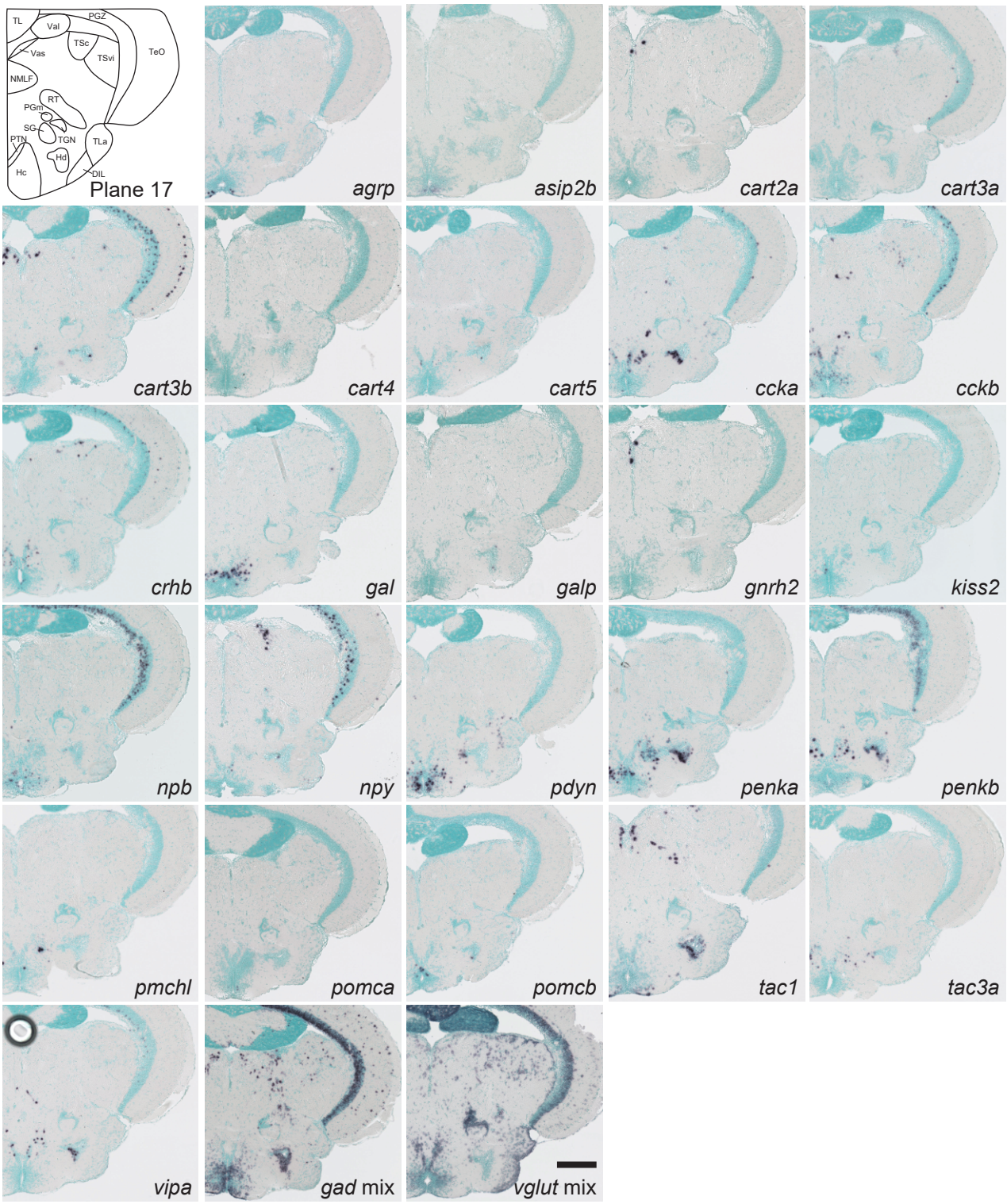


Figure 18

A schematic of plane 17 and microscopic images of genes expressed in the plane 17. Scale bar = 200 μm.

