**Whole brain mapping of neurons innervating extraorbital lacrimal glands in mice and rats of both genders**

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***Background pattern

Description automatically generatedSupplementary material***

**Figure 1 |** Distribution patterns of labelled neurons after 2.5d PRV transmission. (A,B) Representative images showing labelled neurons from male mouse with PRV injection on ELGs. Scale bar: 1mm. (C,D) Representative images showing labelled neurons from female mouse with PRV injection on ELGs. Scale bar: 1mm. Green: GFP; red: dsRed; blue: DAPI. PB: parabrachial nucleus; PBmm: parabrachial nucleus, medial division, medial medial part; PSV: principal sensory nucleus of the trigeminal; MV: medial vestibular nucleus; PARN: parvicellular reticular nucleus; PRNc: potine reticular nucleus, caudal part.

**Supplementary Table 1: Abbreviations**

|  |  |
| --- | --- |
| **Abbreviation** | **Definition** |
| **Isocortex**  SSp  AI (d/v/p)  PL  MO (p/s)  ECT  PERI  AUD (d/v/p)  **OLF**  Pir  TR  TT (d/v)  **STR**  LS(r/c/v)  CEA  MEA  ACB  **PAL**  BST (a/p)  SI  **HY**  LPO  LHA  MPO  MPN  DMH  VMH  PSTN  STN  PH  ZI  **CTXsp**  BLAa  BLAp  BMAp  **MB**  PAG  MRN  VTA  SCm  **Pons**  PRNr  PRNc  PBl  PBmm  PBKF  **Medulla**  PARN  MARN  PGRNl  IRN  GRN  VII  MV  ACN  CMPI  CIL-LC-MS  EA  FA  PBS  RT  TEA  FFAs  TCA  ATP  sPLA2  POCE  NMR  Myo  Cre  Asp  Glx  Gly  Glu  Gln  Tau  GABA  TMSP  NAA | Primary somatosensory area  Agranular insular area  Prelimbic area  Somatomotor areas  Ectorhinal area  Perirhinal area  Auditory areas  **Olfactory areas**  Piriform area  Post-piriform transition area  Taenia tecta  **Striatum**  Lateral septal nucleus  Central amygdalar nucleus  Medial amygdalar nucleus  Nucleus accumbens  **Pallidum**  Bed nuclei of the stria terminalis  Substantia innominata  **Hypothalamus**  Lateral preoptic area  Lateral hypothalamic area  Medial preoptic area  Medial preoptic nucleus  Dorsomedial nucleus of the hypothalamus  Ventromedial nucleus of the hypothalamus  Parasubthalamic nucleus  Subthalamic nucleus  Posterior hypothalamic nucleus  Zona incerta  **Cortical subplate**  Basolateral amygdalar nucleus, anterior  Basolateral amygdalar nucleus, posterior  Basomedial amygdalar nucleus  **Midbrain**  Periaqueductal gray  Midbrain reticular nucleus  Ventral tegmental area  Superior colliculus, motor related  Pontine reticular nucleus  Pontine reticular nucleus, caudal part  Parabrachial nucleus, lateral division  Parabrachial nucleus, medial division, medial medial part  Koelliker-Fuse subnucleus  Parvicellular reticular nucleus  Magnocellular reticular nucleus  Paragigantocellular reticular nucleus, lateral part  Intermediate reticular nucleus  Gigantocellular reticular nucleus  Facial motor nucleus  Medial vestibular nucleus  Acetonitrile  2-chloro-1-methylpyridinium iodide  Chemical isotope labelling-assisted liquid chromatography-mass spectrometry  Ethyl acetate  Formic acid  Phosphate-buffered saline  Retention time  Triethylamine  Free fatty acids  Tricarboxylic acid  Adenosine triphosphate  Secretory phospholipase A2  1H observed/13C-edited  Nuclear magnetic resonance  Myo-inositol  Creatine  Aspartate  Glutamine + glutamate  Glycine  Glutamate  Glutamine  Taurine  γ-aminobutyric acid  3-(Trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt  N-acetylaspartate |

