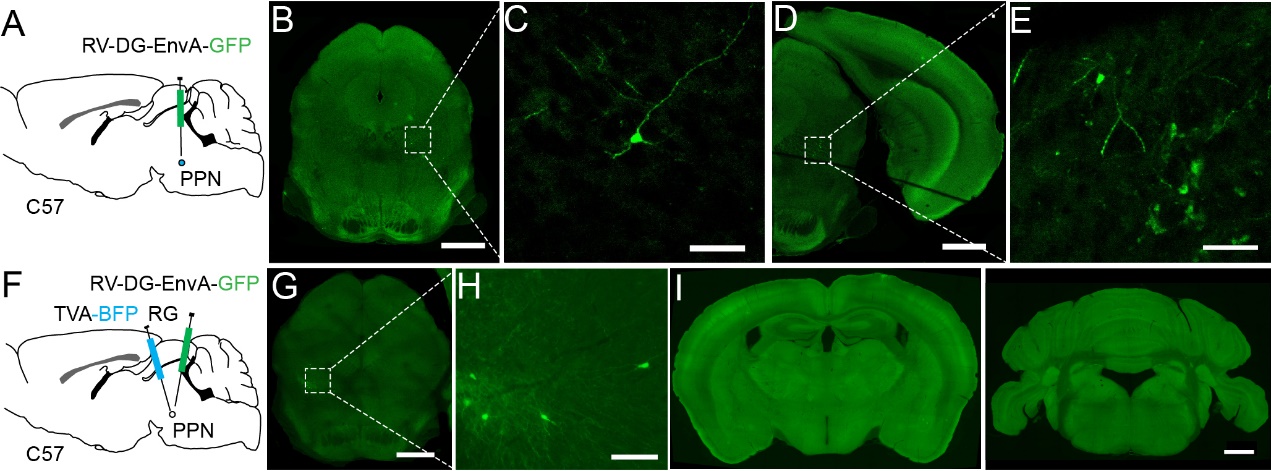
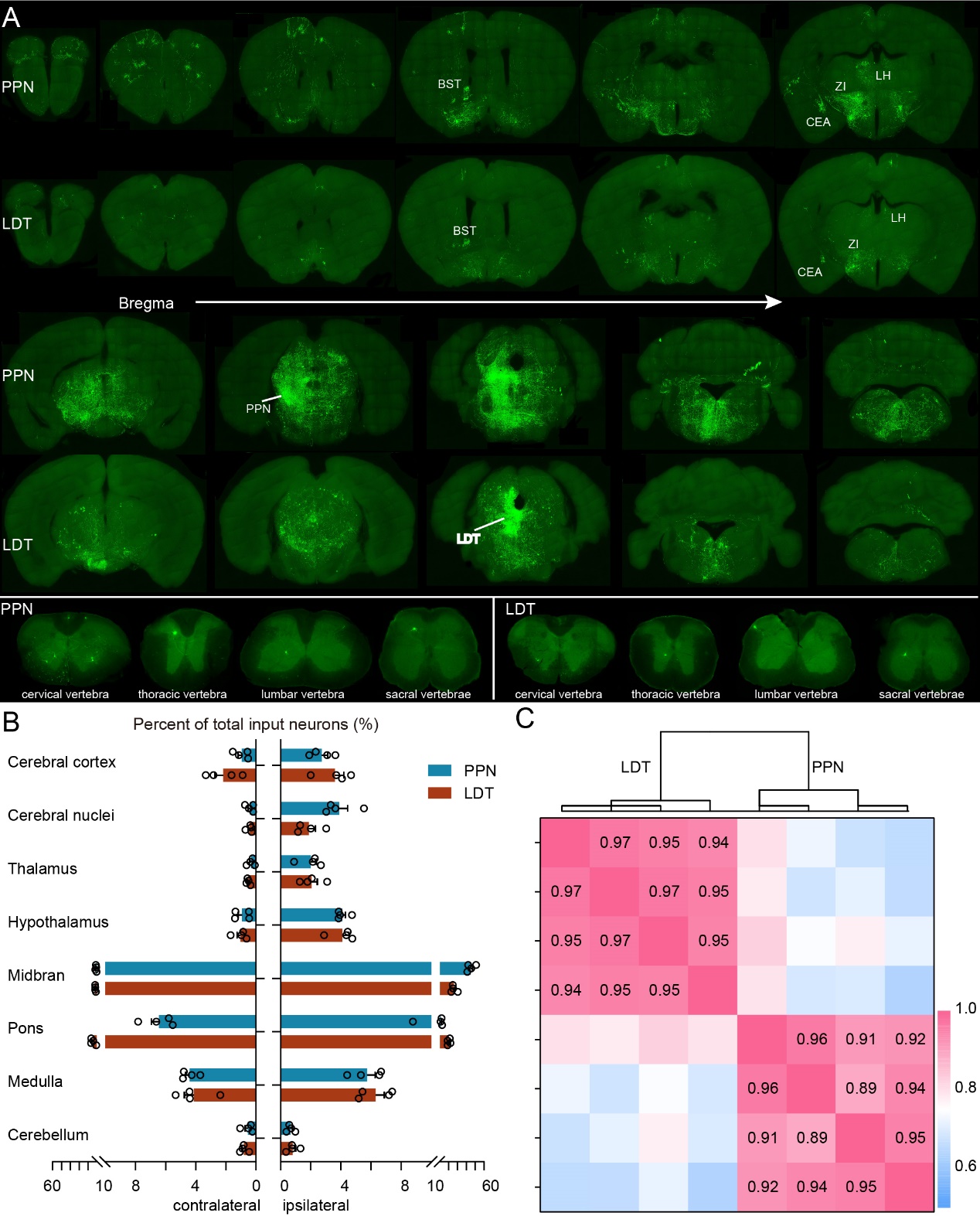
# The Mesoscopic Connectome of Cholinergic Neurons in the mouse Pontomesencephalic Tegmentum

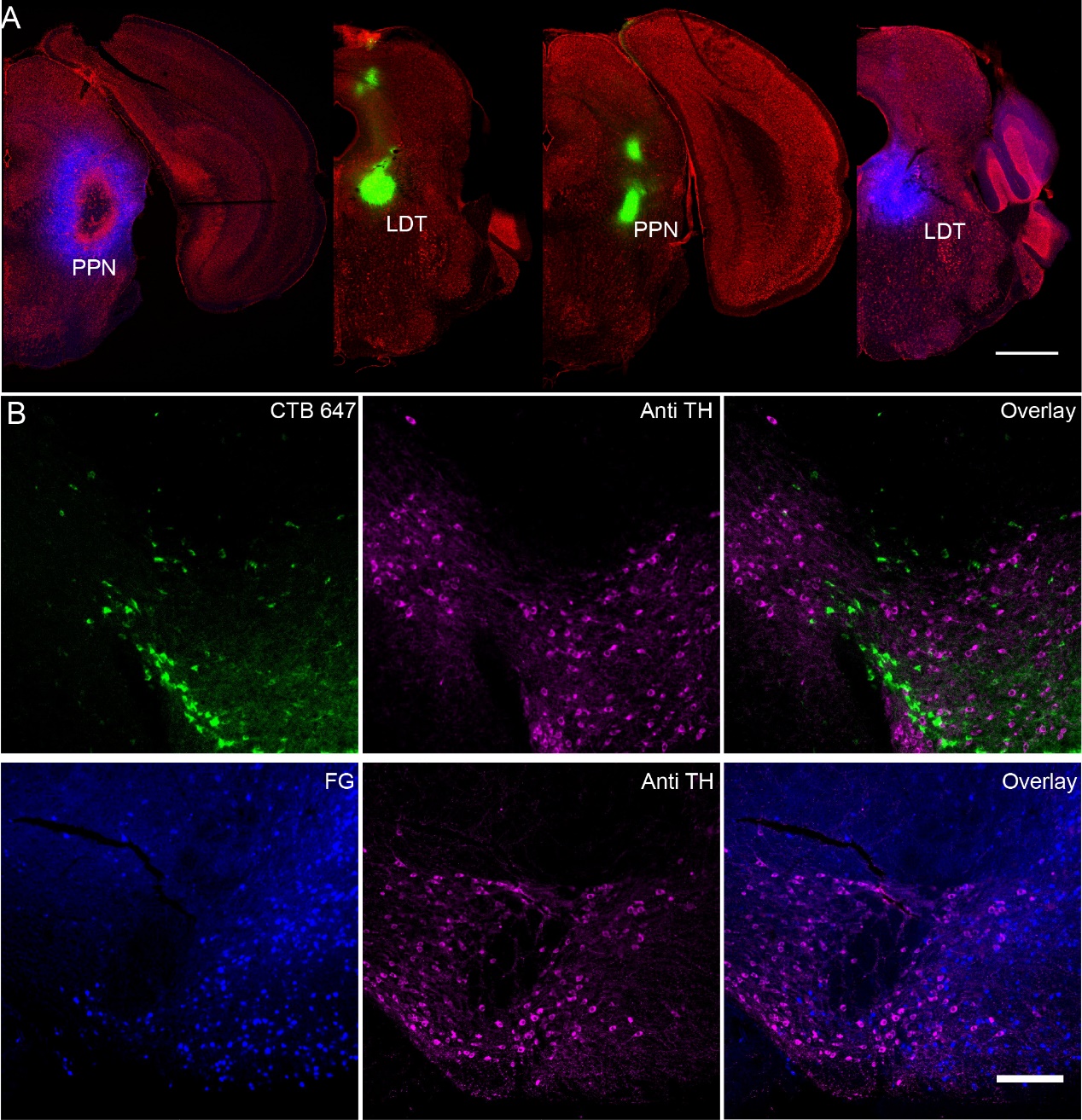
Peilin Zhao1, Huading Wang1, Anan Li1, 2, 3, Guangcai Liu1, Yutong Han1, Qingtao Sun2, Jing Yuan1, 2, Tao Jiang2, Xueyan Jia2, Xiangning Li1, 2 \*, Hui Gong1, 2, 3, \*