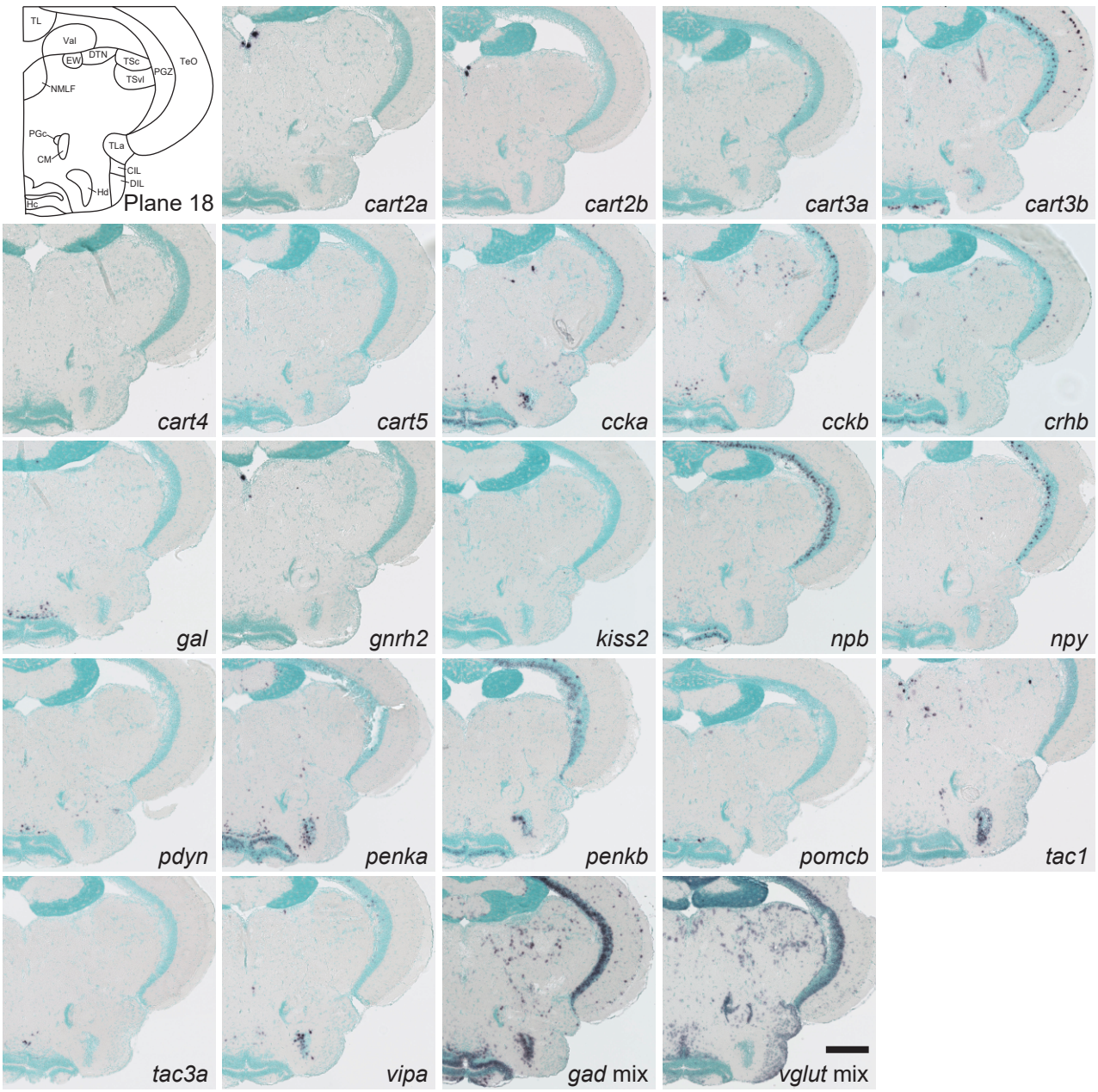


Figure 19

A schematic of plane 18 and microscopic images of genes expressed in the plane 18. Scale bar = 200 μm.

