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**Supplementary information**

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**Reverse-translational identification of a cerebellar satiation network**

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Supplementary Table 1 | Statistical analysis

| Figure | Sample size (n)                                                                              | Statistical test                      | Values & mean ± SEM                                                                     |
|--------|----------------------------------------------------------------------------------------------|---------------------------------------|-----------------------------------------------------------------------------------------|
| 1b     | Controls: 14 subjects<br>Hyperphagia: 14 PWS subjects                                        | Full factorial ANOVA                  |                                                                                         |
|        |                                                                                              | Interaction                           | F(1,52)=15.31, <i>P</i> <0.001; k(E)=85, voxel coordinates (x, y, z)=-24, -49, -38      |
|        |                                                                                              | Post-hoc comparisons                  |                                                                                         |
|        |                                                                                              | Fasting: Hyperphagia vs. Control      | t(26)=2.72, <i>P</i> =0.01                                                              |
|        |                                                                                              | Post-meal: Hyperphagia vs. Control    | t(26)=3.60, <i>P</i> =0.001                                                             |
|        |                                                                                              | Control: Post-meal vs. Fasting        | t(13)=4.73, <i>P</i> <0.001                                                             |
|        |                                                                                              | Hyperphagia: Post-meal vs. Fasting    | t(13)=0.90, <i>P</i> =0.38                                                              |
|        |                                                                                              | Pre-meal (Control)                    | 0.378 ± 0.073                                                                           |
|        |                                                                                              | Post-meal (Control)                   | -0.331 ± 0.124                                                                          |
|        |                                                                                              | Pre-meal (Hyperphagia)                | -0.0550 ± 0.094                                                                         |
|        |                                                                                              | Post-meal (Hyperphagia)               | 0.106 ± 0.093                                                                           |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
| 1e     | Food deprived: 3 mice, 4 brain slices each mouse<br>Refed: 3 mice, 4 brain slices each mouse | Unpaired t-test                       | <i>P</i> =0.029<br>Food dep: 16.1 ± 2.79<br>Refed: 26.4 ± 3.27                          |
| 1f     | Food deprived: 3 mice, 50 neurons each mouse<br>Refed: 3 mice, 50 neurons each mouse         | Unpaired t-test                       | <i>P</i> <0.001<br>Food dep: 594 ± 85<br>Refed: 1224 ± 162                              |
|        |                                                                                              |                                       |                                                                                         |
| 2b     | 19 mice                                                                                      | Paired t-test                         | <i>P</i> <0.001                                                                         |
|        |                                                                                              | Vehicle                               | 0.54 ± 0.027                                                                            |
|        |                                                                                              | CNO                                   | 0.15 ± 0.040                                                                            |
|        |                                                                                              | Difference                            | -0.391 ± 0.042                                                                          |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
| 2d     | 16 mice                                                                                      | Paired t-test                         | <i>P</i> =0.477                                                                         |
|        |                                                                                              | Vehicle                               | 0.53 ± 0.026                                                                            |
|        |                                                                                              | CNO                                   | 0.54 ± 0.021                                                                            |
|        |                                                                                              | Difference                            | -0.0125 ± 0.017                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
| 2f     | aDCN-LAT: 9 mice<br>aDCN-INT: 16 mice<br>aDCN-mCherry (control): 8 mice                      | Two-way ANOVA                         |                                                                                         |
|        |                                                                                              | Interaction                           | F (2, 60) = 4.97, <i>P</i> =0.010                                                       |
|        |                                                                                              | Factor 1: group                       | F (2, 60) = 5.18, <i>P</i> =0.008                                                       |
|        |                                                                                              | Factor 2: treatment                   | F (1, 60) = 4.43, <i>P</i> =0.039                                                       |
|        |                                                                                              | Holm-Sidak's multiple comparison test |                                                                                         |
|        |                                                                                              | aDCN-LAT                              | <i>P</i> =0.002                                                                         |
|        |                                                                                              | aDCN-INT                              | <i>P</i> =0.720                                                                         |
|        |                                                                                              | aDCN mCherry                          | <i>P</i> =0.720                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              | aDCN-LAT (vehicle)                    | 0.292 ± 0.0503                                                                          |
|        |                                                                                              | aDCN-LAT (CNO)                        | 0.034 ± 0.011                                                                           |
|        |                                                                                              | aDCN-INT (vehicle)                    | 0.310 ± 0.372                                                                           |
|        |                                                                                              | aDCN-INT (CNO)                        | 0.271 ± 0.0355                                                                          |
|        |                                                                                              | aDCN mCherry (vehicle)                | 0.280 ± 0.448                                                                           |
|        |                                                                                              | aDCN mCherry (CNO)                    | 0.332 ±0.0571                                                                           |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
| 2g     | aDCN-LAT: 9 mice<br>aDCN-INT: 16 mice<br>aDCN-mCherry (control): 8 mice                      | Two-way ANOVA                         |                                                                                         |
|        |                                                                                              | Interaction                           | F (2, 60) = 1.11, <i>P</i> =0.338                                                       |
|        |                                                                                              | Factor 1: group                       | F (2, 60) = 1.07, <i>P</i> =0.349                                                       |
|        |                                                                                              | Factor 2: treatment                   | F (1, 60) = 1.17, <i>P</i> =0.284                                                       |
|        |                                                                                              | Holm-Sidak's multiple comparison test |                                                                                         |
|        |                                                                                              | aDCN-LAT                              | <i>P</i> =0.286                                                                         |
|        |                                                                                              | aDCN-INT                              | <i>P</i> =0.620                                                                         |
|        |                                                                                              | aDCN mCherry                          | <i>P</i> =0.621                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              | aDCN-LAT (vehicle)                    | 2.22 ± 0.4001                                                                           |
|        |                                                                                              | aDCN-LAT (CNO)                        | 3.00 ± 0.408                                                                            |
|        |                                                                                              | aDCN-INT (vehicle)                    | 2.13 ± 0.211                                                                            |
|        |                                                                                              | aDCN-INT (CNO)                        | 2.44 ± 0.192                                                                            |
|        |                                                                                              | aDCN mCherry (vehicle)                | 2.25 ± 0.366                                                                            |
|        |                                                                                              | aDCN mCherry (CNO)                    | 2.00 ± 0.327                                                                            |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
| 2h     | 13 mice                                                                                      | Paired t-test                         | <i>P</i> =0.016<br>Veh: 0.15 ± 0.017<br>CNO: 0.22 ± 0.028<br>Difference: -0.069 ± 0.025 |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
|        |                                                                                              |                                       |                                                                                         |
| 2j     | aDCN-mCherry (control): EI 7 mice, EE 8 mice<br>aDCN-LAT: EI 8 mice, EE 8 mice               | Two-way ANOVA                         |                                                                                         |
|        |                                                                                              | Interaction                           | F(1, 27) = 26.6, <i>P</i> <0.001                                                        |
|        |                                                                                              | Factor 1: EI vs EE                    | F(1, 27) = 7.66, <i>P</i> =0.01                                                         |
|        |                                                                                              | Factor 2: group                       | F(1, 27) = 130, <i>P</i> <0.001                                                         |
|        |                                                                                              | Holm-Sidak's multiple comparison test |                                                                                         |
|        |                                                                                              | EI aDCN-LAT vs. EI Control            | <i>P</i> <0.001                                                                         |
|        |                                                                                              | EE Control vs. EI Control             | <i>P</i> =0.109                                                                         |
|        |                                                                                              | EE aDCN-LAT vs. EI Control            | <i>P</i> <0.001                                                                         |
|        |                                                                                              | EE Control vs. EI aDCN-LAT            | <i>P</i> <0.001                                                                         |
|        |                                                                                              | EE aDCN-LAT vs. EI aDCN-LAT           | <i>P</i> <0.001                                                                         |
|        |                                                                                              | EE aDCN-LAT vs. EE Control            | <i>P</i> <0.001                                                                         |