**Supplementary Table 2:**

**Quantification of total input cells across the whole brain for males**

|  |  |  |  |
| --- | --- | --- | --- |
|  | 1#mouse | 2#mouse | 3#mouse |
| DsRed+ | 1910 | 959 | 2047 |
| GFP+ | 5216 | 4195 | 1330 |

**Quantification of total input cells across the whole brain for females**

|  |  |  |  |
| --- | --- | --- | --- |
|  | 1#mouse | 2#mouse | 3#mouse |
| DsRed+ | 587 | 3727 | 4263 |
| GFP+ | 2617 | 11267 | 5090 |

**Supplementary Table 3: Statistical analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Figure | Target | Data values | P values | Methods |
| 2B | Isocortex | 4.85%1.79% for males vs females, 4.38% 1.14% | 0.829 | T-tests |
|  | OLF | 4.01%0.89% for males vs females, 1.54%0.53% | **0.037** | T-tests |
|  | STR | 16.79%1.44% for males vs females, 9.85%2.26% | **0.027** | T-tests |
|  | PAL | 1.68%1.04% for males vs females, 3.49%0.84% | 0.207 | T-tests |
|  | HY | 20.30%1.95% for males vs females, 29.93%1.51% | **0.003** | T-tests |
|  | CTXsp | 5.56%1.93% for males vs females,7.58%3.21% | 0.600 | T-tests |
|  | MB | 18.51%3.72% for males vs females, 13.78%1.11% | 0.251 | T-tests |
|  | Pons | 11.87%1.56% for males vs females, 8.19%1.60% | 0.130 | T-tests |
|  | Medulla | 16.41%4.90% for males vs females, 21.28%4.74% | 0.492 | T-tests |
|  |  |  |  |  |
| 2C (GFP+) | Isocortex | 5.10%2.77% for males vs females, 4.50%2.16% | 0.873 | T-tests |
|  | OLF | 4.99%1.39% for males vs females, 2.16%0.82% | 0.155 | T-tests |
|  | STR | 16.61%2.67% for males vs females, 10.31%3.44% | 0.222 | T-tests |
|  | PAL | 1.53%1.49% for males vs females, 3.04%0.57% | 0.398 | T-tests |
|  | HY | 18.52%2.5% for males vs females, 29.43%1.93% | **0.026** | T-tests |
|  | CTXsp | 5.69%2.83% for males vs females, 9.33%5.97% | 0.611 | T-tests |
|  | MB | 20.14%7.04% for males vs females, 13.71%1.32% | 0.420 | T-tests |
|  | Pons | 13.70%2.09% for males vs females, 8.29%3.33% | 0.271 | T-tests |
|  | Medulla | 13.72%6.09% for males vs females, 19.22%8.63% | 0.630 | T-tests |
|  |  |  |  |  |
| 2C (dsRed+) | Isocortex | 4.60%2.9% for males vs females, 4.25%1.36% | 0.919 | T-tests |
|  | OLF | 3.04%1.02% for males vs females, 0.91%0.57% | 0.144 | T-tests |
|  | STR | 16.97%1.79% for males vs females, 9.38%3.66% | 0.136 | T-tests |
|  | PAL | 1.84%1.79% for males vs females, 3.94%1.72% | 0.447 | T-tests |
|  | HY | 22.09%3.09% for males vs females, 30.42%2.73% | 0.113 | T-tests |
|  | CTXsp | 5.42%3.24% for males vs females, 5.83%3.60% | 0.936 | T-tests |
|  | MB | 16.88%4.12% for males vs females,13.84%2.11% | 0.547 | T-tests |
|  | Pons | 10.05%2.11% for males vs females, 8.09%1.30% | 0.473 | T-tests |