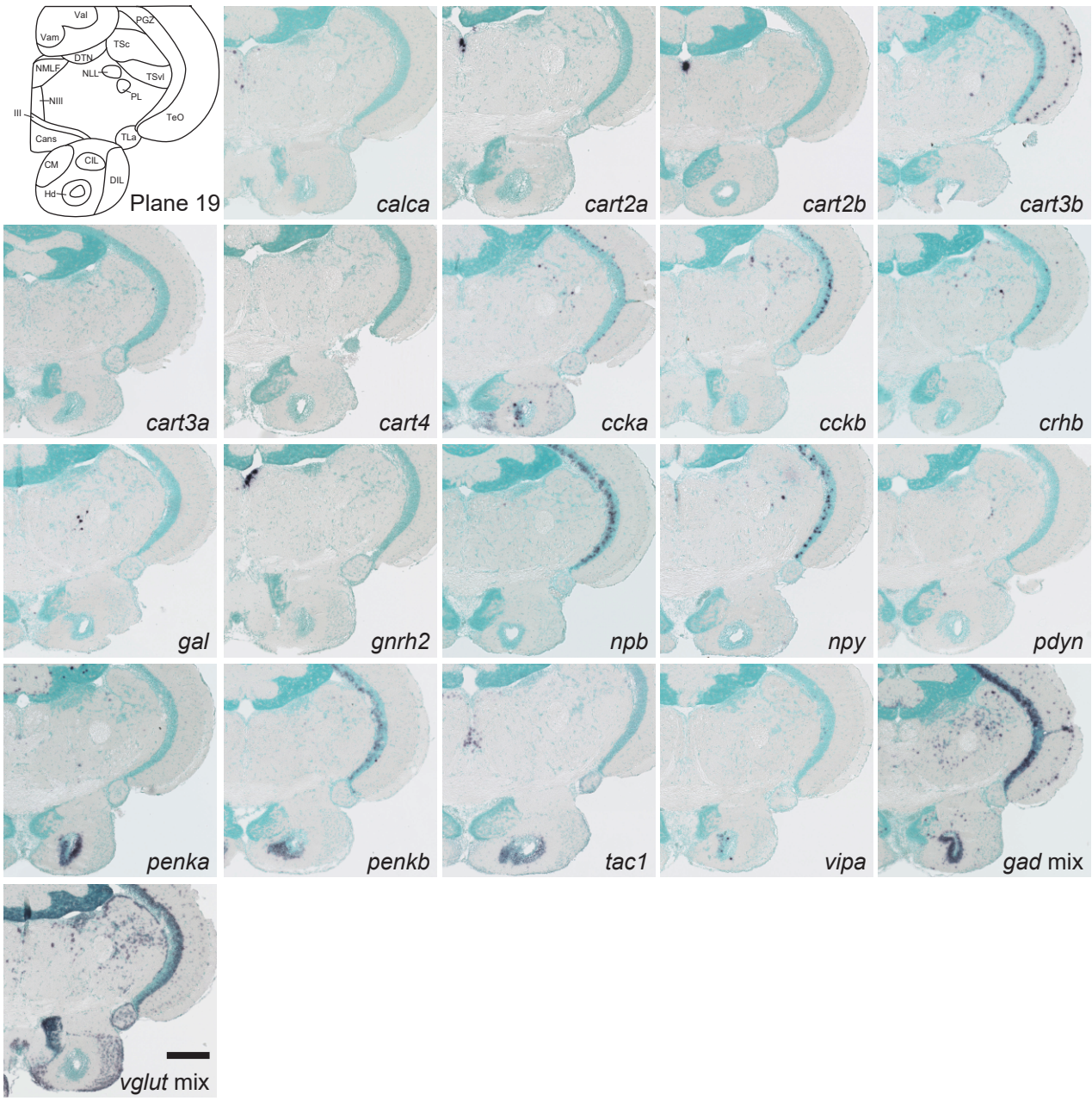


Figure 20

A schematic of plane 19 and microscopic images of genes expressed in the plane 19. Scale bar = 200 μm.