|        |                                                                                              | EI Controls                           | 6.49 ± 0.523                                                                            |
|        |                                                                                              | EI aDCN-LAT                           | 0.895 ± 0.287                                                                           |
|        |                                                                                              | EE Controls                           | 5.69 ± 0.229                                                                            |
|        |                                                                                              | EE aDCN-LAT                           | 3.57 ± 0.294                                                                            |
|        |                                                                                              |                                       |                                                                                         |
| 2k     | aDCN-LAT: 8 mice<br>aDCN-INT: 8 mice                                                         | Two-way ANOVA                         |                                                                                         |
|        |                                                                                              | Interaction                           | F(190, 1995) = 2.75, <i>P</i> <0.001                                                    |
|        |                                                                                              |                                       |                                                                                         |
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|----|--------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
|    | aDCN-mCherry (control):<br>8 mice                            | Factor 1: time<br>Factor 2: group                                                                                                                                                                                                 | F(95, 1995) = 1355, $P<0.001$<br>F(2, 21) = 9.23, $P=0.001$                                                                                              |
|    |                                                              | Holm-Sidak's multiple comparison test<br>aDCN-LAT vs. aDCN-INT<br>aDCN-LAT vs. Controls<br>aDCN-INT vs. Controls                                                                                                                  | $P=0.008$<br>$P=0.002$<br>$P=0.403$                                                                                                                      |
| 2l | 7-8 mice                                                     | EE (0, 6, 12, 24, 48 h)<br>EI (0, 6, 12, 24, 48 h)                                                                                                                                                                                | Mean: 0, 16, 50, 69, 82%<br>Mean: 0, 63, 80, 90, 95%                                                                                                     |
| 2m | 9 mice                                                       | Repeated measures two-way ANOVA<br>Interaction<br>Factor 1: food type<br>Factor 2: treatment                                                                                                                                      | $F(1, 32) = 17.5$ , $P<0.001$<br>$F(1, 32) = 43.6$ , $P<0.001$<br>$F(1, 32) = 93.2$ , $P<0.001$                                                          |
|    |                                                              | Holm-Sidak multiple comparisons<br>Chow (veh versus CNO)<br>HFHS (veh versus CNO)<br>Veh (chow vs HFHS)<br>CNO (chow vs HFHS)                                                                                                     | $P=0.002$<br>$P<0.001$<br>$P<0.001$<br>$P=0.097$                                                                                                         |
|    |                                                              | Chow (vehicle)<br>HFHS (vehicle)<br>Chow (CNO)<br>HFHS (CNO)                                                                                                                                                                      | $4.01 \pm 0.219$<br>$10.6 \pm 0.653$<br>$0.671 \pm 0.263$<br>$2.15 \pm 0.974$                                                                            |
| 2o | aDCN-LAT hM3D(Gq): 9 mice<br>aDCN-mCherry (control): 13 mice | Two-way ANOVA<br>Interaction<br>Factor 1: calorie density<br>Factor 2: group                                                                                                                                                      | $F(1, 40) = 0.0042$ , $P=0.949$<br>$F(1, 40) = 12.9$ , $P<0.001$<br>$F(1, 40) = 85.9$ , $P<0.001$                                                        |
|    |                                                              | Holm-Sidak's multiple comparison test<br>0.9 kcal/ml: mCherry vs. hM3D(Gq)<br>1.5 kcal/ml: mCherry vs. hM3D(Gq)<br>mCherry: 0.9 kcal/ml vs. 1.5 kcal/ml<br>hM3D(Gq): 0.9 kcal/ml vs. 1.5 kcal/ml                                  | $P<0.001$<br>$P<0.001$<br>$P=0.017$<br>$P=0.022$                                                                                                         |
|    |                                                              | 0.9 kcal/ml: mCherry<br>0.9 kcal/ml: hM3D(Gq)<br>1.5 kcal/ml: mCherry<br>1.5 kcal/ml: hM3D(Gq)                                                                                                                                    | $3.02 \pm 0.106$<br>$1.57 \pm 0.141$<br>$2.27 \pm 0.196$<br>$0.917 \pm 0.232$                                                                            |
| 2p | aDCN-LAT hM3D(Gq): 9 mice<br>aDCN-mCherry (control): 13 mice | Two-way ANOVA<br>Interaction<br>Factor 1: calorie density<br>Factor 2: group                                                                                                                                                      | $F(1, 40) = 2.14$ , $P=0.151$<br>$F(1, 40) = 1.16$ , $P=0.288$<br>$F(1, 40) = 43.4$ , $P<0.001$                                                          |
|    |                                                              | Holm-Sidak's multiple comparison test<br>0.9 kcal/ml: control vs hM3D(Gq)<br>1.5 kcal/ml: control vs hM3D(Gq)<br>mCherry (0.9 kcal/ml vs 1.5 kcal/ml)<br>hM3D(Gq) (0.9 kcal/ml vs 1.5 kcal/ml)                                    | $P=0.002$<br>$P<0.001$<br>$P=0.105$<br>$P=0.803$                                                                                                         |
|    |                                                              | 0.9 kcal/ml (vehicle)<br>0.9 kcal/ml (CNO)<br>1.5 kcal/ml (vehicle)<br>1.5 kcal/ml (CNO)                                                                                                                                          | $2.80 \pm 0.098$<br>$1.45 \pm 0.131$<br>$3.63 \pm 0.29$<br>$1.35 \pm 0.491$                                                                              |
| 3d | 3 brains, 4 slices each                                      | One-way ANOVA                                                                                                                                                                                                                     | $F(3, 8) = 41.7$ , $P<0.001$                                                                                                                             |
|    |                                                              | Holm-Sidak's multiple comparisons<br><i>Spp1</i> vs. <i>Celf4</i><br><i>Spp1</i> vs. <i>Spp1/Celf4</i><br><i>Spp1</i> vs. Others<br><i>Celf4</i> vs. <i>Spp1/Celf4</i><br><i>Celf4</i> vs. Others<br><i>Spp1/Celf4</i> vs. Others | $P<0.001$<br>$P<0.001$<br>$P<0.001$<br>$P=0.176$<br>$P=0.829$<br>$P=0.176$                                                                               |
|    |                                                              | <i>Spp1</i><br><i>Celf4</i><br><i>Spp1/Celf4</i><br>Others                                                                                                                                                                        | $77.54 \pm 7.864$<br>$3.000 \pm 1.178$<br>$18.21 \pm 7.708$<br>$1.246 \pm 0.7046$                                                                        |
| 3f | 7 mice                                                       | Repeated measures two-way ANOVA<br>Interaction<br>Factor 1: treatment<br>Factor 2: time                                                                                                                                           | $F(11, 132) = 7.07$ , $P<0.001$<br>$F(1, 12) = 14.7$ , $P=0.002$<br>$F(11, 132) = 3.68$ , $P<0.001$                                                      |
|    |                                                              | Holm-Sidak's multiple comparisons<br>-120 to -90<br>-90 to -60<br>-60 to -30<br>-30 to 0<br>0 to 30<br>30 to 60<br>60 to 90<br>90 to 120<br>120 to 150<br>150 to 180<br>180 to 210<br>210 to 240                                  | $P=0.992$<br>$P=0.992$<br>$P=0.992$<br>$P=0.992$<br>$P=0.768$<br>$P=0.381$<br>$P=0.005$<br>$P=0.002$<br>$P<0.001$<br>$P<0.001$<br>$P<0.001$<br>$P<0.001$ |

|                             |                                                                     |                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------------------|---------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 3h                          | 14 mice                                                             | Paired t-test                                                                       | $P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Vehicle                                                                             | $0.506 \pm 0.0216$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                             |                                                                     | CNO                                                                                 | $0.071 \pm 0.014$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|                             |                                                                     | Difference                                                                          | $-0.436 \pm 0.022$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 3i                          | 12 mice                                                             | Paired t-test                                                                       | $P=0.504$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Vehicle                                                                             | $0.515 \pm 0.0303$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                             |                                                                     | CNO                                                                                 | $0.506 \pm 0.0252$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                             |                                                                     | Difference                                                                          | $-0.00916 \pm 0.0133$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|                             |                                                                     |                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 4c                          | aDCN-LAT hM3D(Gq): 6 mice<br>aDCN-LAT hM3D(Gq) VTA hM4D(Gi): 6 mice | Repeated measures two-way ANOVA<br>Interaction<br>Factor 1: time<br>Factor 2: group | $F(10, 100) = 12.8, P<0.001$<br>$F(10, 100) = 23.8, P<0.001$<br>$F(1, 10) = 15.1, P=0.003$                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|                             |                                                                     | Holm-Sidak's multiple comparisons test:                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                             |                                                                     | Bin 1 (-3-0)                                                                        | $P=0.988$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 2 (0-3)                                                                         | $P=0.988$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 3 (3-6)                                                                         | $P=0.988$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 4 (6-9)                                                                         | $P=0.682$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 5 (9-12)                                                                        | $P=0.089$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 6 (12-15)                                                                       | $P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 7 (15-18)                                                                       | $P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 8 (18-21)                                                                       | $P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 9 (21-24)                                                                       | $P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 10 (24-27)                                                                      | $P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Bin 11 (27-30)                                                                      | $P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 4d                          | 7 mice                                                              | Repeated measures one-way ANOVA                                                     | $F(1.89, 11.3) = 23.2, P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                             |                                                                     | Holm-Sidak's multiple comparisons                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                             |                                                                     | aDCN-LAT hM3D(Gq): vehicle versus CNO                                               | $P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | aDCN-LAT hM3D(Gq) vehicle versus aDCN-LAT hM3D(Gq) VTA hM4D(Gi) CNO                 | $P=0.237$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | CNO: aDCN-LAT hM3D(Gq) versus aDCN-LAT hM3D(Gq) VTA hM4D(Gi)                        | $P=0.005$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | aDCN-LAT hM3D(Gq) (veh)                                                             | $0.526 \pm 0.0354$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                             |                                                                     | aDCN-LAT hM3D(Gq) (CNO)                                                             | $0.164 \pm 0.0471$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                             |                                                                     | aDCN-LAT hM3D(Gq) VTA hM4D(Gi) (CNO)                                                | $0.446 \pm 0.0412$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 4e                          | 13 mice                                                             | Pearson correlation                                                                 | $r=-0.84, P<0.001$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 4h                          | Control: 6 mice<br>aDCN-LAT hM3D(Gq): 7 mice                        | Unpaired t-test                                                                     | $P=0.002$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Control                                                                             | $0.152 \pm 0.0591$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                             |                                                                     | aDCN-LAT hM3D(Gq)                                                                   | $0.0508 \pm 0.0107$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|                             |                                                                     | Difference                                                                          | $0.101 \pm 0.0250$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 4j                          | Control: 7 mice<br>aDCN-LAT hM3D(Gq) VTA hM4D(Gi): 6 mice           | Unpaired t-test                                                                     | $P=0.586$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                             |                                                                     | Control                                                                             | $0.138 \pm 0.0271$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                             |                                                                     | aDCN-LAT hM3D(Gq) VTA hM4D(Gi)                                                      | $0.117 \pm 0.0259$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                             |                                                                     | Difference                                                                          | $-0.0212 \pm 0.0378$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                             |                                                                     |                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Extended Data Figure</b> |                                                                     |                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| ED 2j                       | 9 mice                                                              | Regions:<br>Lat<br>LatPC<br>IntA<br>IntDL<br>IntP<br>IntPPC<br>Med<br>MedL<br>MedDL | Water/ Ensure/ Refed:<br>$10.33 \pm 2.333 / 30.00 \pm 11.67 / 67.50 \pm 2.333$<br>$7.667 \pm 2.333 / 18.75 \pm 5.662 / 22.50 \pm 2.333$<br>$4.667 \pm 1.202 / 20.75 \pm 9.595 / 36.00 \pm 1.202$<br>$1.667 \pm 1.202 / 10.25 \pm 5.618 / 9.500 \pm 1.202$<br>$13.33 \pm 1.856 / 21.00 \pm 4.899 / 42.00 \pm 1.856$<br>$2.333 \pm 1.453 / 7.500 \pm 3.476 / 4.500 \pm 1.453$<br>$6.000 \pm 1.155 / 18.00 \pm 9.174 / 39.50 \pm 1.155$<br>$0.6667 \pm 0.667 / 2.500 \pm 0.957 / 6.00 \pm 0.667$<br>$0.6667 \pm 0.333 / 11.75 \pm 2.594 / 11.50 \pm 0.333$ |
| ED 2cc                      | 9 mice                                                              | Regions:<br>DCN<br>NTS<br>PVH<br>ARC<br>LPBN<br>CEA<br>BNST                         | Non-nutritive / nutritive:<br>$8.33 \pm 1.04 / 68.8 \pm 9.68$<br>$32.9 \pm 7.14 / 83.5 \pm 5.99$<br>$27.0 \pm 5.21 / 72.8 \pm 7.17$<br>$54.9 \pm 6.73 / 69.2 \pm 7.08$<br>$47.1 \pm 7.56 / 75.9 \pm 9.31$<br>$33.7 \pm 5.55 / 76.6 \pm 8.42$<br>$57.1 \pm 4.08 / 86.5 \pm 6.42$                                                                                                                                                                                                                                                                         |
|                             |                                                                     | Regions:<br>DCN<br>NTS<br>PVH<br>ARC<br>LPBN<br>CEA<br>BNST                         | Ensure infusion / refed:<br>$0.651 \pm 0.102 / 0.676 \pm 0.324$<br>$0.827 \pm 0.101 / 0.608 \pm 0.165$<br>$0.573 \pm 0.0980 / 0.737 \pm 0.263$<br>$0.301 \pm 0.0907 / 0.451 \pm 0.549$<br>$0.756 \pm 0.110 / 0.118 \pm 0.348$<br>$0.695 \pm 0.0660 / 0.551 \pm 0.449$<br>$0.892 \pm 0.0576 / 0.272 \pm 0.244$                                                                                                                                                                                                                                           |
|                             |                                                                     |                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| ED 3b                       | aDCN-LAT – 5 mice:<br>377, 282, 192, 156 & 91                       |                                                                                     | aDCN-LAT / aDCN-INT:<br>Lat: $38.15 \pm 8.24 / 11.13 \pm 4.21$<br>LatPC: $30.26 \pm 5.58 / 2.98 \pm 2.04$                                                                                                                                                                                                                                                                                                                                                                                                                                               |

|       |                                                                                      |                                                                        |                                                                                                                                                                                                                                               |
|-------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|       | neurons from each mouse<br>aDCN-INT – 3 mice: 504, 560 & 165 neurons from each mouse |                                                                        | IntDL: 5.03 ± 2.25 / 3.99 ± 2.67<br>IntA: 8.90 ± 3.84 / 43.20 ± 9.97<br>IntP: 15.93 ± 7.56 / 16.12 ± 8.11<br>IntPPC: 1.12 ± 0.70 / 1.84 ± 0.99<br>MedDL: 0.48 ± 0.477 / 12.67 ± 0.58<br>Med: 0 ± 0 / 8.07 ± 2.27<br>MedL: 0.13 ± 0.13 / 0 ± 0 |
| ED 3f | 8 mice                                                                               | Paired t-test                                                          | <i>P</i> =0.539                                                                                                                                                                                                                               |
|       |                                                                                      | Vehicle                                                                | 0.52 ± 0.031                                                                                                                                                                                                                                  |
|       |                                                                                      | CNO                                                                    | 0.55 ± 0.054                                                                                                                                                                                                                                  |
|       |                                                                                      | Difference                                                             | 0.025 ± 0.34                                                                                                                                                                                                                                  |
| ED 3h | 12 mice                                                                              | Paired t-test                                                          | <i>P</i> =0.548                                                                                                                                                                                                                               |
|       |                                                                                      | Vehicle                                                                | 0.48 ± 0.032                                                                                                                                                                                                                                  |
|       |                                                                                      | CNO                                                                    | 0.46 ± 0.039                                                                                                                                                                                                                                  |
|       |                                                                                      | Difference                                                             | -0.025 ± 0.040                                                                                                                                                                                                                                |
| ED 3i | 8 mice                                                                               | Paired t-test                                                          | <i>P</i> =0.722                                                                                                                                                                                                                               |
|       |                                                                                      | Vehicle                                                                | 0.51 ± 0.052                                                                                                                                                                                                                                  |
|       |                                                                                      | CNO                                                                    | 0.53 ± 0.42                                                                                                                                                                                                                                   |
|       |                                                                                      | Difference                                                             | 0.016 ± 0 .044                                                                                                                                                                                                                                |
| <hr/> |                                                                                      |                                                                        |                                                                                                                                                                                                                                               |
| ED 4b | aDCN-LAT: 9 mice<br>aDCN-INT: 16 mice<br>aDCN-mCherry (control): 8 mice              | Two-way ANOVA<br>Interaction<br>Factor 1: group<br>Factor 2: treatment | <i>F</i> (2, 60) = 8.55, <i>P</i> <0.001<br><i>F</i> (2, 60) = 9.04, <i>P</i> =0.001<br><i>F</i> (1, 60) = 8.44, <i>P</i> =0.005                                                                                                              |
|       |                                                                                      | Holm-Sidak's multiple comparison test                                  |                                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN-LAT                                                               | <i>P</i> <0.001                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN-INT                                                               | <i>P</i> >0.999                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN mCherry                                                           | <i>P</i> >0.999                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN-LAT (vehicle)                                                     | 27.9 ± 7.10                                                                                                                                                                                                                                   |
|       |                                                                                      | aDCN-LAT (CNO)                                                         | 450 ± 169                                                                                                                                                                                                                                     |
|       |                                                                                      | aDCN-INT (vehicle)                                                     | 26.7 ± 4.14                                                                                                                                                                                                                                   |
|       |                                                                                      | aDCN-INT (CNO)                                                         | 21.4 ± 3.99                                                                                                                                                                                                                                   |
|       |                                                                                      | Control (vehicle)                                                      | 12.9 ± 3.13                                                                                                                                                                                                                                   |
|       |                                                                                      | Control (CNO)                                                          | 13.8 ± 4.56                                                                                                                                                                                                                                   |