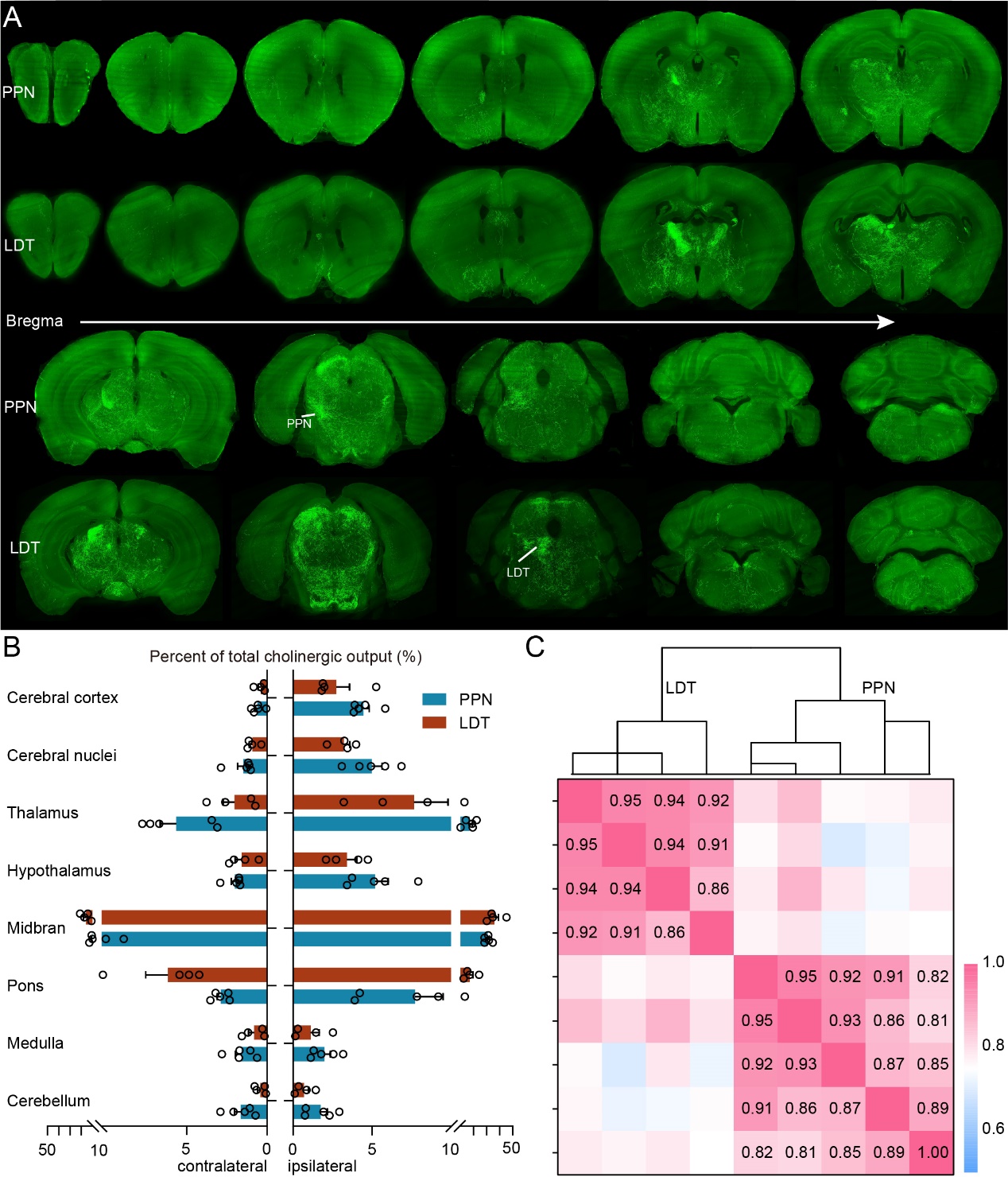
**Supplementary figure S1. The specificity of virus.** **(A)** RV was performed in the PPN of C57 mice directly. **(B) (D)** Several neurons were labeled in the inject site. **(C) (E)** Clear view of the labeled neurons. **(F)** AAV helpers were injected in the PPN of C57 mice and subsequent with modified RV 3 weeks later. **(G)** A few neurons presented in the inject site. **(H)** Clear view of the labeled neurons. (I) No neuron was infected in other input regions. Scale bar, **(B, D, G, I)** 1000 μm; **(C, E, H)** 100μm.



**Supplementary figure S2. Overview of whole-brain input distribution**. **(A)** whole-brain input to PPN and LDT. **(B)** Quantitative statistical proportion of eight input regions to whole-brain input neurons. Left side of vertical axis represents proportion of input neurons in the contralateral hemispheres, and the right side represents ipsilateral hemispheres. Data shown as Mean ± SEM. **(C)** Clustering analysis of all counted input samples according to the Pearson's correlation.