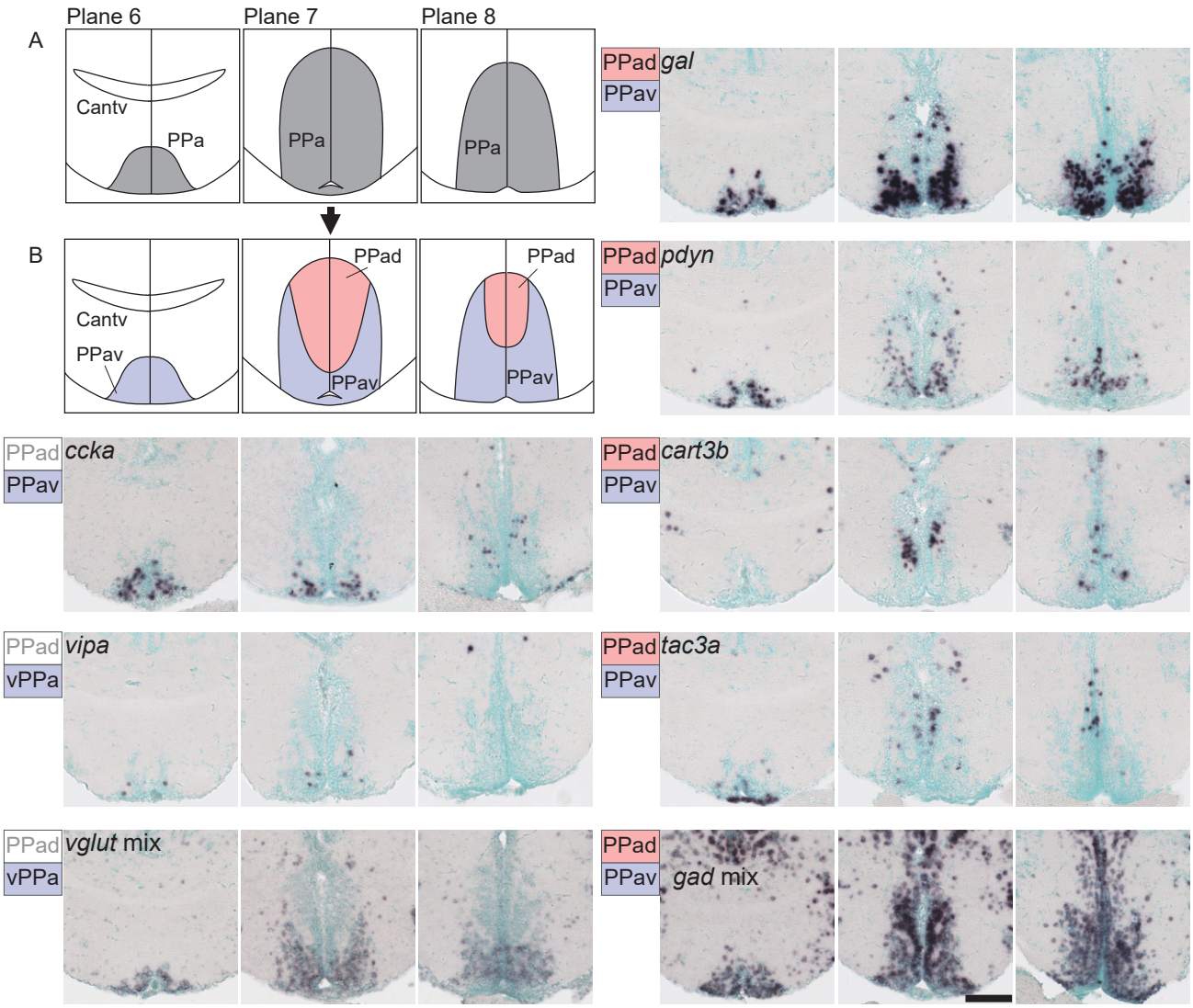


Figure 21

Subnuclei in the anterior part of parvocellular preoptic nucleus (PPa). (A) The PPa (grey) in the original zebrafish brain atlas. (B) The PPa can be divided into 2 subnuclei: the ventral part of PPa (PPav, pink) and the dorsal part of PPa (PPad, purple). Neuropeptides such as *ccka* and *vipa* and *vglut* mix are exclusively expressed in the PPav. *gal* and *pdyn* are expressed in both the PPad and PPav, but predominantly in the PPav. *cart3b* and *tac3a* are also expressed in the PPav and PPad, but predominantly in the PPad. *gad* mix is expressed in both the PPad and PPav. Scale bar = 100 µm.