| ED 4c | aDCN-LAT: 9 mice<br>aDCN-INT: 16 mice<br>aDCN-mCherry (control): 8 mice              | Two-way ANOVA<br>Interaction<br>Factor 1: group<br>Factor 2: treatment | <i>F</i> (2, 60) = 5.78, <i>P</i> =0.005<br><i>F</i> (2, 60) = 5.60, <i>P</i> =0.006<br><i>F</i> (1, 60) = 13.3, <i>P</i> <0.001                                                                                                              |
|       |                                                                                      | Holm-Sidak's multiple comparison test                                  |                                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN-LAT                                                               | <i>P</i> <0.001                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN-INT                                                               | <i>P</i> =0.248                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN mCherry                                                           | <i>P</i> =0.891                                                                                                                                                                                                                               |
|       |                                                                                      | Vehicle: aDCN-LAT                                                      | 2348 ± 287.9                                                                                                                                                                                                                                  |
|       |                                                                                      | Vehicle: aDCN-INT                                                      | 2404 ± 162.7                                                                                                                                                                                                                                  |
|       |                                                                                      | Vehicle: aDCN mCherry                                                  | 2256 ± 284.1                                                                                                                                                                                                                                  |
|       |                                                                                      | CNO: aDCN-LAT                                                          | 584.6 ± 289.3                                                                                                                                                                                                                                 |
|       |                                                                                      | CNO: aDCN-INT                                                          | 1974 ± 176.2                                                                                                                                                                                                                                  |
|       |                                                                                      | CNO: aDCN mCherry                                                      | 2201 ± 252.5                                                                                                                                                                                                                                  |
| ED 4d | aDCN-LAT: 9 mice<br>aDCN-INT: 16 mice<br>aDCN-mCherry (control): 8 mice              | Two-way ANOVA<br>Interaction<br>Factor 1: group<br>Factor 2: treatment | <i>F</i> (2, 60) = 0.292, <i>P</i> =0.748<br><i>F</i> (2, 60) = 1.89, <i>P</i> =0.160<br><i>F</i> (1, 60) = 0.114, <i>P</i> =0.737                                                                                                            |
|       |                                                                                      | Holm-Sidak's multiple comparison test                                  |                                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN-LAT                                                               | <i>P</i> =0.951                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN-INT                                                               | <i>P</i> =0.752                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN mCherry                                                           | <i>P</i> =0.951                                                                                                                                                                                                                               |
|       |                                                                                      | Vehicle: aDCN-LAT                                                      | 0.796 ± 0.0635                                                                                                                                                                                                                                |
|       |                                                                                      | Vehicle: aDCN-INT                                                      | 0.890 ± 0.0821                                                                                                                                                                                                                                |
|       |                                                                                      | Vehicle: aDCN mCherry                                                  | 0.736 ± 0.127                                                                                                                                                                                                                                 |
|       |                                                                                      | CNO: aDCN-LAT                                                          | 0.833 ± 0.210                                                                                                                                                                                                                                 |
|       |                                                                                      | CNO: aDCN-INT                                                          | 1.03 ± 0.134                                                                                                                                                                                                                                  |
|       |                                                                                      | CNO: aDCN mCherry                                                      | 0.673 ± 0.129                                                                                                                                                                                                                                 |
| ED 4f | aDCN-LAT: 9 mice<br>aDCN-INT: 16 mice<br>aDCN-mCherry (control): 8 mice              | Two-way ANOVA<br>Interaction<br>Factor 1: treatment<br>Factor 2: group | <i>F</i> (2, 60) = 7.77, <i>P</i> =0.001<br><i>F</i> (1, 60) = 8.11, <i>P</i> =0.006<br><i>F</i> (2, 60) = 0.926, <i>P</i> =0.402                                                                                                             |
|       |                                                                                      | LatPC (CNO) vs. LatPC (vehicle)                                        | <i>P</i> <0.001                                                                                                                                                                                                                               |
|       |                                                                                      | aDCN-INT (CNO) vs. aDCN-INT (vehicle)                                  | <i>P</i> =0.997                                                                                                                                                                                                                               |
|       |                                                                                      | Control (CNO) vs. Control (vehicle)                                    | <i>P</i> >0.999                                                                                                                                                                                                                               |
|       |                                                                                      | Vehicle: aDCN-LAT                                                      | 0.110 ± 0.0251                                                                                                                                                                                                                                |
|       |                                                                                      | Vehicle: aDCN-INT                                                      | 0.0806 ± 0.0106                                                                                                                                                                                                                               |
|       |                                                                                      | Vehicle: aDCN mCherry                                                  | 0.0463 ± 0.0124                                                                                                                                                                                                                               |
|       |                                                                                      | CNO: aDCN-LAT                                                          | 0.001 ± 0.001                                                                                                                                                                                                                                 |
|       |                                                                                      | CNO: aDCN-INT                                                          | 0.0637 ± 0.0126                                                                                                                                                                                                                               |
|       |                                                                                      | CNO: aDCN mCherry                                                      | 0.0638 ± 0.0189                                                                                                                                                                                                                               |
| ED 4g | aDCN-LAT: 9 mice<br>aDCN-INT: 16 mice<br>aDCN-mCherry (control): 8 mice              | Two-way ANOVA<br>Interaction<br>Factor 1: treatment<br>Factor 2: group | <i>F</i> (2, 60) = 29.9, <i>P</i> <0.001<br><i>F</i> (1, 60) = 35.8, <i>P</i> <0.001<br><i>F</i> (2, 60) = 20.6, <i>P</i> <0.001                                                                                                              |
|       |                                                                                      | Holm-Sidak's multiple comparison test                                  |                                                                                                                                                                                                                                               |
|       |                                                                                      | CNO:aDCN-LAT vs. Veh:aDCN-LAT                                          | <i>P</i> <0.001                                                                                                                                                                                                                               |

|       |                                                             |                                                                                         |                                                                                                                                    |
|-------|-------------------------------------------------------------|-----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|
|       |                                                             | CNO:aDCN-INT vs. Veh:aDCN-INT                                                           | <i>P</i> =0.990                                                                                                                    |
|       |                                                             | CNO:Control vs. Veh:Control                                                             | <i>P</i> =0.924                                                                                                                    |
|       |                                                             | aDCN-LAT: vehicle                                                                       | 422 ± 107                                                                                                                          |
|       |                                                             | aDCN-LAT: CNO                                                                           | 3425 ± 175                                                                                                                         |
|       |                                                             | aDCN-INT: vehicle                                                                       | 827 ± 209                                                                                                                          |
|       |                                                             | aDCN-INT: CNO                                                                           | 725 ± 151                                                                                                                          |
|       |                                                             | Control: vehicle                                                                        | 384 ± 93                                                                                                                           |
|       |                                                             | Control: CNO                                                                            | 714 ± 416                                                                                                                          |
