Supplemental Information

Transcriptional and Spatial Resolution of Cell Types in the Mammalian Habenula

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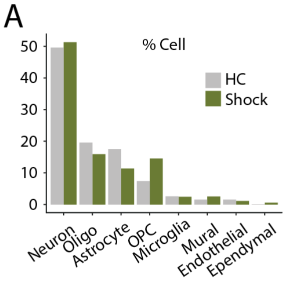
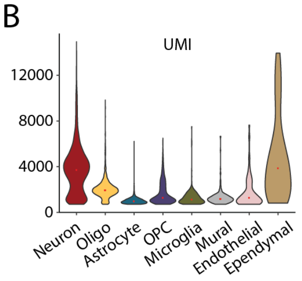
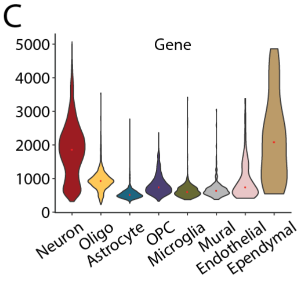
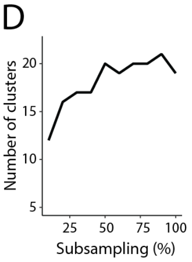
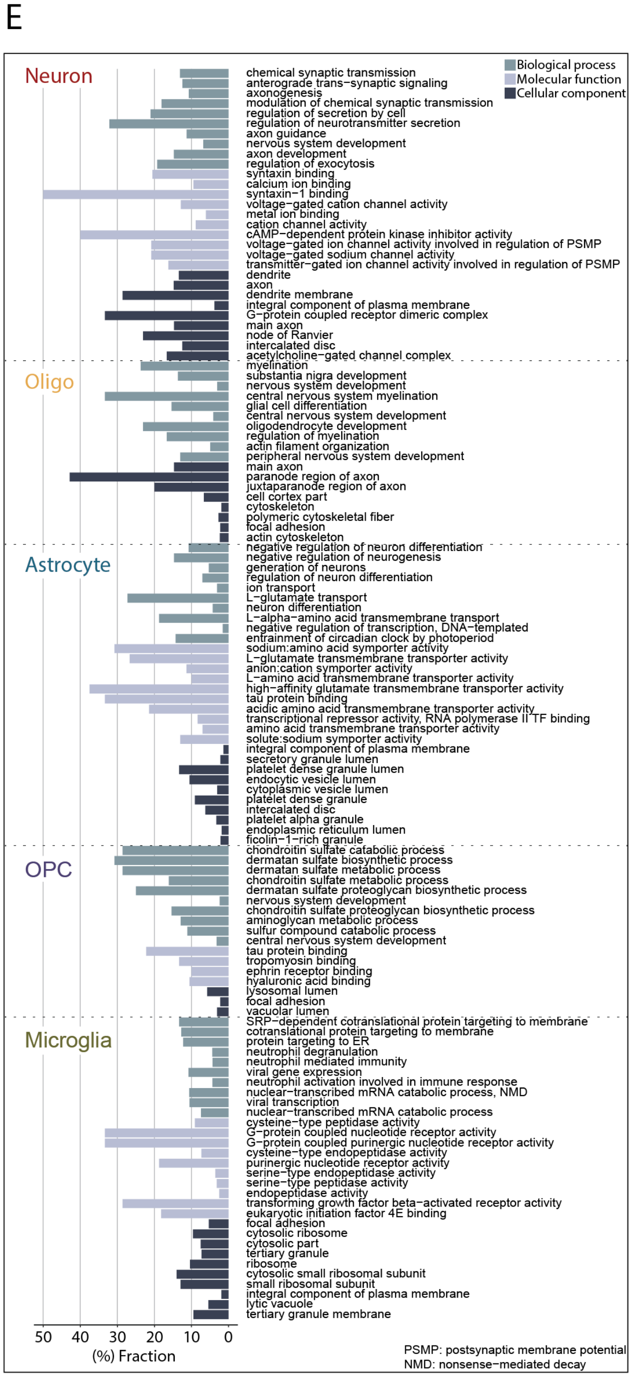
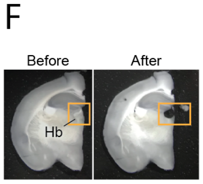

A
% Cell
50
40
30
20
10
0
HC
Shock
Neuron
Oligo
Astrocyte
OPC
Microglia
Mural
Endothelial
Ependymal

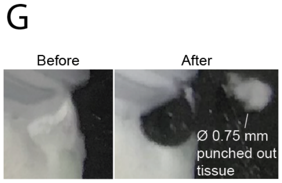

B
UMI
12000
8000
4000
0
Neuron
Oligo
Astrocyte
OPC
Microglia
Mural
Endothelial
Ependymal


C
Gene
5000
4000
3000
2000
1000
0
Neuron
Oligo
Astrocyte
OPC
Microglia
Mural
Endothelial
Ependymal


D
Number of clusters
20
15
10
5
25
50
75
100
Subsampling (%)