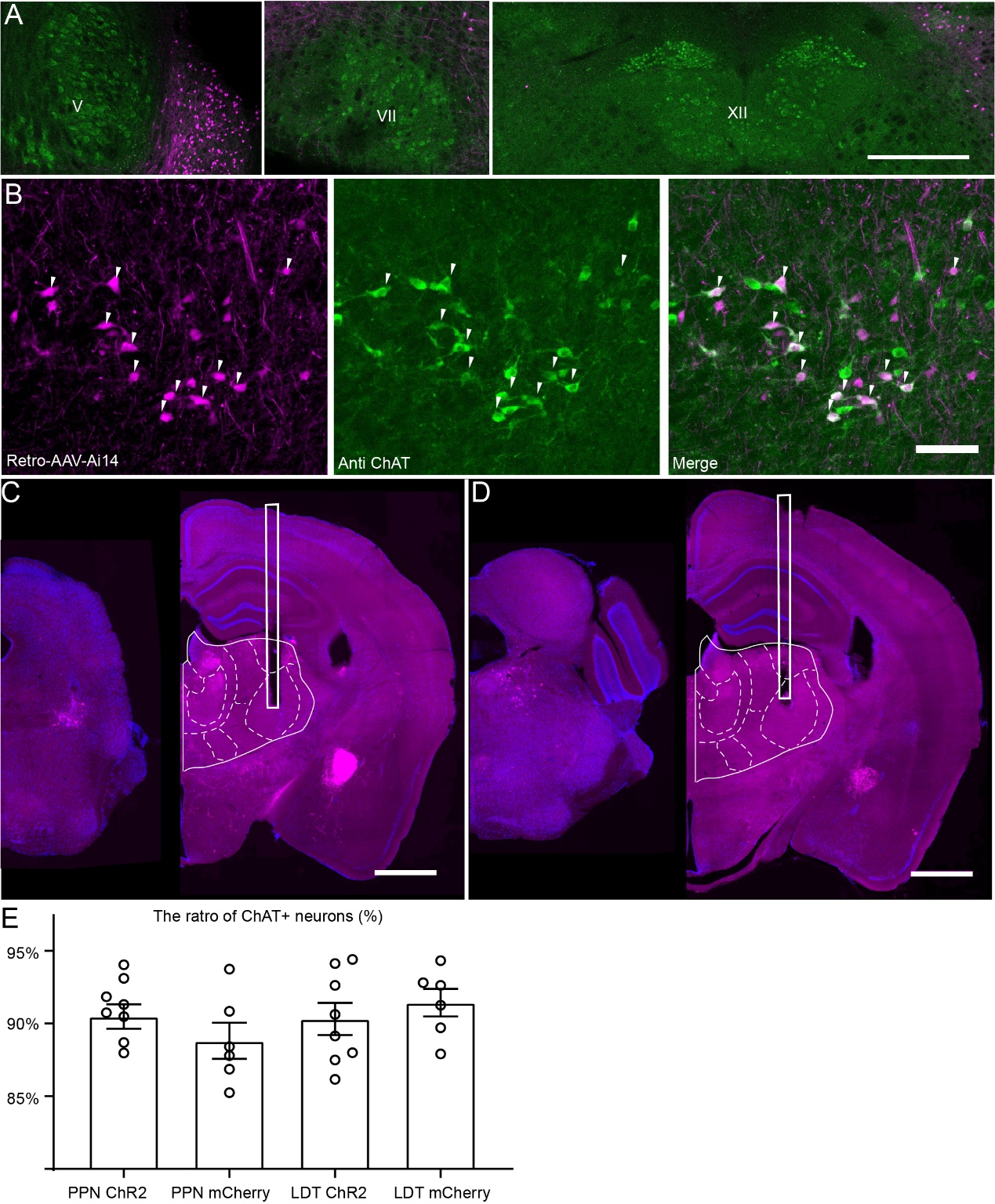
**Supplementary figure S3. Retrograde tracing with CTB and FG.** (A) 75nl 4% FG (blue) or CTB 647 (green) were injected in the PPN or LDT. **(B)** Immunofluorescence staining of labeled in the VTA. All neurons labeled with CTB or FG present TH-negative. Scale bar, **(A)** 1000 μm; **(B)** 200 μm.



**Supplementary figure S4. Overview of cholinergic fibers in the whole brain.** **(A)** The whole brain projection of cholinergic neurons from PPN and LDT. **(B)** Quantitative statistical proportion of eight main target regions to whole-brain input neurons. Left side of vertical axis represents proportion of input neurons in the contralateral hemispheres, and the right side represents ipsilateral hemispheres. Data shown as Mean ± SEM. **(C)** Clustering analysis of all counted output samples according to the Pearson's correlation.