| ED 4h | pDCN hM3D(Gq): 12 mice<br>pDCN mCherry (control): 8 mice    | Two-way ANOVA<br>Interaction<br>Factor 1: treatment<br>Factor 2: group                  | <i>F</i> (1, 36) = 0.866, <i>P</i> =0.358<br><i>F</i> (1, 36) = 0.497, <i>P</i> =0.485<br><i>F</i> (1, 36) = 1.66, <i>P</i> =0.206 |
|       |                                                             | pDCN mCherry Vehicle                                                                    | 0.035 ± 0.00598                                                                                                                    |
|       |                                                             | pDCN mCherry CNO                                                                        | 0.0713 ± 0.0248                                                                                                                    |
|       |                                                             | pDCN hM3D Vehicle                                                                       | 0.0842 ± 0.0145                                                                                                                    |
|       |                                                             | pDCN hM3D CNO                                                                           | 0.0792 ± 0.0282                                                                                                                    |
| ED 4j | 7-8 mice                                                    | Unpaired t-test                                                                         | <i>P</i> =0.004                                                                                                                    |
|       |                                                             | aDCN-mCherry (control)aDCN-LAT                                                          | 42.9 ± 2.61                                                                                                                        |
|       |                                                             | Difference                                                                              | 39.0 ± 1.56<br>-3.93 ± 1.13                                                                                                        |
| ED 4k | 7-8 mice                                                    | Repeated measures two-way ANOVA<br>Interaction<br>Factor 1: treatment<br>Factor 2: time | <i>F</i> (12, 112) = 11.1, <i>P</i> <0.001<br><i>F</i> (3, 28) = 17.6, <i>P</i> <0.001<br><i>F</i> (2, 66)=18.62, <i>P</i> <0.001  |
|       |                                                             | Holm-Sidak's multiple comparisons<br>aDCN-mCherry (control) vs aDCN-LAT (EE)            |                                                                                                                                    |
|       |                                                             | 6 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 12 h                                                                                    | <i>P</i> =0.009                                                                                                                    |
|       |                                                             | 24 h                                                                                    | <i>P</i> =0.283                                                                                                                    |
|       |                                                             | 48 h                                                                                    | <i>P</i> =0.430                                                                                                                    |
|       |                                                             | aDCN-mCherry (control)vs aDCN-LAT (EI)                                                  |                                                                                                                                    |
|       |                                                             | 6 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 12 h                                                                                    | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 24 h                                                                                    | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 48 h                                                                                    | <i>P</i> =0.006                                                                                                                    |
| <hr/> |                                                             |                                                                                         |                                                                                                                                    |
| ED 5a | aDCN-mCherry (control): 8 mice<br>aDCN-LAT hM3D(Gq): 9 mice | Repeated measures two-way ANOVA<br>Interaction<br>Factor 1: time<br>Factor 2: treatment | <i>F</i> (8, 72) = 4.35, <i>P</i> <0.001<br><i>F</i> (8, 72) = 68.1, <i>P</i> <0.001<br><i>F</i> (1, 72) = 158, <i>P</i> <0.001    |
|       |                                                             | Holm-Sidak's multiple comparisons                                                       |                                                                                                                                    |
|       |                                                             | 0.5 h                                                                                   | <i>P</i> =0.094                                                                                                                    |
|       |                                                             | 1 h                                                                                     | <i>P</i> =0.008                                                                                                                    |
|       |                                                             | 2 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 4 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 6 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 8 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 10 h                                                                                    | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 12 h                                                                                    | <i>P</i> <0.001                                                                                                                    |
| ED 5b | 9 mice                                                      | Paired t-test                                                                           | <i>P</i> =0.008                                                                                                                    |
|       |                                                             | Vehicle                                                                                 | 10.80 ± 0.99                                                                                                                       |
|       |                                                             | CNO                                                                                     | 6.08 ± 0.84                                                                                                                        |
|       |                                                             | Difference                                                                              | -4.72 ± 1.35                                                                                                                       |
| ED 5c | 8 mice                                                      | Paired t-test                                                                           | <i>P</i> =0.391                                                                                                                    |
|       |                                                             | Vehicle                                                                                 | 10.14 ± 0.55                                                                                                                       |
|       |                                                             | CNO                                                                                     | 9.74 ± 0.65                                                                                                                        |
|       |                                                             | Difference                                                                              | -0.40 ± 0.437                                                                                                                      |
| ED 5d | 9 mice                                                      | Paired t-test                                                                           | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | Vehicle                                                                                 | 4.01 ± 0.22                                                                                                                        |
|       |                                                             | CNO                                                                                     | 0.67 ± 0.26                                                                                                                        |
|       |                                                             | Difference                                                                              | -3.34 ± 0.40                                                                                                                       |
| ED 5e | 8 mice                                                      | Paired t-test                                                                           | <i>P</i> =0.223                                                                                                                    |
|       |                                                             | Vehicle                                                                                 | 3.607 ± 0.202                                                                                                                      |
|       |                                                             | CNO                                                                                     | 3.36 ± 0.155                                                                                                                       |
|       |                                                             | Difference                                                                              | -0.244 ± 0.183                                                                                                                     |
| ED 5f | aDCN-mCherry (control): 8 mice<br>aDCN-LAT hM3D(Gq): 9 mice | Repeated measures two-way ANOVA<br>Interaction<br>Factor 1: time<br>Factor 2: treatment | <i>F</i> (8, 72) = 8.45, <i>P</i> <0.001<br><i>F</i> (8, 72) = 57.6, <i>P</i> <0.001<br><i>F</i> (1, 72) = 445, <i>P</i> <0.001    |
|       |                                                             | Holm-Sidak's multiple comparisons                                                       |                                                                                                                                    |
|       |                                                             | 0.5 h                                                                                   | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 1 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 2 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 4 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 6 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 8 h                                                                                     | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 10 h                                                                                    | <i>P</i> <0.001                                                                                                                    |
|       |                                                             | 12 h                                                                                    | <i>P</i> <0.001                                                                                                                    |

|       |                                                                                                                           |                                                                                                  |                                                                                              |
|-------|---------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| ED 5g | 9 mice                                                                                                                    | Paired t-test                                                                                    | $P<0.001$                                                                                    |
|       |                                                                                                                           | Vehicle                                                                                          | $19.57 \pm 0.713$                                                                            |
|       |                                                                                                                           | CNO                                                                                              | $13.53 \pm 0.99$                                                                             |
| ED 5h | 8 mice                                                                                                                    | Difference                                                                                       | $-6.04 \pm 0.668$                                                                            |
|       |                                                                                                                           | Paired t-test                                                                                    | $P=0.527$                                                                                    |
|       |                                                                                                                           | Vehicle                                                                                          | $16.67 \pm 0.74$                                                                             |
| ED 5i | 9 mice                                                                                                                    | CNO                                                                                              | $15.69 \pm 1.11$                                                                             |