E
Biological process
Molecular function
Cellular component
Neuron
chemical synaptic transmission
anterograde trans−synaptic signaling
axonogenesis
modulation of chemical synaptic transmission
regulation of secretion by cell
regulation of neurotransmitter secretion
axon guidance
nervous system development
axon development
regulation of exocytosis
syntaxin binding
calcium ion binding
syntaxin−1 binding
voltage−gated cation channel activity
metal ion binding
cation channel activity
cAMP−dependent protein kinase inhibitor activity
voltage−gated ion channel activity involved in regulation of PSMP
voltage−gated sodium channel activity
transmitter−gated ion channel activity involved in regulation of PSMP
dendrite
axon
dendrite membrane
integral component of plasma membrane
G−protein coupled receptor dimeric complex
main axon
node of Ranvier
intercalated disc
acetylcholine−gated channel complex
Oligo
myelination
substantia nigra development
nervous system development
central nervous system myelination
glial cell differentiation
central nervous system development
oligodendrocyte development
regulation of myelination
actin filament organization
peripheral nervous system development
main axon
paranode region of axon
juxtaparanode region of axon
cell cortex part
cytoskeleton
polymeric cytoskeletal fiber
focal adhesion
actin cytoskeleton
Astrocyte
negative regulation of neuron differentiation
negative regulation of neurogenesis
generation of neurons
regulation of neuron differentiation
ion transport
L−glutamate transport
neuron differentiation
L−alpha−amino acid transmembrane transport
negative regulation of transcription, DNA−templated
entrainment of circadian clock by photoperiod
sodium:amino acid symporter activity
L−glutamate transmembrane transporter activity
anion:cation symporter activity
L−amino acid transmembrane transporter activity
high−affinity glutamate transmembrane transporter activity
tau protein binding
acidic amino acid transmembrane transporter activity
transcriptional repressor activity, RNA polymerase II TF binding
amino acid transmembrane transporter activity
solute:sodium symporter activity
integral component of plasma membrane
secretory granule lumen
platelet dense granule lumen
endocytic vesicle lumen
cytoplasmic vesicle lumen
platelet dense granule
intercalated disc
platelet alpha granule
endoplasmic reticulum lumen
ficolin−1−rich granule
OPC
chondroitin sulfate catabolic process
dermatan sulfate biosynthetic process
dermatan sulfate metabolic process
chondroitin sulfate metabolic process
dermatan sulfate proteoglycan biosynthetic process
nervous system development
chondroitin sulfate proteoglycan biosynthetic process
aminoglycan metabolic process
sulfur compound catabolic process
central nervous system development
tau protein binding
tropomyosin binding
ephrin receptor binding
hyaluronic acid binding
lysosomal lumen
focal adhesion
vacuolar lumen
Microglia
SRP−dependent cotranslational protein targeting to membrane
cotranslational protein targeting to membrane
protein targeting to ER
neutrophil degranulation
neutrophil mediated immunity
viral gene expression
neutrophil activation involved in immune response
nuclear−transcribed mRNA catabolic process, NMD
viral transcription
nuclear−transcribed mRNA catabolic process
cysteine−type peptidase activity
G−protein coupled nucleotide receptor activity
G−protein coupled purinergic nucleotide receptor activity
cysteine−type endopeptidase activity
purinergic nucleotide receptor activity
serine−type endopeptidase activity
serine−type peptidase activity
endopeptidase activity
transforming growth factor beta−activated receptor activity
eukaryotic initiation factor 4E binding
focal adhesion
cytosolic ribosome
cytosolic part
tertiary granule
ribosome
cytosolic small ribosomal subunit
small ribosomal subunit
integral component of plasma membrane
lytic vacuole
tertiary granule membrane
50
40
30
20
10
0
(%) Fraction
PSMP: postsynaptic membrane potential
NMD: nonsense−mediated decay


F
Before
After
Hb


G
Before
After
Ø 0.75 mm
punched out
tissue

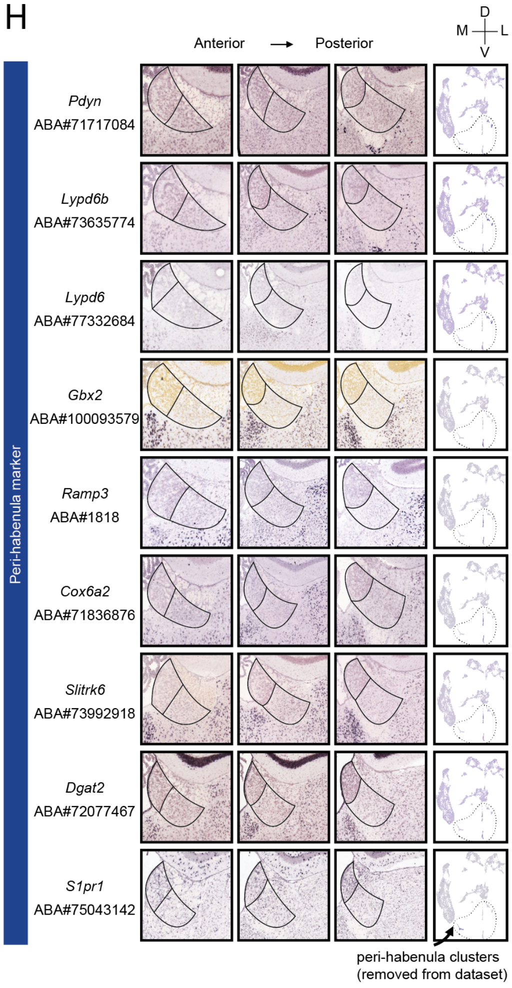

H
Anterior
Posterior
D
M
L
V
Peri-habenula marker
Pdyn
ABA#71717084
Lypd6b
ABA#73635774
Lypd6
ABA#77332684
Gbx2
ABA#100093579
Ramp3
ABA#1818
Cox6a2
ABA#71836876
Slitrk6
ABA#73992918
Dgat2
ABA#72077467
S1pr1
ABA#75043142
peri-habenula clusters
(removed from dataset)

Figure S1. Additional information for scRNAseq experiments, related to Figure 1. A. Percentage of cells in each cell type from home cage (HC) or shock groups. **B and C**. Violin plot showing the UMI (**B**) and gene (**C**) distributions in each cell type. **D.** The number of clusters of sub-sampled habenula cells. **E.** Enriched gene ontology terms and fractions of all genes belonging to each term for major cell types. Only terms with adjusted $p<0.1$ were shown. GO Biological Process 2018, GO Molecular Function 2018, and GO Cellular Component 2018 were referenced. **F, G.** Representative images showing tissue punching of the habenula. **H.** Representative images from the Allen Brain Atlas (left 3 images) showing the expressions of peri-habenular marker genes and their expression levels in the UMAP (most right images). Based on the expressions of the peri-habenular markers, peri-habenula clusters (dotted line) were removed from downstream analysis.