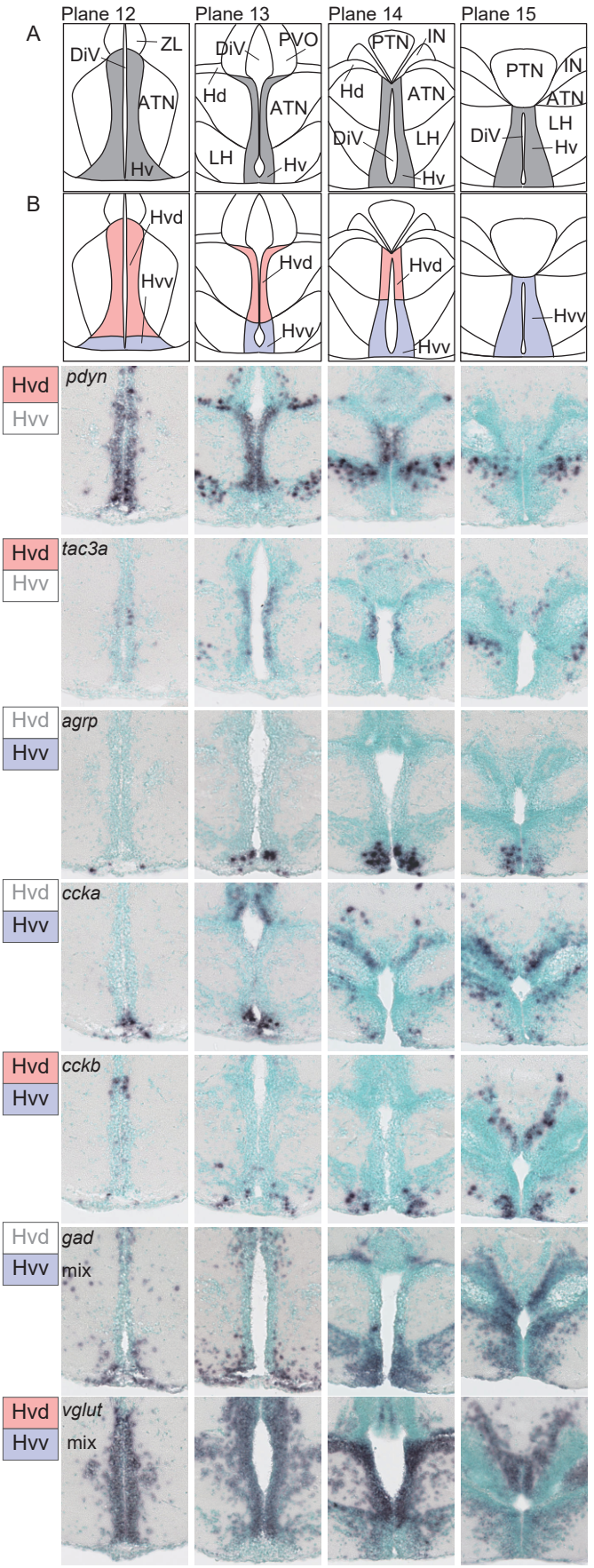


Figure 22

Subnuclei in the ventral zone of periventricular hypothalamus (Hv). (A) The Hv (grey) in the original zebrafish brain atlas. (B) The Hv can be divided into 2 subnuclei: the ventral part of Hv (Hvv, purple) and the dorsal part of Hv (Hvd, pink). Neuropeptides such as *pdyn* and *tac3a* are expressed only in the Hvd, whereas *agrp* and *ccka* are expressed only in the Hvv. *cckb* is expressed in both the Hvd and Hvv. *gad* mix is predominantly expressed in the Hvv and the *vglut* mix is more densely expressed in the Hvd. Scale bar = 100 µm.