|       |                                                                                                                           | Difference                                                                                       | $-0.976 \pm 1.467$                                                                           |
|       |                                                                                                                           | Paired t-test                                                                                    | $P<0.001$                                                                                    |
| ED 5j | 8 mice                                                                                                                    | Vehicle                                                                                          | $10.60 \pm 0.65$                                                                             |
|       |                                                                                                                           | CNO                                                                                              | $2.15 \pm 0.97$                                                                              |
|       |                                                                                                                           | Difference                                                                                       | $-8.45 \pm 0.92$                                                                             |
|       |                                                                                                                           | Paired t-test                                                                                    | $P=0.686$                                                                                    |
|       |                                                                                                                           | Vehicle                                                                                          | $6.615 \pm 0.55$                                                                             |
|       |                                                                                                                           | CNO                                                                                              | $6.12 \pm 0.66$                                                                              |
|       |                                                                                                                           | Difference                                                                                       | $-0.50 \pm 1.18$                                                                             |
| ED 6i | 3 mice each group                                                                                                         | Two-way ANOVA                                                                                    |                                                                                              |
|       |                                                                                                                           | Interaction                                                                                      | $F(2, 18) = 0.850, P=0.444$                                                                  |
|       |                                                                                                                           | Factor 1: neuronal type                                                                          | $F(1, 18) = 4.99, P=0.038$                                                                   |
|       |                                                                                                                           | Factor 2: state                                                                                  | $F(2, 18) = 18.5, P<0.001$                                                                   |
|       |                                                                                                                           | Holm-Sidak's multiple comparisons                                                                |                                                                                              |
|       |                                                                                                                           | Refed - Food dep.:                                                                               |                                                                                              |
|       |                                                                                                                           | <i>vGluT2</i> <sup>+</sup>                                                                       | $P=0.034$                                                                                    |
|       |                                                                                                                           | <i>vGAT</i> <sup>+</sup>                                                                         | $P=0.500$                                                                                    |
|       |                                                                                                                           | <i>GlyT2</i> <sup>+</sup>                                                                        | $P=0.655$                                                                                    |
|       |                                                                                                                           | <i>vGluT2</i> <sup>+</sup> (food dep.)                                                           | $968.4 \pm 115.8$                                                                            |
|       |                                                                                                                           | <i>vGluT2</i> <sup>+</sup> (refed)                                                               | $1521 \pm 232.1$                                                                             |
|       |                                                                                                                           | <i>vGAT</i> <sup>+</sup> (food dep.)                                                             | $454.0 \pm 39.90$                                                                            |
|       |                                                                                                                           | <i>vGAT</i> <sup>+</sup> (refed)                                                                 | $754.2 \pm 69.16$                                                                            |
|       |                                                                                                                           | <i>GlyT2</i> <sup>+</sup> (food dep.)                                                            | $205.9 \pm 29.39$                                                                            |
|       |                                                                                                                           | <i>GlyT2</i> <sup>+</sup> (refed)                                                                | $331.6 \pm 40.01$                                                                            |
| ED 6j | 3 mice each group                                                                                                         | Two-way ANOVA                                                                                    |                                                                                              |
|       |                                                                                                                           | Interaction                                                                                      | $F(2, 12) = 1.58, P=0.247$                                                                   |
|       |                                                                                                                           | Factor 1: neuronal type                                                                          | $F(1, 12) = 9.53, P=0.009$                                                                   |
|       |                                                                                                                           | Factor 2: state                                                                                  | $F(2, 12) = 24.2, P<0.001$                                                                   |
|       |                                                                                                                           | Holm-Sidak's multiple comparisons                                                                |                                                                                              |
|       |                                                                                                                           | Refed - Food dep.:                                                                               |                                                                                              |
|       |                                                                                                                           | <i>vGluT2</i> <sup>+</sup>                                                                       | $P=0.023$                                                                                    |
|       |                                                                                                                           | <i>vGAT</i> <sup>+</sup>                                                                         | $P=0.370$                                                                                    |
|       |                                                                                                                           | <i>GlyT2</i> <sup>+</sup>                                                                        | $P=0.434$                                                                                    |
|       |                                                                                                                           | <i>vGluT2</i> <sup>+</sup> (food dep.)                                                           | $37.00 \pm 7.371$                                                                            |
|       |                                                                                                                           | <i>vGAT</i> <sup>+</sup> (food dep.)                                                             | $14.67 \pm 3.756$                                                                            |
|       |                                                                                                                           | <i>GlyT2</i> <sup>+</sup> (food dep.)                                                            | $8.333 \pm 2.603$                                                                            |
|       |                                                                                                                           | <i>vGluT2</i> <sup>+</sup> (refed)                                                               | $63.33 \pm 10.48$                                                                            |
|       |                                                                                                                           | <i>vGAT</i> <sup>+</sup> (refed)                                                                 | $25.67 \pm 3.712$                                                                            |
|       |                                                                                                                           | <i>GlyT2</i> <sup>+</sup> (refed)                                                                | $15.00 \pm 2.082$                                                                            |
| ED 6k | 3 mice each group<br>Lateral, 70 neurons each mouse<br>Interposed, 92 neurons each mouse<br>Medial, 60 neurons each mouse | Two-way ANOVA                                                                                    |                                                                                              |
|       |                                                                                                                           | Interaction                                                                                      | $F(2, 1326) = 2.93, P=0.054$                                                                 |
|       |                                                                                                                           | Factor 1: DCN regions                                                                            | $F(2, 1326) = 8.43, P<0.001$                                                                 |
|       |                                                                                                                           | Factor 2: state                                                                                  | $F(1, 1326) = 6.24, P=0.013$                                                                 |
|       |                                                                                                                           | Holm-Sidak's multiple comparisons                                                                |                                                                                              |
|       |                                                                                                                           | Refed - Food dep.:                                                                               |                                                                                              |
|       |                                                                                                                           | Lateral                                                                                          | $P=0.006$                                                                                    |
|       |                                                                                                                           | Interposed                                                                                       | $P=0.914$                                                                                    |