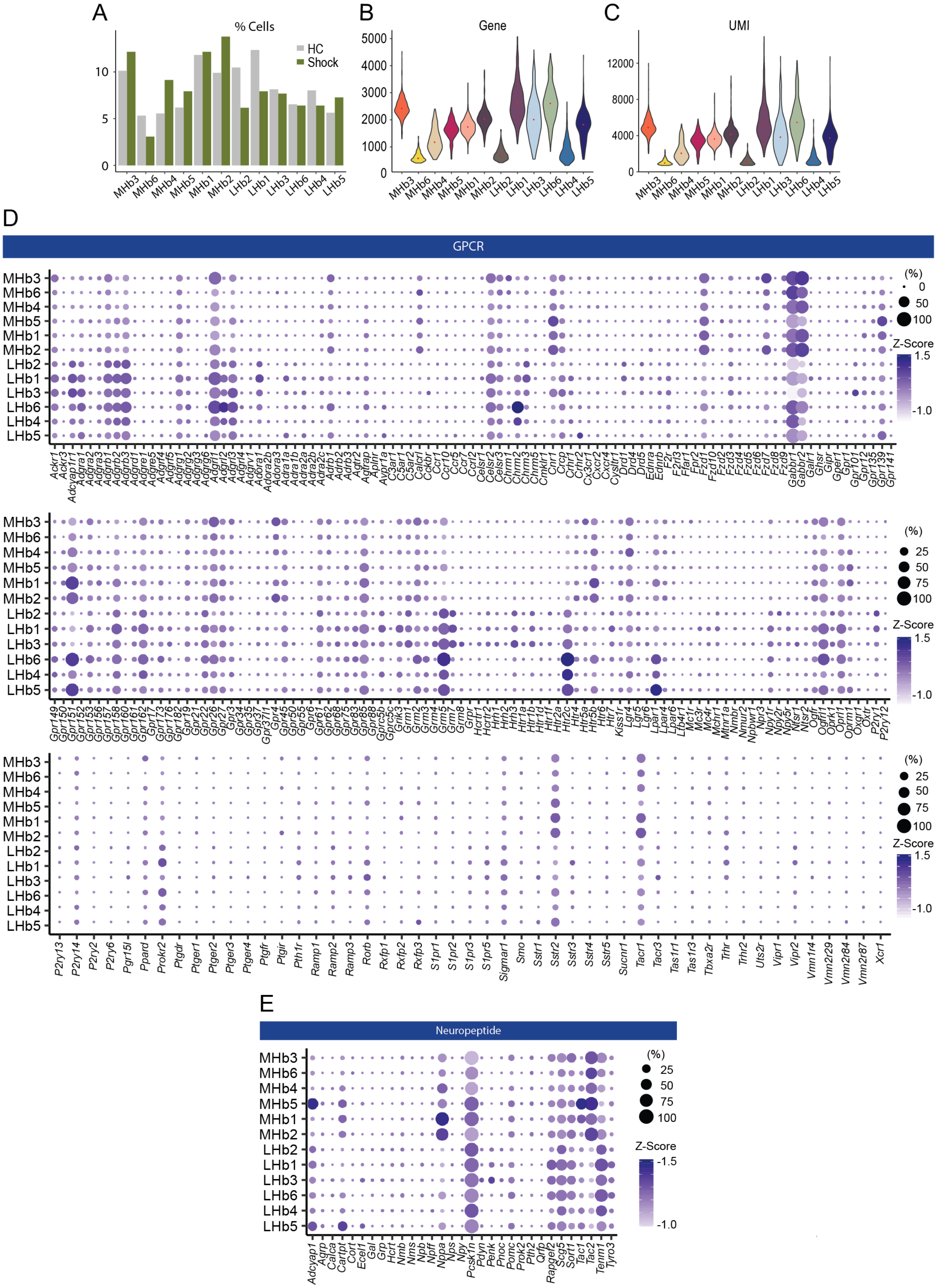

A
% Cells
HC
Shock
0
5
10
MHb3
MHb6
MHb4
MHb5
MHb1
MHb2
LHb2
LHb1
LHb3
LHb6
LHb4
LHb5
B
Gene
0
1000
2000
3000
4000
5000
MHb3
MHb6
MHb4
MHb5
MHb1
MHb2
LHb2
LHb1
LHb3
LHb6
LHb4
LHb5
C
UMI
0
4000
8000
12000
MHb3
MHb6
MHb4
MHb5
MHb1
MHb2
LHb2
LHb1
LHb3
LHb6
LHb4
LHb5
D
GPCR
MHb3
MHb6
MHb4
MHb5
MHb1
MHb2
LHb2
LHb1
LHb3
LHb6
LHb4
LHb5
Ackr1
Ackr3
Adcyap1r1
Adgra1
Adgra2
Adgra3
Adgrb1
Adgrb2
Adgrb3
Adgrd1
Adgre1
Adgre5
Adgrf4
Adgrf5
Adgrg1
Adgrg2
Adgrg3
Adgrg6
Adgrl1
Adgrl2
Adgrl3
Adgrl4
Adgrv1
Adora1
Adora2b
Adora3
Adra1a
Adra1b
Adra2a
Adra2b
Adra2c
Adrb1
Adrb2
Adrb3
Agtr2
Agtrap
Aplnr
Avpr1a
C3ar1
C5ar1
C5ar2
Calcrl
Cckbr
Ccr1
Ccr10
Ccr5
Ccr7
Ccrl2
Celsr1
Celsr2
Celsr3
Chrm1
Chrm2
Chrm3
Chrm5
Cmklr1
Cnr1
Crcp
Crhr1
Crhr2
Cx3cr1
Cxcr2
Cxcr4
Cysltr1
Drd1
Drd4
Drd5
Ednra
Ednrb
F2r
F2rl3
Ffar1
Fpr2
Fzd1
Fzd10
Fzd2
Fzd3
Fzd4
Fzd5
Fzd6
Fzd7
Fzd8
Fzd9
Gabbr1
Gabbr2
Galr1
Ghsr
Gipr
Gper1
Gpr1
Gpr101
Gpr12
Gpr135
Gpr139
Gpr141
(%)
0
50
100
Z-Score
1.5
-1.0
MHb3
MHb6
MHb4
MHb5
MHb1
MHb2
LHb2
LHb1
LHb3
LHb6
LHb4
LHb5
Gpr149
Gpr150
Gpr151
Gpr152
Gpr153
Gpr156
Gpr157
Gpr158
Gpr160
Gpr161
Gpr162
Gpr17
Gpr173
Gpr176
Gpr182
Gpr19
Gpr21
Gpr22
Gpr26
Gpr27
Gpr3
Gpr34
Gpr35
Gpr37
Gpr37l1
Gpr4
Gpr45
Gpr50
Gpr55
Gpr6
Gpr61
Gpr62
Gpr68
Gpr75
Gpr83
Gpr85
Gpr88
Gprc5b
Gprc5c
Grik3
Grm1
Grm2
Grm3
Grm4
Grm5
Grm7
Grm8
Grpr
Hcrtr1
Hcrtr2
Hrh1
Hrh2
Hrh3
Htr1a
Htr1b
Htr1d
Htr1f
Htr2a
Htr2c
Htr4
Htr5a
Htr5b
Htr6
Htr7
Kiss1r
Lgr4
Lgr5
Lgr6
Lpar1
Lpar4
Lpar6
Ltb4r1
Mc1r
Mc3r
Mc4r
Mchr1
Mtnr1a
Nmbr
Nmur2
Npbwr1
Npr3
Npy1r
Npy2r
Npy5r
Ntsr1
Ntsr2
Ogfr
Ogfrl1
Oprk1
Oprl1
Oprm1
Oxgr1
Oxtr
P2ry1
P2ry12
(%)
25
50
75
100
Z-Score
1.5
-1.0
MHb3
MHb6
MHb4
MHb5
MHb1
MHb2
LHb2
LHb1
LHb3
LHb6
LHb4
LHb5
P2ry13
P2ry14
P2ry2
P2ry6
Pgr15l
Ppard
Prokr2
Ptgdr
Ptger1
Ptger2
Ptger3
Ptger4
Ptgfr
Ptgir
Pth1r
Ramp1
Ramp2
Ramp3
Rorb
Rxfp1
Rxfp2
Rxfp3
S1pr1
S1pr2
S1pr3
S1pr5
Sigmar1
Smo
Sstr1
Sstr2
Sstr3
Sstr4
Sstr5
Sucnr1
Tacr1
Tacr3
Tas1r1
Tas1r3
Tbxa2r
Trhr
Trhr2
Uts2r
Vipr1
Vipr2
Vmn1r4
Vmn2r29
Vmn2r84
Vmn2r87
Xcr1
(%)
25
50
75
100
Z-Score
1.5
-1.0
E
Neuropeptide
MHb3
MHb6
MHb4
MHb5
MHb1
MHb2
LHb2
LHb1
LHb3
LHb6
LHb4
LHb5
Adcyap1
Agrp
Calca
Cartpt
Cort
Ecel1
Gal
Grp
Hcrt
Nmb
Nms
Npb
Npff
Nppa
Nps
Npy
Pcsk1n
Pdyn
Penk
Pnoc
Pomc
Prok2
Pth2
Qrfp
Rapgef2
Scg5
Sort1
Tac1
Tac2
Tenm1
Tyro3
(%)
25
50
75
100
Z-Score
-1.5
-1.0