|  | Medulla | 19.11%8.70% for males vs females, 23.33%5.82% | 0.707 | T-tests |
|  |  |  |  |  |
| 2D | SSp | 0.09%0.07% for males vs females, 0.47%0.19% | 0.077 | T-tests |
|  | AI | 3.04%1.30% for males vs females, 1.16%0.38% | 0.196 | T-tests |
|  | PL | 0.03%0.02% for males vs females, 0.11%0.05% | 0.137 | T-tests |
|  | MO | 0.25%0.15% for males vs females, 0.39%0.20% | 0.572 | T-tests |
|  | ECT | 0.38%0.26% for males vs females, 1.04%0.34% | 0.153 | T-tests |
|  | PERI | 1.07%0.46% for males vs females, 0.85%0.31% | 0.697 | T-tests |
|  | AUD | 0 for males vs females, 0.36%0.21% | 0.113 | T-tests |
|  | Pir | 0.94%0.35% for males vs females, 0.76%0.17% | 0.658 | T-tests |
|  | TR | 3.03%1.08% for males vs females, 0.50%0.37% | **0.052** | T-tests |
|  | TT | 0.04%0.02% for males vs females, 0.27%0.13% | 0.124 | T-tests |
|  | LS | 0.76%0.35% for males vs females, 1.00%0.19% | 0.554 | T-tests |
|  | CEA | 11.16%1.72% for males vs females, 7.16%2.59% | 0.227 | T-tests |
|  | MEA | 4.65%2.59% for males vs females, 1.64%0.37% | 0.276 | T-tests |
|  | ACB | 0.22%0.14% for males vs females, 0.05%0.03% | 0.281 | T-tests |
|  | BST | 1.50%0.94% for males vs females, 3.17%0.87% | 0.223 | T-tests |
|  | SI | 0.18%0.13% for males vs females, 0.32%0.12% | 0.444 | T-tests |
|  | LPO | 0.09%0.05% for males vs females, 0.87%0.29% | **0.025** | T-tests |
|  | LHA | 8.31%2.31% for males vs females,9.83 %1.04% | 0.561 | T-tests |
|  | MPO | 1.18%0.53% for males vs females, 2.07%0.27% | 0.162 | T-tests |
|  | MPN | 0.77%0.56% for males vs females, 1.41%0.48% | 0.403 | T-tests |
|  | DMH | 1.33%0.45% for males vs females, 1.54%0.26% | 0.691 | T-tests |
|  | VMH | 0.33%0.22% for males vs females, 1.09%0.44% | 0.152 | T-tests |
|  | PSTN | 4.39%1.84% for males vs females, 9.31%0.96% | **0.040** | T-tests |
|  | STN | 0.70%0.24% for males vs females, 0.43%0.20% | 0.402 | T-tests |
|  | PH | 1.49%0.49% for males vs females, 1.75%0.47% | 0.716 | T-tests |
|  | ZI | 1.70%1.05% for males vs females, 1.62%0.72% | 0.946 | T-tests |
|  | BLAa | 5.18%2.04% for males vs females, 3.66%1.47% | 0.559 | T-tests |
|  | BLAp | 0.25%0.18% for males vs females, 1.11%0.43% | 0.093 | T-tests |
|  | BMAp | 0.13%0.08% for males vs females,2.82 %1.57% | 0.118 | T-tests |
|  | PAG | 12.42%2.21% for males vs females, 9.64%0.65% | 0.255 | T-tests |
|  | MRN | 5.23%1.31% for males vs females, 3.35%0.42% | 0.202 | T-tests |
|  | VTA | 0.78%0.42% for males vs females, 0.49%0.31% | 0.575 | T-tests |
|  | SCm | 0.07%0.05% for males vs females, 0.30%0.13% | 0.140 | T-tests |