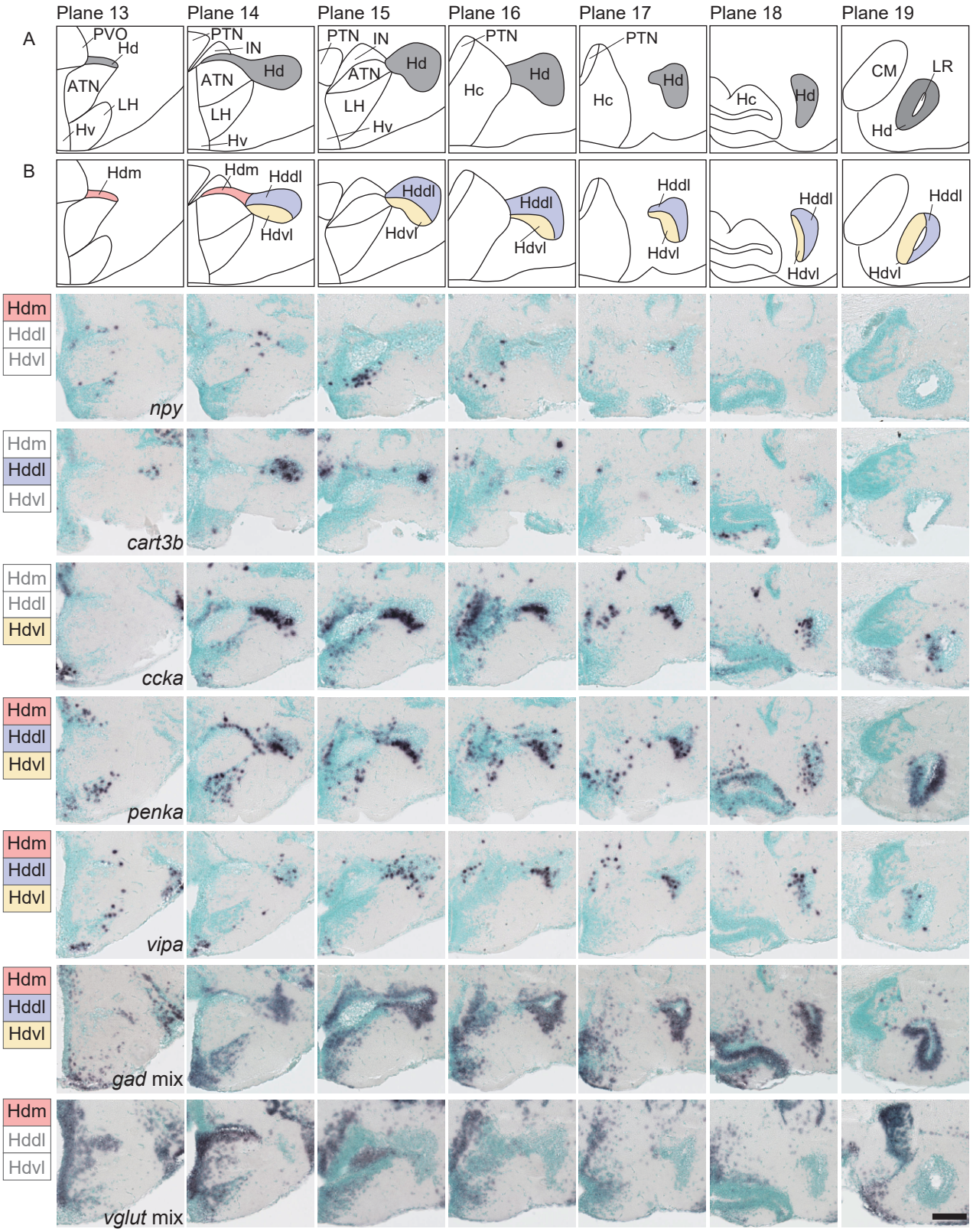


Figure 23

Subnuclei in the dorsal zone of the periventricular hypothalamus (Hd). (A) The Hd (grey) in the original zebrafish brain atlas. (B) The Hd can be divided into 3 subnuclei: the medial part of Hd (Hdm, pink), the dorsolateral part of Hd (Hddl, purple), and the ventrolateral part of Hd (Hdvl, yellow). Some neuropeptides are expressed solely in the Hdm, Hddl, or Hdvl (represented by *npy*, *cart*, and *ccka*, respectively). Neuropeptides such as *penka* and *vipa* are expressed in all three subnuclei. *gad* mix is also expressed in all subnuclei. *vglut* mix is absent in all subnuclei. Scale bar = 100 μm.