Figure S2. Additional information for scRNAseq experiments on neuronal cells, related to Figure 2. A. Percentage of cells in each cell type from home cage (HC) or shock groups. **B and C**. Violin plot showing the gene (**B**) and UMI (**C**) distributions in each cell type. **D, E.** Dot plot illustrating scaled expression levels (color) and the proportions of expressing cells (dot size) of GPCR **(D)** and neuropeptide **(E)** in each cluster.

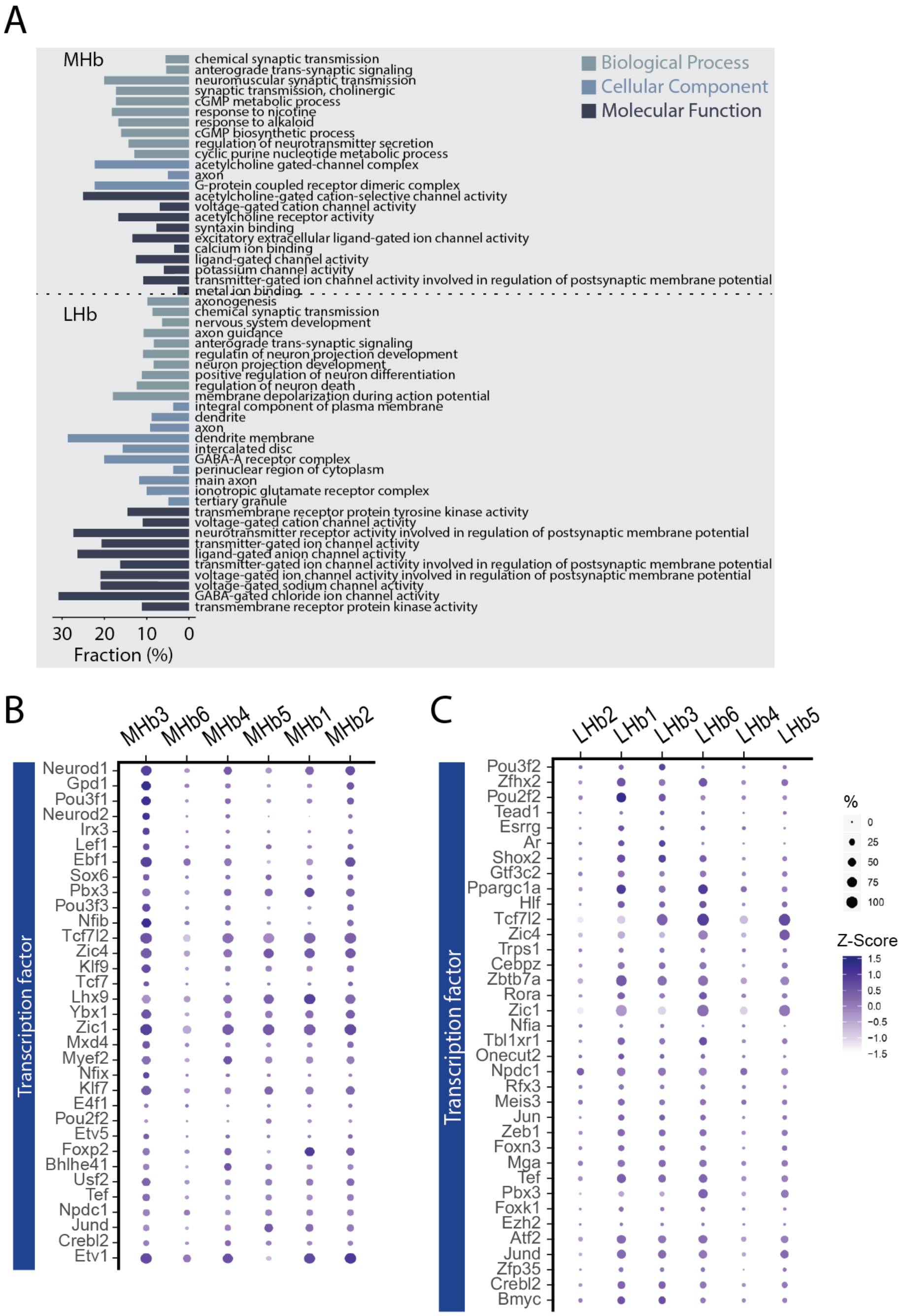


Figure S3. Additional information for scRNAseq experiments on neuronal cells, related to Figure 3. A. Enriched gene ontology terms and fractions of all genes belonging to each term in the MHb and LHb. Only terms with adjusted p<0.1 were shown. GO Biological Process 2018, GO Molecular Function 2018, and GO Cellular Component 2018 were referenced. **B and C**. Dot plot illustrating scaled expression levels (color) and the proportions of expressing cells (dot size) of transcription factors in MHb (**B**) and LHb (**C**) clusters. 33 (MHb) and 36 (LHb) TFs, top selective TFs regulating >80% of marker genes, were shown.