|  | PRNr | 0.32%0.11% for males vs females, 0.67%0.25% | 0.225 | T-tests |
|  | PRNc | 2.93%0.65% for males vs females, 3.83%1.38% | 0.570 | T-tests |
|  | PBI | 4.29%0.66% for males vs females, 2.45%0.74% | 0.092 | T-tests |
|  | PBmm | 3.93%1.08% for males vs females, 0.80%0.59% | **0.029** | T-tests |
|  | PBKF | 0.41%0.29% for males vs females, 0.44%0.28% | 0.962 | T-tests |
|  | PARN | 3.55%1.56% for males vs females, 4.20%1.23% | 0.750 | T-tests |
|  | MARN | 2.93%1.03% for males vs females, 6.48%1.59% | 0.089 | T-tests |
|  | PGRNI | 0.18%0.11% for males vs females, 1.95%1.33% | 0.216 | T-tests |
|  | IRN | 4.31%1.35% for males vs females, 3.79%1.12% | 0.774 | T-tests |
|  | GRN | 0.69%0.17% for males vs females, 2.21%0.81% | 0.099 | T-tests |
|  | VII | 3.01%1.20% for males vs females, 1.16%0.62% | 0.201 | T-tests |
|  | MV | 1.75%1.14% for males vs females, 1.48%0.78% | 0.850 | T-tests |
|  |  |  |  |  |
| 3D | LPO | 0.41%0.35% for males vs females, 3.79%1.01% | **0.033** | T-tests |
|  | LHA | 41.99%% for males vs females, 34.53%3.37% | 0.719 | T-tests |
|  | MPO | 5.94%3.52% for males vs females, 6.96%1.17% | 0.797 | T-tests |
|  | MPN | 4.05%3.16% for males vs females, 4.82%2.51% | 0.859 | T-tests |
|  | DMH | 7.17%3.78% for males vs females, 5.22%0.94% | 0.641 | T-tests |
|  | VMH | 1.56%1.56% for males vs females, 3.89%2.51% | 0.476 | T-tests |
|  | PSTN | 20.48%11.90% for males vs females, 29.29%2.50% | 0.254 | T-tests |
|  | STN | 3.95%2.20% for males vs females, 1.37%0.10% | 0.345 | T-tests |
|  | PH | 6.58%3.11% for males vs females, 5.55%2.21% | 0.801 | T-tests |
|  | ZI | 7.87%7.57% for males vs females, 5.36%3.44% | 0.778 | T-tests |
|  |  |  |  |  |
| 4D | Pir | 37.73%28.31% for males vs females, 59.57%18.97% | 0.557 | T-tests |
|  | TR | 60.10%28.86% for males vs females, 26.54%15.96% | 0.366 | T-tests |
|  | TT | 2.85%0.48% for males vs females, 13.89%6.46% | 0.163 | T-test |
|  |  |  |  |  |
| 5D | LS | 4.48%2.00% for males vs females, 11.85%6.08% | 0.313 | T-tests |
|  | CEA | 72.49%16.02% for males vs females, 64.36%14.07% | 0.723 | T-tests |
|  | MEA | 21.90%18.22% for males vs females, 23.06%9.86% | 0.958 | T-tests |
|  | ACB | 1.14%0.94% for males vs females, 0.73%0.31% | 0.702 | T-tests |
|  |  |  |  |  |
| 6D | PRNr | 2.49%1.05% for males vs females, 12.29%6.62% | 0.217 | T-tests |
|  | PRNc | 26.99%9.25% for males vs females, 33.59%18.22% | 0.758 | T-tests |
|  | PBI | 33.99%1.95% for males vs females, 29.75%4.68% | 0.451 | T-tests |
|  | PBmm | 30.85%8.25% for males vs females, 19.60%17.70% | 0.595 | T-tests |
|  | PBKF | 5.68%5.37% for males vs females, 4.77%4.21% | 0.900 | T-tests |