**Supplementary figure S5. The clustering relation of nuclei strongly interconnect with PMT.** **(A)** Clustering relation of input circuits. **(B)** Clustering relation of output circuits. **(C)** Clustering relation of input and output circuits for PPN. **(D)** Clustering relation of input and output circuits for LDT.



**Supplementary figure S6. Tracing the cholinergic input of VP. (A)** None of cholinergic neurons in the V, VII and XII projected to the VP directly. **(B)** A three-panel presentation with AAV-Ai14, anti-CHAT, and merged, arrows point out ChAT-positive neurons. **(C, D)** Inject site of PPN and LDT and tipical coronal plane of VP embedded with ceramic ferrule. **(E)** Statistical proportion of ChAT+ neurons labeled with ChR2 or mCherry. Scale bar, **(A)** 500 μm; **(B)** 100 μm; **(C, D)** 1000 μm.

**Supplementary Table S1 The abbreviation of brain regions**

|  |  |
| --- | --- |
| **Abbrevition** | **Full name** |
| AI | Agranular insular area |
| AON | Anterior olfactory nucleus |
| ATN | Anterior group of the dorsal thalamus |
| AuD | Auditory areas |
| B | Barrington's nucleus |
| BF | basal forebrain |
| CBN | Cerebellar nuclei |
| CBX | Cerebellar cortex |
| CP | Caudoputamen |
| CS | Superior central nucleus raphe |
| CTXpl | cortical plate |
| CTXsp | cortical subplate |
| DORpm | Thalamus, polymodal association cortex related |
| DORsm | Thalamus, sensory-motor cortex related |
| DR | Dorsal nucleus raphe |
| DTN | Dorsal tegmental nucleus |
| ECT | Ectorhinal area |
| EPI | Epithalamus |
| GENd | Geniculate group, dorsal thalamus |
| GENv | Geniculate group, ventral thalamus |
| GRN | Gigantocellular reticular nucleus |
| GU | Gustatory areas |
| IC | Inferior colliculus |
| ILM | Intralaminar nuclei of the dorsal thalamus |
| IRN | Linear nucleus of the medulla |
| LAT | Lateral group of the dorsal thalamus |
| LDT | Laterodorsal tegmental nucleus |
| LHA | Lateral hypothalamic area |
| LSX | Lateral septal complex |
| LZ | Hypothalamic lateral zone |
| MARN | Magnocellular reticular nucleus |
| MBmot | Midbrain, motor related |
| MBsen | Midbrain, sensory related |
| MBsta | Midbrain, behavioral state related |
| MED | Medial group of the dorsal thalamus |
| MEZ | Hypothalamic medial zone |
| MOp | Primary motor area |
| MOs | Secondary motor area |
| mPFC | medial prefrontal cortex |
| MRN | Midbrain reticular nucleus |
| MY- mot | Medulla, motor related |
| MY-sat | Medulla, behavioral state related |
| MY-sen | Medulla, sensory related |
| NI | Nucleus incertus |
| OLF | olfactory areas |
| ORB | orbital area |
| PAG | Periaqueductal gray |
| PAL | pallidum |
| PALd | Pallidum, dorsal region |
| PALm | Pallidum, medial region |
| PALv | Pallidum, ventral region |
| PARN | Parvicellular reticular nucleus |
| PB | Parabrachial nucleus |
| PCG | Pontine central gray |
| PERI | Perirhinal area |
| PFL | Paraflocculus |
| PG | Pontine gray |
| P-mot | Pons, motor related |