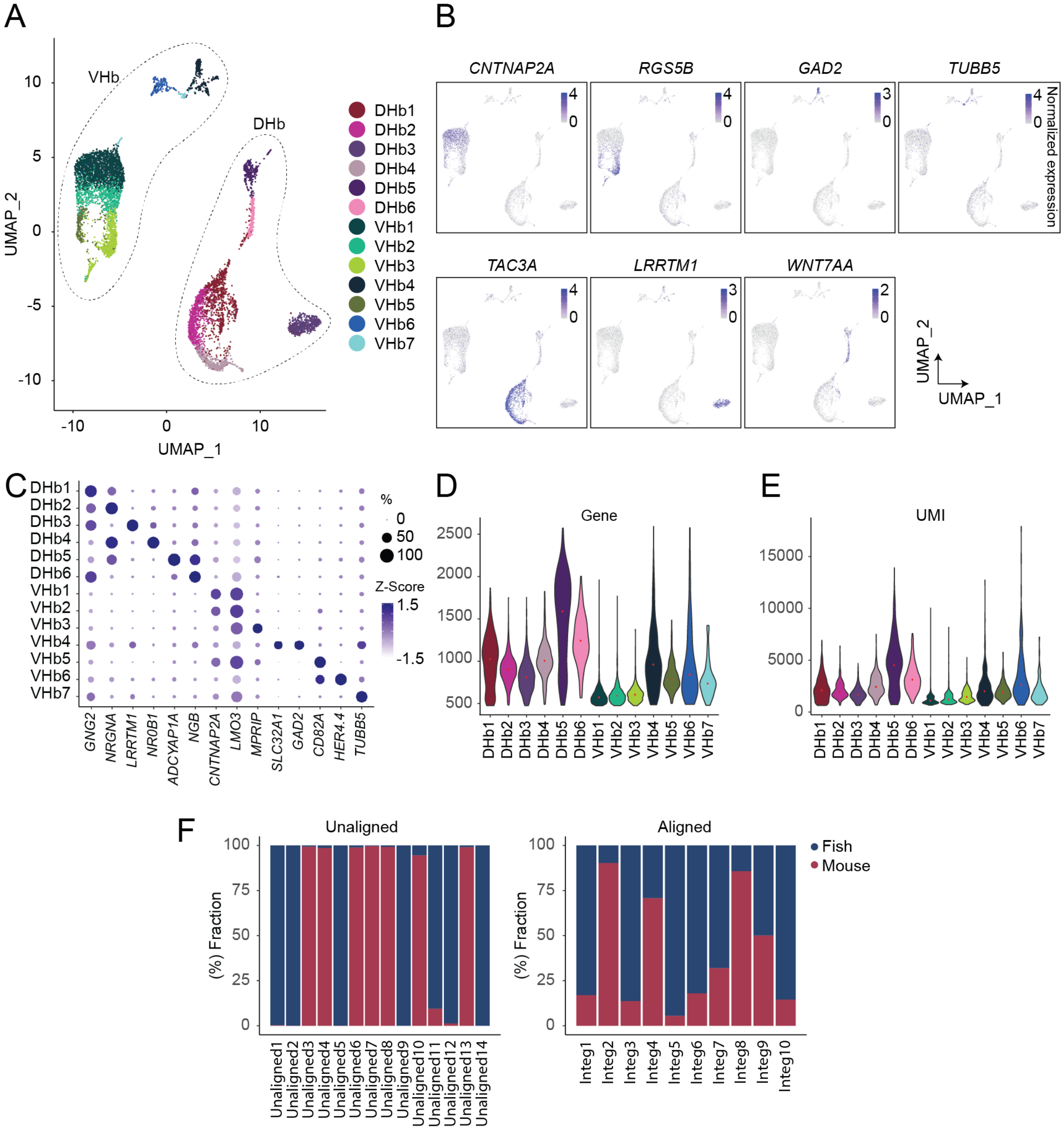


Figure S4. Additional information for scRNAseq analysis on adult zebrafish habenula neurons, related to Figure 4. A. UMAP visualization of neuronal clusters of zebrafish habenula. **B.** Expression plots illustrating expression levels of marker genes for dorsal or ventral parts of zebrafish habenula. **C.** Dot plot illustrating scaled expression levels (color) and the proportions of expressing cells (dot size) of discriminative marker genes for clusters. **D and E**. Violin plot showing the gene (**D**) and UMI (**E**) distributions in each cell type. **F.** Proportion of zebrafish or mouse cells in each unaligned (Left) and aligned (Right) cluster.