**Supplementary Table 4:** **Content of the detected potential fatty acids in ELG**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Time | RI | m/z (DMED-labeled) | m/z (d4-DMED-labeled) | Intensity (cps) d4-DMED-labeled | Formula (DMED-labeled) | Formula (unlabeled) | Molecular weight | Name | CASNo. | KEGG ID |
| 1 | 3.38931 | 600.3782 | 187.1807 | 191.2058 | 88328893.18 | C10H23ON2 | C6H12O2 | 116.0837 | Isocaproic acid | 646-07-1 | - |
| 2 | 3.55929 | 611.7983 | 199.1808 | 203.2058 | 59488947.13 | C11H23ON2 | C7H12O2 | 128.0837 | Cyclohexanecarboxylic acid | 98-89-5 | C09822 |
| 3 | 5.1612 | 675.3815 | 281.2226 | 285.2477 | 2415725.277 | C16H29O2N2 | C12H18O3 | 210.1256 | (±)-Jasmonic Acid | 77026-92-7 | C08491 |
| 4 | 5.68599 | 697.5957 | 273.2175 | 277.2424 | 8681027.886 | C14H29O3N2 | C10H18O4 | 202.1205 | Sebacic acid | 111-20-6 | C08277 |
| 5 | 5.79772 | 703.4345 | 279.1701 | 283.1957 | 1115418.753 | C15H23O3N2 | C11H12O4 | 208.0736 | 3-(3,4-Dimethoxyphenyl)-2-propenoic acid | 14737-89-4 | - |
| 6 | 5.7478 | 700.313 | 201.1965 | 205.2214 | 33134540.24 | C11H25ON2 | C7H14O2 | 130.0994 | Heptanoic acid | - | C17714 |
| 7 | 6.18516 | 727.6614 | 215.2121 | 219.2373 | 6020427.921 | C12H27ON2 | C8H16O2 | 144.115 | Valproic acid | 99-66-1 | C07185 |
| 8 | 7.06233 | 782.5108 | 213.1963 | 217.2216 | 6685916.595 | C12H25ON2 | C8H14O2 | 142.0994 | 2-Octenoic acid | 1871-67-6 | C16653 |
| 9 | 7.26086 | 794.9251 | 299.2331 | 303.2583 | 9534159.567 | C16H31O3N2 | C12H20O4 | 228.1361 | Traumatic acid | 6402-36-4 | C16308 |
| 10 | 7.359 | 801.1323 | 215.2119 | 219.2369 | 126892753.5 | C12H27ON2 | C8H16O2 | 144.115 | Caprylic acid | 124-07-2 | C06423 |
| 11 | 8.24825 | 860.4397 | 259.2385 | 263.2634 | 945941.031 | C14H31O2N2 | C10H20O3 | 188.1412 | 3-Hydroxycapric acid | 14292-26-3 | - |
| 12 | 8.23034 | 859.2451 | 479.3847 | 483.4093 | 658001283.5 | C28H51O4N2 | C24H40O5 | 408.2876 | Ursocholic acid | 2955-27-3 | - |
| 13 | 8.69582 | 890.2892 | 321.2538 | 325.279 | 927330.471 | C19H33O2N2 | C15H22O3 | 250.1569 | Gemfibrozil | 25812-30-0 | C07020 |
| 14 | 8.65198 | 887.3655 | 315.2645 | 319.2897 | 627467.205 | C17H35O3N2 | C13H24O4 | 244.1675 | 1,11-Undecanedicarboxylic acid | 505-52-2 | - |
| 15 | 8.86027 | 901.5199 | 477.3691 | 481.394 | 360059635.9 | C28H49O4N2 | C24H38O5 | 406.2719 | 7-Ketodeoxycholic acid | 911-40-0 | - |
| 16 | 8.845 | 900.2886 | 229.2276 | 233.2525 | 269705482.6 | C13H29ON2 | C9H18O2 | 158.1306 | Nonanoic acid | 112-05-0 | C01601 |
| 17 | 8.93115 | 907.2338 | 463.3898 | 467.4148 | 23192434.53 | C28H51O3N2 | C24H40O4 | 392.2627 | Isoursodeoxycholic acid | 78919-26-3 | - |
| 18 | 9.68509 | 967.8825 | 405.3114 | 409.3365 | 9801175.464 | C24H41O3N2 | C20H30O4 | 334.2144 | PGA2 | 13345-50-1 | C05953 |
| 19 | 10.0853 | 1000.31 | 243.2433 | 247.2683 | 226298335.1 | C14H31ON2 | C10H20O2 | 172.1463 | Capric acid | 334-48-5 | C01571 |
| 20 | 10.0718 | 999.1933 | 255.2434 | 259.2685 | 17200363.13 | C15H31ON2 | C11H20O2 | 184.1463 | Undecylenic acid | 112-38-9 | C12522 |