| PPN | Pedunculopontine nucleus |
| PRNc | Pontine reticular nucleus, caudal part |
| PRNr | Pontine reticular nucleus |
| PRT | Pretectal region |
| P-sat | Pons, behavioral state related |
| P-sen | Pons, sensory related |
| PVR | periventricular region |
| PVZ | periventricular zone |
| RR | Midbrain reticular nucleus, retrorubral area |
| RT | Reticular nucleus of the thalamus |
| SAG | Nucleus sagulum |
| sAMY | Striatum-like amygdalar nuclei |
| SCm | Superior colliculus, motor related |
| SCs | Superior colliculus, sensory related |
| SLC | Subceruleus nucleus |
| SLD | Sublaterodorsal nucleus |
| SNr | Substantia nigra, reticular part |
| STR | striatum |
| STRv | Striatum ventral region |
| Tea | Temporal association areas |
| TRN | Tegmental reticular nucleus |
| VENT | Ventral group of the dorsal thalamus |
| VISC | Visceral area |
| VNC | Vestibular nuclei |
| VP | Ventral posterior complex of the thalamus |
| VTA | Ventral tegmental area |
| ZI | Zona incerta |

**Supplementary Table S2 Detail P values of significance analysis**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| PPN VS LDT | Input (P values) | | PPN VS LDT | Output (P values) | |
| Ipsilateral | Contralateral | Ipsilateral | Contralateral |
| ORB | ns | ns | OLF | ns | ns |
| mPFC | 0.0436 | ns | STRv | 0.0474 | ns |
| Mos | 0.0292 | ns | CP | 0.0369 | ns |
| CP | 0.0082 | ns | sAMY | ns | ns |
| sAMY | ns | ns | LSX | ns | ns |
| VENT | ns | ns | PALv | ns | ns |
| EPI | ns | ns | PALd | 0.0469 | ns |
| MEZ | 0.0294 | ns | PALm | ns | ns |
| ZI | ns | ns | RT | 0.0297 | ns |
| LHA | ns | ns | VP | 0.0110 | 0.0140 |
| DR | ns | ns | VENT | 0.0082 | 0.0107 |
| PPN | <0.0001 | ns | GENd | ns | ns |
| IC | ns | ns | MED | 0.0470 | ns |
| RR | ns | ns | LAT | 0.0335 | 0.0038 |
| CUN | 0.0043 | 0.0370 | ILM | 0.0225 | ns |
| VTA | 0.4290 | ns | GENv | ns | ns |
| SNr | 0.0418 | ns | ATN | ns | ns |
| SCm | 0.0017 | ns | MEZ | ns | ns |
| PRT | 0.4387 | ns | ZI | 0.0055 | 0.0423 |
| PAG | ns | ns | LHA | ns | ns |
| MRN | 0.0104 | ns | IPN | ns | ns |
| PB | ns | ns | PPN | 0.0001 | ns |
| SLD | 0.0407 | ns | SAG | 0.0206 | ns |
| PRNr | ns | ns | SCs | 0.0094 | 0.0137 |
| NI | 0.0115 | 0.0166 | IC | ns | ns |
| LDT | 0.0039 | 0.0102 | RR | ns | ns |
| CS | 0.0124 | 0.0155 | CUN | ns | ns |
| DTN | 0.0160 | 0.0022 | VTA | ns | ns |
| B | 0.0048 | 0.0079 | SCm | 0.0002 | 0.0016 |
| PRNc | 0.0371 | 0.0032 | PRT | ns | ns |
| PCG | <0.0001 | 0.0001 | PAG | ns | ns |
| PARN | 0.0102 | 0.0283 | MRN | 0.0332 | ns |
| MARN | ns | ns | PB | ns | ns |
| IRN | ns | ns | SLD | 0.0246 | ns |
| GRN | ns | ns | SLC | ns | ns |
| VNC | ns | ns | PRNr | ns | ns |
| PGRN | ns | ns | CS | 0.0211 | 0.0192 |
| CBN | ns | ns | PG | ns | ns |
|  |  |  | DTN | 0.0114 | ns |
|  |  |  | B | 0.0364 | ns |
|  |  |  | TRN | ns | ns |
|  |  |  | PRNc | ns | ns |
|  |  |  | PCG | 0.0001 | 0.0002 |
|  |  |  | CBX | 0.0408 | 0.0083 |