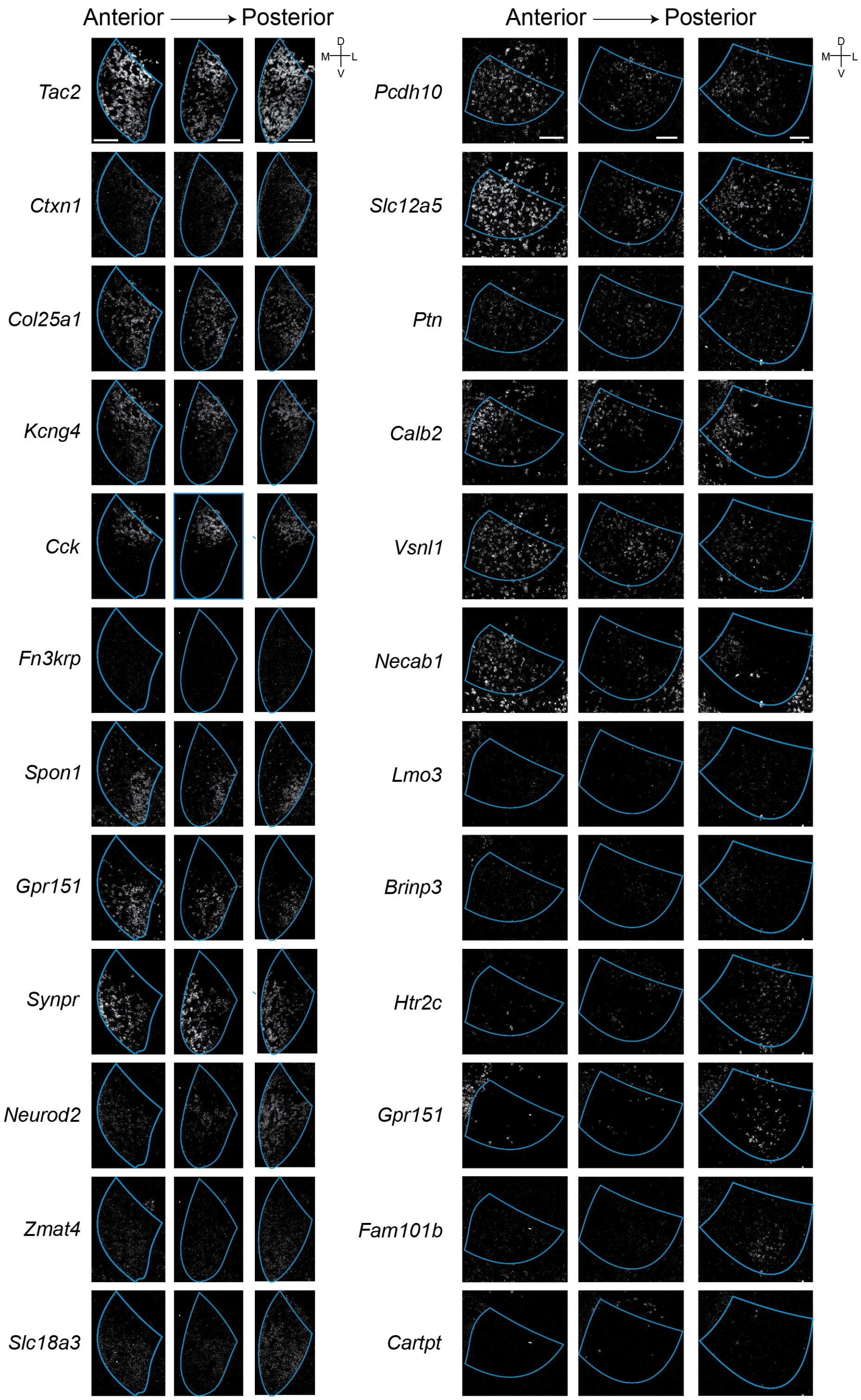


Figure S5. Additional information for HiPlex experiments, related to Figure 5. Representative FISH images of all 12 detected genes for the MHb (Left) and the LHb (Right). Scale bar: 100 µm.

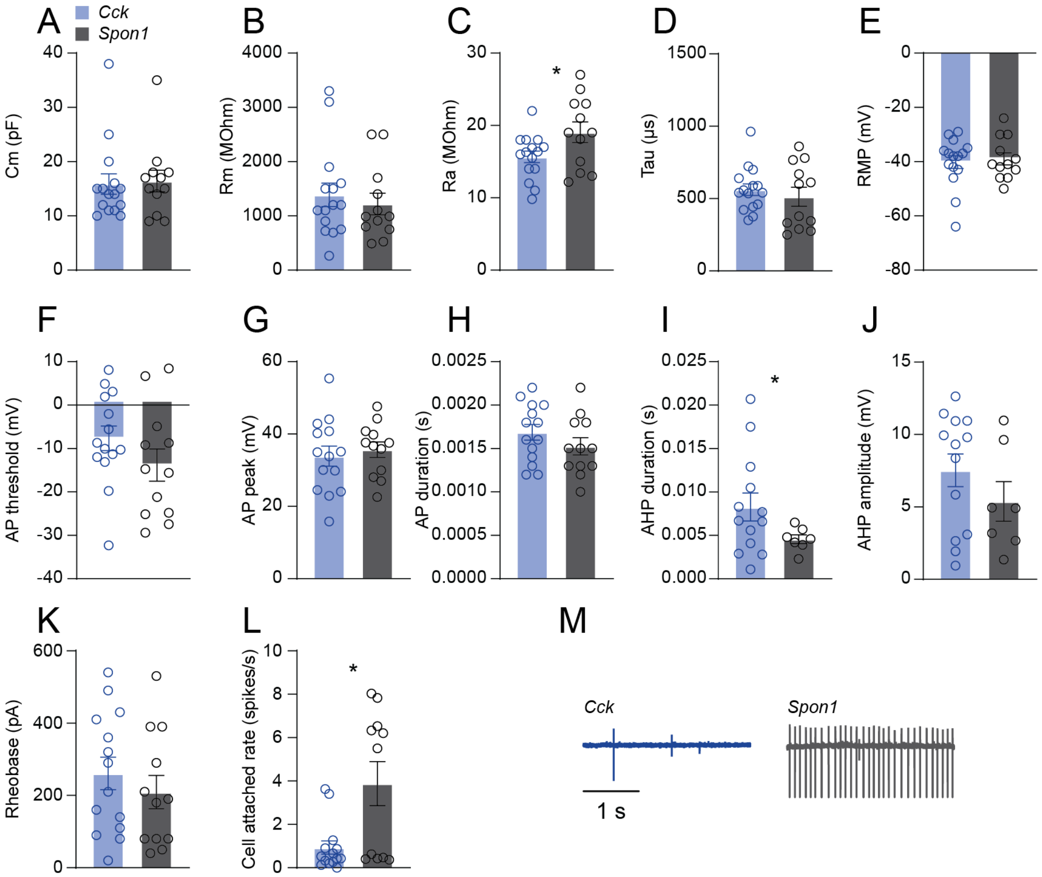


Figure S6. Additional information for slice physiology experiments, related to Figure 6. A. Membrane capacitance (Cm). t-test: t(25)=0.20, p=0.84.**B.** Membrane resistance (Rm). t-test: t(25)=0.56, p=0.58. **C.** Access Resistance (Ra). t-test: t(25)=2.19, p=0.04.**D.** Tau. t-test: t(25)=0.66, p=0.51.**E.** Resting membrane potential (RMP). t-test: t(25)=0.36, p=0.72. **F.** Action potential (AP) threshold. t-test: t(24)=1.35, p=0.19. **G.** Action potential (AP) peak. t-test: t(24)=0.49, p=0.63. **H.** Action potential (AP) duration. t-test: t(24)=1.21, p=0.24. **I.** Afterhyperpolarization (AHP) duration. t-test with Welch's correction for unequal variance: t(14.21)=2.18, p=0.046. **J.** Afterhyperpolarization (AHP) amplitude. t-test: t(18)=1.16, p=0.26.**K.** Rheobase. t-test: t(24)=0.80, p=0.43.**L.** Firing rate recorded in cell-attached mode: t-test with Welch's correction for unequal variance: t(11.84)=2.79, p=0.016.**M.** Example cell-attached recordings from *Cck*- (Left) and *Spon1*- (Right) expressing cells.