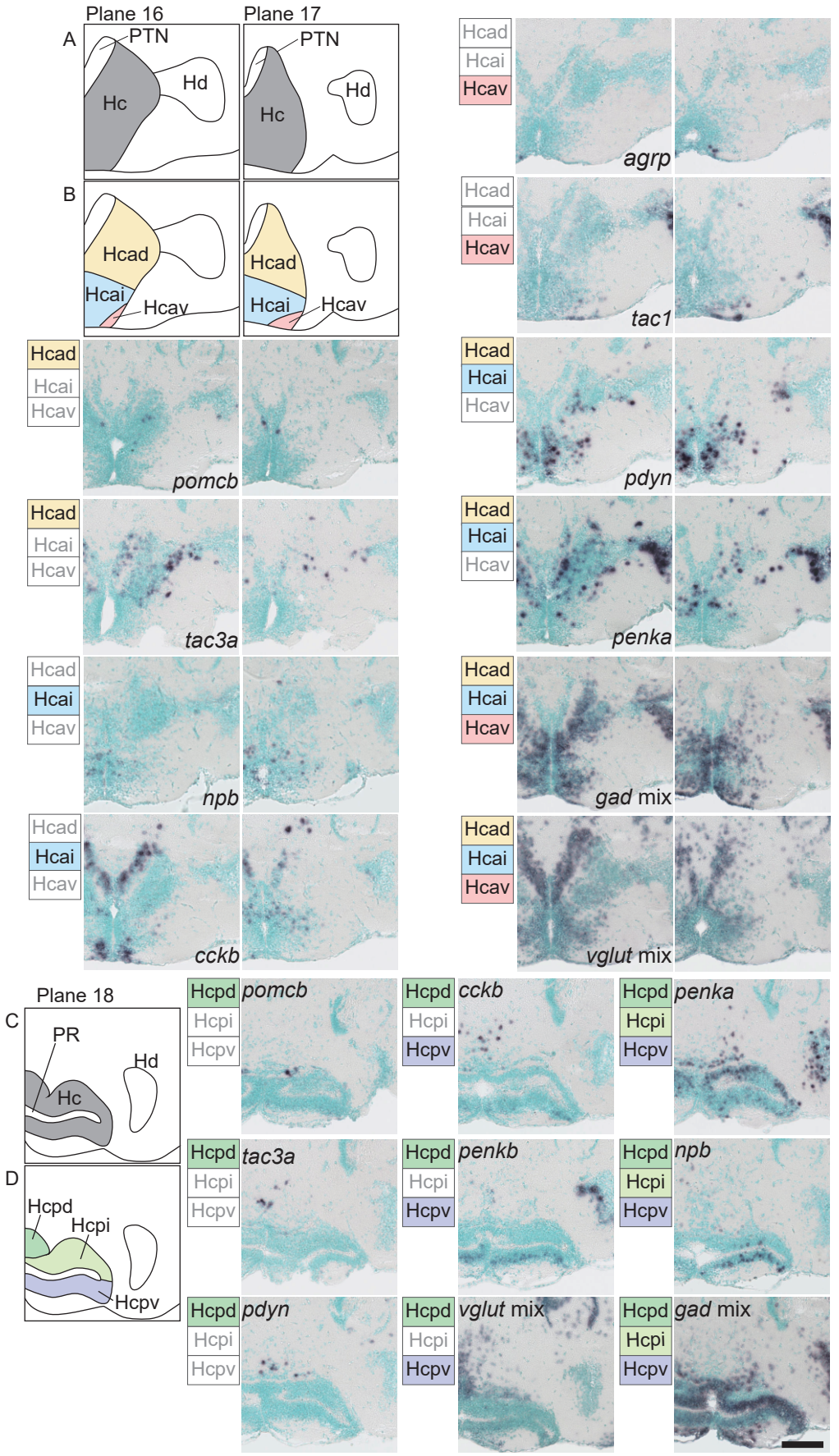
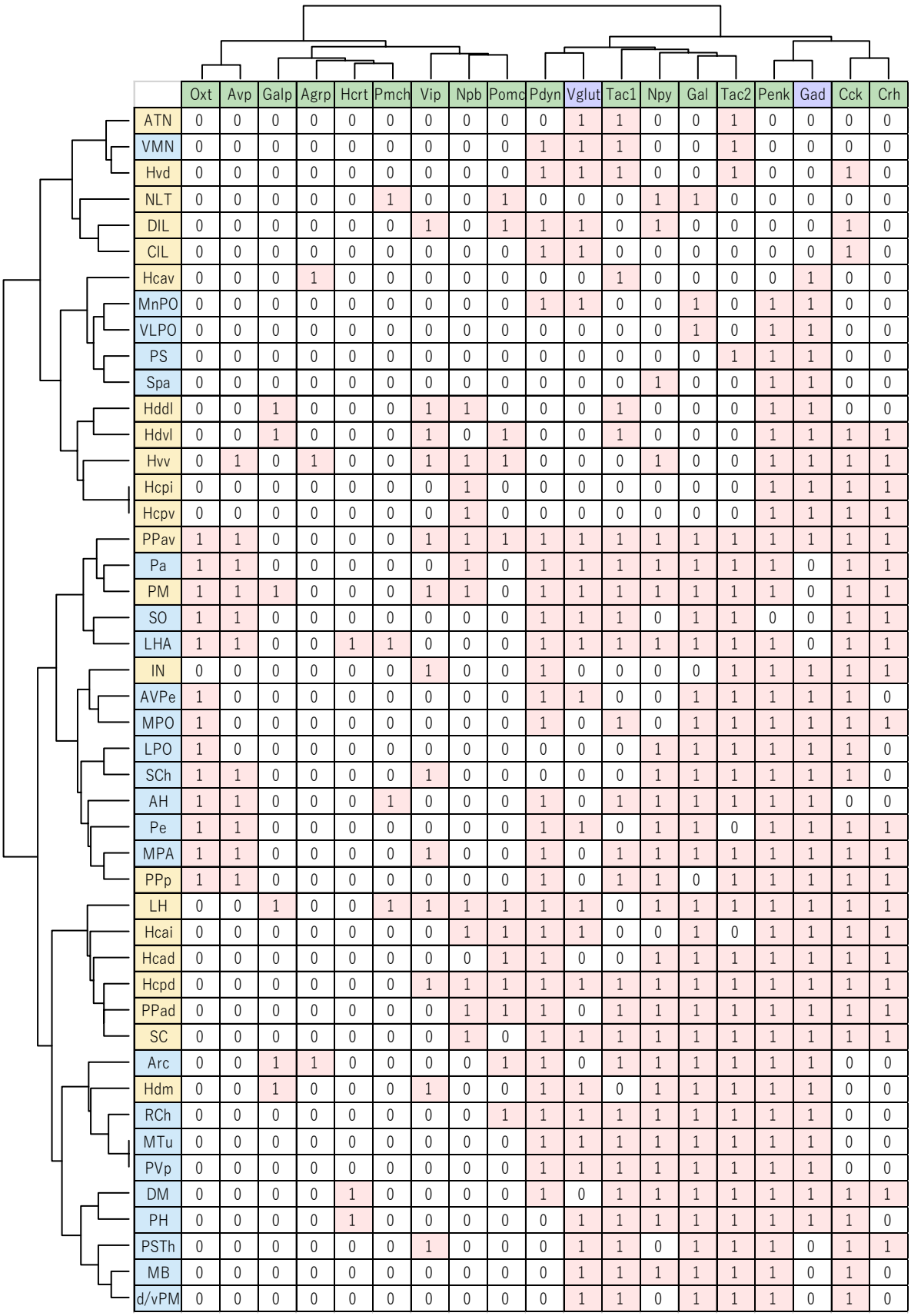