|       |                                                                                                                           | Medial                                                                                           | $P=0.433$                                                                                    |
|       |                                                                                                                           | Lateral (food dep.)                                                                              | $1025 \pm 103$                                                                               |
|       |                                                                                                                           | Interposed (food dep.)                                                                           | $1014 \pm 88.4$                                                                              |
|       |                                                                                                                           | Medial (food dep.)                                                                               | $1426 \pm 250$                                                                               |
|       |                                                                                                                           | Lateral (refed)                                                                                  | $1635 \pm 152$                                                                               |
|       |                                                                                                                           | Interposed (refed)                                                                               | $996 \pm 77.1$                                                                               |
|       |                                                                                                                           | Medial (refed)                                                                                   | $1671 \pm 157$                                                                               |
| ED 6l | 3 mice each group,<br>60 neurons in each mouse analyzed for >1000 <i>homer1a</i> expression level                         | Two-way ANOVA                                                                                    |                                                                                              |
|       |                                                                                                                           | Interaction                                                                                      | $F(2, 12) = 2.33, P=0.140$                                                                   |
|       |                                                                                                                           | Factor 1: neuronal type                                                                          | $F(2, 12) = 3.35, P=0.070$                                                                   |
|       |                                                                                                                           | Factor 2: state                                                                                  | $F(1, 12) = 9.44, P=0.010$                                                                   |
|       |                                                                                                                           | Holm-Sidak's multiple comparisons                                                                |                                                                                              |
|       |                                                                                                                           | Refed - Food dep.:                                                                               |                                                                                              |
|       |                                                                                                                           | Lateral                                                                                          | $P=0.025$                                                                                    |
|       |                                                                                                                           | Interposed                                                                                       | $P=0.900$                                                                                    |
|       |                                                                                                                           | Medial                                                                                           | $P=0.122$                                                                                    |
|       |                                                                                                                           | Lateral (food dep.)                                                                              | $21.00 \pm 0.5774$                                                                           |
|       |                                                                                                                           | Interposed (food dep.)                                                                           | $19.33 \pm 4.485$                                                                            |
|       |                                                                                                                           | Medial (food dep.)                                                                               | $19.67 \pm 2.906$                                                                            |
|       |                                                                                                                           | Lateral (refed)                                                                                  | $37.33 \pm 4.631$                                                                            |
|       |                                                                                                                           | Interposed (refed)                                                                               | $20.00 \pm 5.033$                                                                            |
|       |                                                                                                                           | Medial (refed)                                                                                   | $30.33 \pm 2.333$                                                                            |
| ED 7d | 3 brains; aDCN 319 neurons, pDCN 323 neurons                                                                              | Two-way ANOVA<br>Interaction<br>Factor 1: aDCN vs. pDCN<br>Factor 2: <i>Spp1</i> vs <i>Celf4</i> | $F(1, 1280) = 73.1, P<0.001$<br>$F(1, 1280) = 2.92, P=0.088$<br>$F(1, 1280) = 1.10, P=0.295$ |

|       |                                          |                                                                                                                                                                                                                                                                                                               |                                                                            |
|-------|------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
|       |                                          | Holm-Sidak's multiple comparison test<br>aDCN: <i>Celf4</i> vs. aDCN: <i>Spp1</i><br>pDCN: <i>Spp1</i> vs. aDCN: <i>Spp1</i><br>pDCN: <i>Celf4</i> vs. aDCN: <i>Spp1</i><br>pDCN: <i>Spp1</i> vs. aDCN: <i>Celf4</i><br>pDCN: <i>Celf4</i> vs. aDCN: <i>Celf4</i><br>pDCN: <i>Celf4</i> vs. pDCN: <i>Spp1</i> | $P<0.001$<br>$P<0.001$<br>$P=0.640$<br>$P=0.100$<br>$P<0.001$<br>$P<0.001$ |
|       |                                          | aDCN: <i>Spp1</i><br>pDCN: <i>Spp1</i><br>aDCN: <i>Celf4</i><br>pDCN: <i>Celf4</i>                                                                                                                                                                                                                            | $20.1 \pm 1.33$<br>$9.51 \pm 1.09$<br>$12.4 \pm 0.708$<br>$19.5 \pm 0.927$ |
| ED 7g | 1,434 neurons                            | One-way ANOVA                                                                                                                                                                                                                                                                                                 | $F(2, 1431) = 30.6, P<0.001$                                               |
|       |                                          | Holm-Sidak's multiple comparisons<br>Lateral vs. Interposed<br>Lateral vs. Medial<br>Interposed vs. Medial                                                                                                                                                                                                    | $P=0.165$<br>$P<0.001$<br>$P<0.001$                                        |
|       |                                          | Lateral<br>Interposed<br>Medial                                                                                                                                                                                                                                                                               | $6207 \pm 419.1$<br>$5716 \pm 174.6$<br>$3132 \pm 168.2$                   |
| ED 7h | 1,434 neurons                            | One-way ANOVA                                                                                                                                                                                                                                                                                                 | $F(2, 1431) = 21.3, P<0.001$                                               |
|       |                                          | Holm-Sidak's multiple comparisons<br>Lateral vs. Interposed<br>Lateral vs. Medial<br>Interposed vs. Medial                                                                                                                                                                                                    | $P=0.003$<br>$P=0.006$<br>$P<0.001$                                        |
|       |                                          | Lateral<br>Interposed<br>Medial                                                                                                                                                                                                                                                                               | $6531 \pm 272.6$<br>$7483 \pm 160.9$<br>$5506 \pm 228.5$                   |
| ED 7i | 2,261 neurons                            | One-way ANOVA                                                                                                                                                                                                                                                                                                 | $F(2, 2258) = 96.1, P<0.001$                                               |
|       |                                          | Holm-Sidak's multiple comparisons<br>Lateral vs. Interposed<br>Lateral vs. Medial<br>Interposed vs. Medial                                                                                                                                                                                                    | $P<0.001$<br>$P<0.001$<br>$P<0.001$                                        |
|       |                                          | Lateral<br>Interposed<br>Medial                                                                                                                                                                                                                                                                               | $2151 \pm 89.49$<br>$3287 \pm 110.4$<br>$6134 \pm 299.0$                   |
| ED 7j | 2,261 neurons                            | One-way ANOVA                                                                                                                                                                                                                                                                                                 | $F(2, 2258) = 56.1, P<0.001$                                               |
|       |                                          | Holm-Sidak's multiple comparisons<br>Lateral vs. Interposed<br>Lateral vs. Medial<br>Interposed vs. Medial                                                                                                                                                                                                    | $P<0.001$<br>$P<0.001$<br>$P<0.001$                                        |
|       |                                          | Lateral<br>Interposed<br>Medial                                                                                                                                                                                                                                                                               | $2692 \pm 127.6$<br>$3942 \pm 103.8$<br>$5524 \pm 214.8$                   |
| ED 7k | 3 mice, 61 neurons each mouse            | Unpaired t-test                                                                                                                                                                                                                                                                                               | $P<0.001$                                                                  |
|       |                                          | <i>Spp1</i><br><i>Celf4</i><br>Difference                                                                                                                                                                                                                                                                     | $22.3 \pm 1.67$<br>$9.62 \pm 0.667$<br>$-12.7 \pm 1.80$                    |
| ED 7l | 3 mice, 61 neurons each mouse            | Unpaired t-test                                                                                                                                                                                                                                                                                               | $P<0.001$                                                                  |
|       |                                          | <i>Spp1</i><br><i>Celf4</i><br>Difference                                                                                                                                                                                                                                                                     | $11.2 \pm 1.66$<br>$18.3 \pm 1.20$<br>$7.05 \pm 2.04$                      |