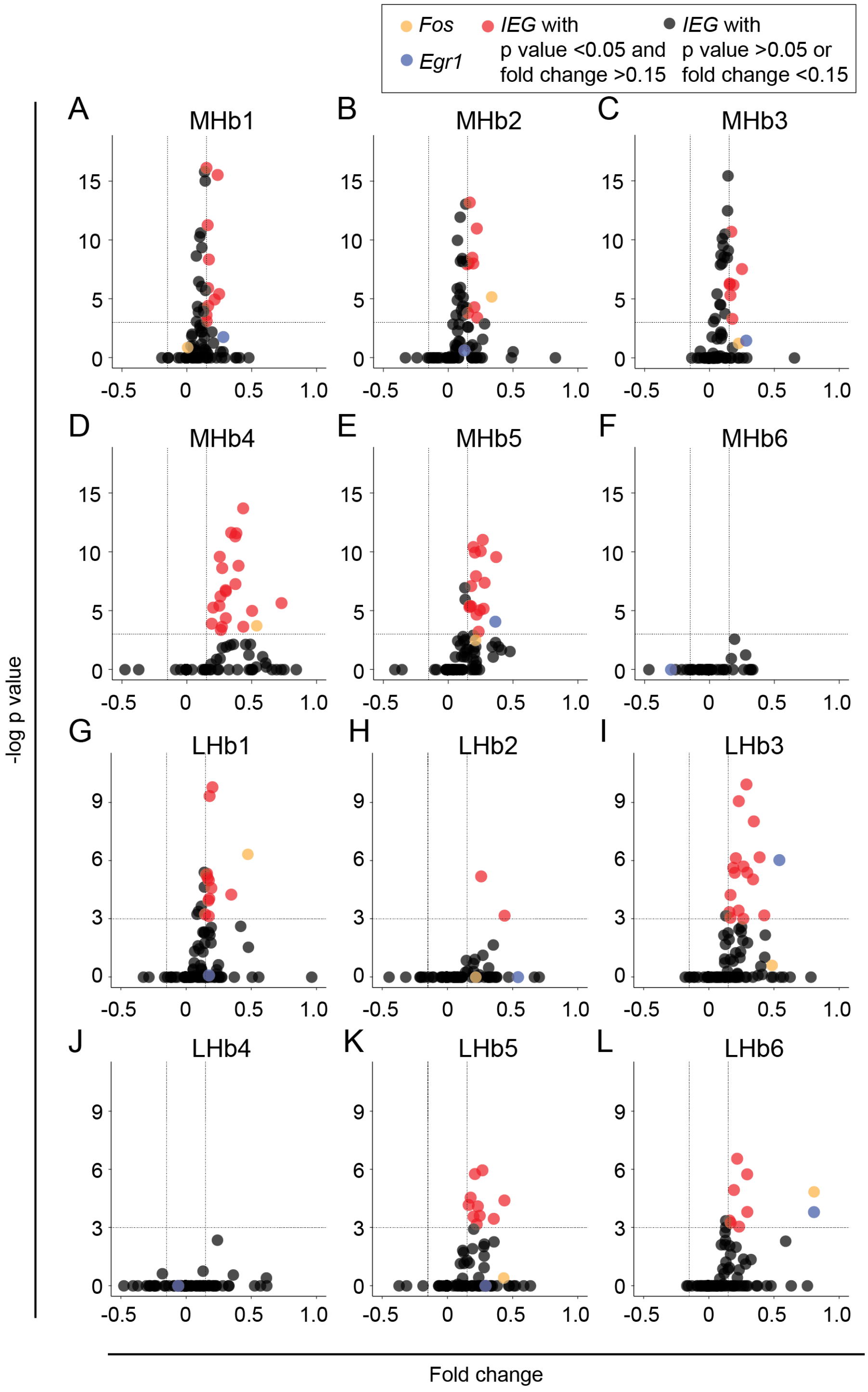


Figure S7. Additional information for *IEG* analysis, related to Figure 7. Scatter plots showing fold change (x-axis) and –log p value (y-axis) of 139 *IEG*s comparing foot shock and home cage groups at each transcriptional cluster.