Plane 16
Plane 17
A
PTN
Hc
Hd
B
Hcad
Hcai
Hcav
agrp
tac1
pomcb
pdyn
tac3a
penka
npb
gad mix
cckb
vglut mix
Plane 18
C
PR
D
Hcpd
Hcpi
Hcpv
penkb

Figure 24

Subnuclei in the caudal zone of the periventricular hypothalamus (Hc). (A) The anterior part of Hc (grey) in the original zebrafish brain atlas. (B) The anterior part of Hc can be divided into 3 subnuclei: the anterodorsal part of Hc (Hcad, yellow), the anterointermediate part of Hc (Hcai, blue), and the anteroventral part of Hc (Hcav, pink). Neuropeptides such as *pomcb* and *tac3a* are solely expressed in the Hcad. Neuropeptides such as *npb* and *cckb* are expressed exclusively in the Hcai. *agrp* and *tac1* are only expressed in the Hcav. Neuropeptides such as *cckb*, *pdyn*, and *penka* are expressed across multiple subnuclei. *gad* mix and *vglut* mix are expressed in all three subnuclei. (C) Posterior part of Hc (grey) in original zebrafish brain atlas. (D) The posterior part of Hc can be divided into 3 subnuclei: the posterodorsal part of Hc (Hcpd, green), the posterointermediate part of Hc (Hcpi, light green), and the posteroventral part of Hc (Hcpv, purple). Neuropeptides such as *pomcb*, *tac3a*, and *pdyn* are solely expressed in the Hcpd. *cckb*, *penkb*, and *vglut* mix are expressed in the Hcpd and Hcpv. *penka*, *npb*, and *gad* mix are expressed in all three subnuclei. Scale bar = 100 μm.



	Oxt	Avp	Galp	Agrp	Hcrt	Pmch	Vip	Npb	Pomc	Pdyn	Vglut	Tac1	Npy	Gal	Tac2	Penk	Gad	Cck	Crh
ATN	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
VMN	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0
Hvd	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	0
NLT	0	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0
DIL	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	0	0	1	0
CIL	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0
Hcav	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
MnPO	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1	0	0
VLPO	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0
PS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
Spa	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0
Hddl	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	1	1	0	0
Hdvl	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0	1	1	1	1
Hvv	0	1	0	1	0	0	1	1	1	0	0	0	1	0	0	1	1	1	1
Hcpi	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1
Hcpv	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1
PPav	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Pa	1	1	0	0	0	0	0	1	0	1	1	1	1	1	1	1	0	1	1
PM	1	1	1	0	0	0	1	1	0	1	1	1	1	1	1	1	0	1	1
SO	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	1	1
LHA	1	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	0	1	1
IN	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	1	1	1
AVPe	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	0
MPO	1	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	1	1	1
LPO	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0
SCh	1	1	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	1	0
AH	1	1	0	0	0	1	0	0	0	1	0	1	1	1	1	1	1	0	0
Pe	1	1	0	0	0	0	0	0	0	1	1	0	1	1	0	1	1	1	1
MPA	1	1	0	0	0	0	1	0	0	1	0	1	1	1	1	1	1	1	1
PPp	1	1	0	0	0	0	0	0	0	1	0	1	1	0	1	1	1	1	1
LH	0	0	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1
Hcai	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0	1	1	1	1
Hcad	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	1	1
Hcpd	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
PPad	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	1
SC	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1
Arc	0	0	1	1	0	0	0	0	1	1	0	1	1	1	1	1	1	0	0
Hdm	0	0	1	0	0	0	1	0	0	1	1	0	1	1	1	1	1	0	0
RCh	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0
MTu	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0
PVp	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0
DM	0	0	0	0	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1
PH	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0
PSTh	0	0	0	0	0	0	1	0	0	0	1	1	0	1	1	1	0	1	1
MB	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0
d/vPM	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	0

Figure 25

Comparison of neuropeptide/neurotransmitter gene expression in the hypothalamus between zebrafish and mouse. Expression of neuropeptides (green) and neurotransmitter markers (purple) in the hypothalamic nuclei of zebrafish (light yellow) and mice (light blue) were extracted from ISH results (zebrafish) and the Allen Brain Atlas and previous papers (mouse) and summarized in a table (expressed = 1, not expressed = 0). Zebrafish and mouse nuclei and expressed genes were hierarchically clustered by Ward's method.