| 21 | 10.6005 | 1045.931 | 287.2697 | 291.2947 | 1797016.917 | C16H35O2N2 | C12H24O3 | 216.1725 | 3-Hydroxydodecanoic acid | 1883-13-2 | - |
| 22 | 11.2136 | 1100.247 | 257.2591 | 261.2841 | 36445392.9 | C15H33ON2 | C11H22O2 | 186.162 | Undecanoic acid | 112-37-8 | C17715 |
| 23 | 11.1527 | 1094.835 | 357.3117 | 361.3366 | 1297140.783 | C20H41O3N2 | C16H30O4 | 286.2144 | Hexadecanedioic acid | 505-54-4 | C08260 |
| 24 | 12.2634 | 1200.63 | 271.2747 | 275.2995 | 631796857.4 | C16H35ON2 | C12H24O2 | 200.1776 | Lauric acid | 143-07-7 | C02679 |
| 25 | 12.6565 | 1238.7 | 389.3166 | 393.3415 | 10534660.64 | C24H41O2N2 | C20H30O3 | 318.2194 | Leukotriene A4 | 72059-45-1 | C00909 |
| 26 | 13.0005 | 1271.895 | 297.2901 | 301.3152 | 209708440.8 | C18H37ON2 | C14H26O2 | 226.1933 | Myristoleic acid | - | C08322 |
| 27 | 13.2949 | 1300.387 | 285.2903 | 289.3153 | 178623349.7 | C17H37ON2 | C13H26O2 | 214.1933 | Tridecanoic acid | 638-53-9 | C17076 |
| 28 | 13.8206 | 1355.243 | 347.3059 | 351.3309 | 382413033.4 | C22H39ON2 | C18H28O2 | 276.2089 | Stearidonic Acid | 20290-75-9 | C16300 |
| 29 | 14.2578 | 1400.819 | 299.3056 | 303.3303 | 3768894996 | C18H39ON2 | C14H28O2 | 228.2089 | Myristic acid | 544-63-8 | C06424 |
| 30 | 14.7605 | 1451.241 | 349.3215 | 353.3459 | 5404017102 | C22H41ON2 | C18H30O2 | 278.2246 | Alpha-Linolenic acid | 463-40-1 | C06427 |
| 31 | 15.251 | 1500.435 | 313.3218 | 317.3466 | 1951178740 | C19H41ON2 | C15H30O2 | 242.2246 | Pentadecylic acid | 1002-84-2 | C16537 |
| 32 | 15.4501 | 1520.333 | 327.3372 | 331.3622 | 27447912.53 | C20H43ON2 | C16H32O2 | 256.2402 | Isopalmitic acid | 32844-67-0 | - |
| 33 | 16.5989 | 1635.161 | 353.353 | 357.3772 | 23365852943 | C22H45ON2 | C18H34O2 | 282.2559 | Oleic acid | 112-80-1 | C00712 |
| 34 | 17.0331 | 1678.563 | 403.3687 | 407.3935 | 275477409.8 | C26H47ON2 | C22H36O2 | 332.2715 | Adrenic Acid | 28874-58-0 | C16527 |
| 35 | 17.2509 | 1700.337 | 341.3531 | 345.3779 | 784668140.5 | C21H45ON2 | C17H34O2 | 270.2559 | Margaric acid | 506-12-7 | - |
| 36 | 17.2683 | 1702.098 | 379.3685 | 383.3936 | 513217501.7 | C24H47ON2 | C20H36O2 | 308.2715 | Eicosadienoic acid | 2091-39-6 | C16525 |
| 37 | 18.0716 | 1783.407 | 355.3687 | 359.3933 | 5234439295 | C22H47ON2 | C18H36O2 | 284.2715 | Stearic acid | 57-11-4 | C01530 |
| 38 | 18.5381 | 1830.634 | 381.3843 | 385.4089 | 2586073263 | C24H49ON2 | C20H38O2 | 310.2872 | Cis-8-Eicosenoic acid | 5561-99-9 | C16526 |
| 39 | 20.1271 | 2000.403 | 383.4001 | 387.4248 | 1175004095 | C24H51ON2 | C20H40O2 | 312.3028 | Arachidic acid | 506-30-9 | C06425 |
| 40 | 20.3048 | 2020.325 | 409.4156 | 413.4403 | 1297628909 | C26H53ON2 | C22H42O2 | 338.3185 | Erucic acid | 112-86-7 | C08316 |
| 41 | 21.0163 | 2100.104 | 397.4155 | 401.4406 | 329740227.1 | C25H53ON2 | C21H42O2 | 326.3185 | Heneicosanoic acid | 2363-71-5 | - |
| 42 | 21.7877 | 2200.987 | 411.4315 | 415.4561 | 932694976 | C26H55ON2 | C22H44O2 | 340.3341 | Docosanoic acid | 112-85-6 | C08281 |