Gene	Fig	ABA experiment number (mouse, adult, ISH, coronal, Passed QC)	
Tac2	2D, 2E	72339556	http://mouse.brain-map.org/experiment/show/72339556
Pcdh10	2D, 2E	NA	NA
Slc17a6	2D, 2E	73818754	https://mouse.brain-map.org/experiment/show?id=73818754
Slc17a7	2D, 2E	70436317	https://mouse.brain-map.org/experiment/show?id=70436317
Gad1	2D, 2E	479	https://mouse.brain-map.org/experiment/show?id=479
Gad2	2D, 2E	79591669	https://mouse.brain-map.org/experiment/show?id=79591669
Slc32a1	2D, 2E	1098	https://mouse.brain-map.org/experiment/show?id=1098
Gpr151	2D, 2E	74724649	https://mouse.brain-map.org/experiment/show?id=74724649
Pou4f1	2D, 2E	74641304	https://mouse.brain-map.org/experiment/show?id=74641304
Nr4a2	2D, 2E	732	https://mouse.brain-map.org/experiment/show?id=732
Slc18a3	2D, 2E	73521822	https://mouse.brain-map.org/experiment/show?id=73521822
Chat	2D, 2E	252	https://mouse.brain-map.org/experiment/show?id=252
Tac1	2D, 2E	1038	https://mouse.brain-map.org/experiment/show?id=1038
Calb1	2D, 2E	71717640	https://mouse.brain-map.org/experiment/show?id=71717640
Rapgef4	2D, 2E	74749895	https://mouse.brain-map.org/experiment/show?id=74749895
Kcng4	2D, 2E	72081560	https://mouse.brain-map.org/experiment/show?id=72081560
Calb2	2D, 2E	79556662	https://mouse.brain-map.org/experiment/show?id=79556662
Lynx1	2D, 2E	655	https://mouse.brain-map.org/experiment/show?id=655
Neurod2	2D, 2E	75651126	https://mouse.brain-map.org/experiment/show?id=75651126
Plcxd2	2D, 2E	79591579	https://mouse.brain-map.org/experiment/show?id=79591579
Syndig1l	2D, 2E	NA	NA
Slc5a7	2D, 2E	73929608	https://mouse.brain-map.org/experiment/show?id=73929608
Ntm	2D, 2E	573	https://mouse.brain-map.org/experiment/show?id=573
Fn3krp	2D, 2E	74819239	https://mouse.brain-map.org/experiment/show?id=74819239
Abhd2	2D, 2E	NA	NA
Hsf2	2D, 2E	NA	NA
Cck	2D, 2E	77869074	https://mouse.brain-map.org/experiment/show?id=77869074
Adcyap1	2D, 2E	74511882	https://mouse.brain-map.org/experiment/show?id=74511882
Rasd2	2D, 2E	73636089	http://mouse.brain-map.org/experiment/show/73636089
Wif1	2D, 2E	1111	https://mouse.brain-map.org/experiment/show?id=1111
Fam81a	2D, 2E	73732150	https://mouse.brain-map.org/experiment/show?id=73732150
Gnb4	2D, 2E	74047771	https://mouse.brain-map.org/experiment/show?id=74047771
Spon1	2D, 2E	71836797	https://mouse.brain-map.org/experiment/show?id=71836797
Kcnmb4os2	2D, 2E	NA	NA
Cytip	2D, 2E	1270	https://mouse.brain-map.org/experiment/show?id=1270
Vangl1	2D, 2E	71670709	https://mouse.brain-map.org/experiment/show?id=71670709
Zmat4	2D, 2E	71249750	https://mouse.brain-map.org/experiment/show?id=71249750
Lmo1	2D, 2E	1373	https://mouse.brain-map.org/experiment/show?id=1373
Prokr2	2D, 2E	74511780	https://mouse.brain-map.org/experiment/show?id=74511780
Vav2	2D, 2E	74583149	https://mouse.brain-map.org/experiment/show?id=74583149
Nhlh2	2D, 2E	74657929	https://mouse.brain-map.org/experiment/show?id=74657929
Syt6	2D, 2E	1032	https://mouse.brain-map.org/experiment/show?id=1032
Cacna2d1	2D, 2E	75042246	https://mouse.brain-map.org/experiment/show?id=75042246
Slc6a1	2D, 2E	79591685	https://mouse.brain-map.org/experiment/show?id=79591685
Lpar1	2D, 2E	79556595	https://mouse.brain-map.org/experiment/show?id=79556595
Ptn	2D, 2E	2507	https://mouse.brain-map.org/experiment/show?id=2507
Edil3	2D, 2E	NA	NA
Arpp21	2D, 2E	71587781	https://mouse.brain-map.org/experiment/show?id=71587781
Pou2f2	2D, 2E	NA	NA
Sox1	2D, 2E	NA	NA
Iqsec3	2D, 2E	73636153	https://mouse.brain-map.org/experiment/show?id=73636153
Kcnk2	2D, 2E	75147764	https://mouse.brain-map.org/experiment/show?id=75147764
Rbfox1	2D, 2E	NA	NA
Lmo3	2D, 2E	73616037	https://mouse.brain-map.org/experiment/show?id=73616037
Sv2c	2D, 2E	74357583	https://mouse.brain-map.org/experiment/show?id=74357583
Fam101b	2D, 2E	NA	NA
Chrm2	2D, 2E	70560343	https://mouse.brain-map.org/experiment/show?id=70560343
Cnih3	2D, 2E	39	https://mouse.brain-map.org/experiment/show?id=39
Maob	2D, 2E	71670489	https://mouse.brain-map.org/experiment/show?id=71670489
Htr2c	2D, 2E	73636098	https://mouse.brain-map.org/experiment/show?id=73636098
Kcnmb4	2D, 2E	72283793	https://mouse.brain-map.org/experiment/show?id=72283793
Cartpt	2D, 2E	72077479	https://mouse.brain-map.org/experiment/show?id=72077479
Foxp2	2D, 2E	72079884	https://mouse.brain-map.org/experiment/show?id=72079884
Fstl1	2D, 2E	71016596	https://mouse.brain-map.org/experiment/show?id=71016596

Table S1. List of habenula marker genes reported in Allen brain atlas, related to Figure 2.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
RNAscope® HiPlex Probe- Mm-Zmat4-T3	ACDBio	Cat#578011-T3
RNAscope® HiPlex Probe- Mm-Kcng4-T5	ACDBio	Cat#316931-T5
RNAscope® HiPlex Probe- Mm-Fn3krp-T6	ACDBio	Cat#583881-T6
RNAscope® HiPlex Probe- Mm-Ctxn1-T7	ACDBio	Cat#467041-T7
RNAscope® HiPlex Probe- Mm-Tac2-T8	ACDBio	Cat#446391-T8
RNAscope® HiPlex Probe- Mm-Col25a1-T9	ACDBio	Cat#538511-T9
RNAscope® HiPlex Probe- Mm-Cck-T10	ACDBio	Cat#402271-T10
RNAscope® HiPlex Probe- Mm-Gpr151-T11	ACDBio	Cat#317321-T11
RNAscope® HiPlex Probe- Mm-Synpr-T12	ACDBio	Cat#500961-T12
RNAscope® HiPlex Probe- Mm-Necab1-T1	ACDBio	Cat#428541-T1
RNAscope® HiPlex Probe- Mm-Rflnb-T2	ACDBio	Cat#524091-T2
RNAscope® HiPlex Probe- Mm-Cartpt-T3	ACDBio	Cat#432001-T3
RNAscope® HiPlex Probe- Mm-Vsnl1-T5	ACDBio	Cat#583871-T5
RNAscope® HiPlex Probe- Mm-Lmo3-T6	ACDBio	Cat#497631-T6
RNAscope® HiPlex Probe- Mm-Brinp3-T7	ACDBio	Cat#583861-T7
RNAscope® HiPlex Probe- Mm-SLC12A5-T8	ACDBio	Cat#311901-T8
RNAscope® HiPlex Probe- Mm-Pcdh10-T9	ACDBio	Cat#477781-T9
RNAscope® HiPlex Probe- Mm-Ptn-T10	ACDBio	Cat#486381-T10
RNAscope® Probe- Mm-Slc18a3-C3	ACDBio	Cat#448771-C3
RNAscope® Probe- Mm-Htr2c	ACDBio	Cat#401001
RNAscope® Probe- Mm-Calb2-C3	ACDBio	Cat#313641-C3
RNAscope® HiPlex Probe- Mm-Neurod2-O1-T1	ACDBio	Cat#537171-T1
RNAscope® HiPlex Probe- Mm-Spon1-T2	ACDBio	Cat#492671-T2
RNAscope® Probe- Mm-Fos	ACDBio	Cat#316921
RNAscope® Probe- Mm-Fos-C2	ACDBio	Cat#316921-C2
RNAscope® Probe- Mm-Fos-C3	ACDBio	Cat#316921-C3
RNAscope® Probe- Mm-Cck	ACDBio	Cat#402271
RNAscope® Probe- Mm-Spon1-C3	ACDBio	Cat#492671-C3
RNAscope® Probe- Mm-Lmo3-C3	ACDBio	Cat#497631-C3
RNAscope® Probe- Mm-Sv2c	ACDBio	Cat#545001-C2

Table S2. RNAscope® probe information, Related to KEY RESOURCES TABLE.