| ED 7m | 3 mice each group, 61 neurons each brain | One-way ANOVA                                                                                                                                                                                                                                                                                                 | $F(2, 546) = 13.6, P<0.001$                                                |
|       |                                          | Holm-Sidak's multiple comparisons<br>Interposed vs. lateral<br>Medial vs. lateral<br>Medial vs. interposed                                                                                                                                                                                                    | $P=0.946$<br>$P<0.001$<br>$P<0.001$                                        |
|       |                                          | Lateral<br>Interposed<br>Medial                                                                                                                                                                                                                                                                               | $21941631 \pm 3379383$<br>$22052476 \pm 3465711$<br>$10945808 \pm 1599521$ |
| ED 7n | 3 mice each group, 61 neurons each brain | One-way ANOVA                                                                                                                                                                                                                                                                                                 | $F(2, 546) = 19.3, P<0.001$                                                |
|       |                                          | Holm-Sidak's multiple comparisons<br>Interposed vs. lateral<br>Medial vs. lateral<br>Medial vs. interposed                                                                                                                                                                                                    | $P=0.026$<br>$P<0.001$<br>$P<0.001$                                        |
|       |                                          | Lateral<br>Interposed<br>Medial                                                                                                                                                                                                                                                                               | $7023844 \pm 761270$<br>$5293495 \pm 1132057$<br>$10042043 \pm 3470704$    |
| ED 7o | 3 mice                                   | One-way ANOVA                                                                                                                                                                                                                                                                                                 | $F(2, 6) = 22.2, P=0.002$                                                  |
|       |                                          | Holm-Sidak's multiple comparisons<br>Lateral vs. Interposed<br>Lateral vs. Medial<br>Interposed vs. Medial                                                                                                                                                                                                    | $P=0.190$<br>$P=0.005$<br>$P=0.002$                                        |

|        |                                                             |                                                                     |                                                                  |
|--------|-------------------------------------------------------------|---------------------------------------------------------------------|------------------------------------------------------------------|
| ED 7p  | 3 mice                                                      | Lateral                                                             | 66.59 ± 2.419                                                    |
|        |                                                             | Interposed                                                          | 79.54 ± 7.658                                                    |
|        |                                                             | Medial                                                              | 23.68 ± 7.129                                                    |
|        |                                                             | One-way ANOVA                                                       | F(2, 6) = 40.0, <i>P</i> <0.001                                  |
|        |                                                             | Holm-Sidak's multiple comparisons                                   |                                                                  |
|        |                                                             | Lateral vs. Interposed                                              | <i>P</i> =0.982                                                  |
|        |                                                             | Lateral vs. Medial                                                  | <i>P</i> <0.001                                                  |
|        |                                                             | Interposed vs. Medial                                               | <i>P</i> <0.001                                                  |
| ED 7q  | 3 mice                                                      | Lateral                                                             | 4.835 ± 2.167                                                    |
|        |                                                             | Interposed                                                          | 4.923 ± 2.189                                                    |
|        |                                                             | Medial                                                              | 34.48 ± 3.527                                                    |
|        |                                                             | One-way ANOVA                                                       | F(2, 6) = 3.20, <i>P</i> =0.113                                  |
|        |                                                             | Holm-Sidak's multiple comparisons                                   |                                                                  |
|        |                                                             | Lateral vs. Interposed                                              | <i>P</i> =0.618                                                  |
|        |                                                             | Lateral vs. Medial                                                  | <i>P</i> =0.206                                                  |
|        |                                                             | Interposed vs. Medial                                               | <i>P</i> =0.150                                                  |
| ED 7r  | 3 mice                                                      | Lateral                                                             | 10.99 ± 2.385                                                    |
|        |                                                             | Interposed                                                          | 6.857 ± 5.958                                                    |
|        |                                                             | Medial                                                              | 25.76 ± 7.165                                                    |
|        |                                                             | One-way ANOVA                                                       | F(2, 6) = 2.96, <i>P</i> =0.128                                  |
|        |                                                             | Holm-Sidak's multiple comparisons                                   |                                                                  |
|        |                                                             | Lateral vs. Interposed                                              | <i>P</i> =0.178                                                  |
|        |                                                             | Lateral vs. Medial                                                  | <i>P</i> =0.714                                                  |
|        |                                                             | Interposed vs. Medial                                               | <i>P</i> =0.204                                                  |
|        |                                                             | Lateral                                                             | 17.6 ± 3.40                                                      |
|        |                                                             | Interposed                                                          | 8.68 ± 1.33                                                      |
|        |                                                             | Medial                                                              | 16.1 ± 3.12                                                      |
| ED 8bb | 7 mice                                                      | One-way ANOVA                                                       | F (1.42, 8.52) = 22.7, <i>P</i> <0.001                           |
|        |                                                             | Holm-Sidak's multiple comparison test                               |                                                                  |
|        |                                                             | <i>Ad libitum</i> fed chow vs. food dep. chow                       | <i>P</i> =0.006                                                  |
|        |                                                             | <i>Ad libitum</i> fed chow vs. non-food Food dep. chow vs. non-food | <i>P</i> =0.475<br><i>P</i> =0.003                               |
| ED 8cc | 7 mice                                                      | One-way ANOVA                                                       | F (1.42, 8.52) = 22.7, <i>P</i> <0.001                           |
|        |                                                             | Holm-Sidak's multiple comparison test                               |                                                                  |
|        |                                                             | <i>Ad libitum</i> fed chow vs. food dep. chow                       | <i>P</i> =0.009                                                  |
|        |                                                             | <i>Ad libitum</i> fed chow vs. non-food Food dep. chow vs. non-food | <i>P</i> =0.651<br><i>P</i> =0.011                               |
| ED 9d  | 11 mice                                                     | Repeated measures one-way ANOVA                                     | F (1.49, 14.9) = 94.1, <i>P</i> =0.001                           |
|        |                                                             | Holm-Sidak's multiple comparison test                               |                                                                  |
|        |                                                             | AgRP (ad lib.) vs. aDCN (food dep.)                                 | <i>P</i> <0.001                                                  |
|        |                                                             | AgRP (ad lib.) vs. aDCN & AgRP (food dep.)                          | <i>P</i> <0.001                                                  |
|        |                                                             | aDCN (food dep.) vs. aDCN & AgRP (food dep.)                        | <i>P</i> =0.024                                                  |
| ED 10e | aDCN-LAT hM3D(Gq): 6 mice<br>aDCN-mCherry (control): 6 mice | Two-way ANOVA                                                       |                                                                  |
|        |                                                             | Interaction                                                         | F(30, 220) = 9.22, <i>P</i> <0.001                               |
|        |                                                             | Factor 1: time                                                      | F(10, 220) = 1.84, <i>P</i> =0.055                               |
|        |                                                             | Factor 2: group                                                     | F(3, 220) = 154, <i>P</i> <0.001                                 |
|        |                                                             | Holm-Sidak's multiple comparisons                                   | hM3D(Gq) CNO to hM3D(Gq) vehicle / control CNO/ control vehicle: |
|        |                                                             | Bin 1 (0-179 s)                                                     | <i>P</i> >0.999 / >0.999/ >0.999                                 |
|        |                                                             | Bin 2 (180-359 s)                                                   | <i>P</i> >0.999 / >0.999/ >0.999                                 |
|        |                                                             | Bin 3 (360-539 s)                                                   | <i>P</i> >0.999 / >0.999/ >0.999                                 |
|        |                                                             | Bin 4 (540-719 s)                                                   | <i>P</i> >0.999 / =0.945/ >0.999                                 |
|        |                                                             | Bin 5 (720-899 s)                                                   | <i>P</i> =0.088 / =0.010/ =0.034                                 |
|        |                                                             | Bin 6 (900-1079 s)                                                  | <i>P</i> =0.027/ =0.397/ =0.625                                  |
|        |                                                             | Bin 7 (1080-1259 s)                                                 | <i>P</i> <0.001/ <0.001/ <0.001                                  |
|        |                                                             | Bin 8 (1260-1439 s)                                                 | <i>P</i> <0.001/ <0.001/ <0.001                                  |
|        |                                                             | Bin 9 (1440-1619 s)                                                 | <i>P</i> <0.001/ <0.001/ <0.001                                  |
|        |                                                             | Bin 10 (1620-1799 s)                                                | <i>P</i> <0.001/ <0.001/ <0.001                                  |
|        |                                                             | Bin 11 (1800-1959 s)                                                | <i>P</i> <0.001/ <0.001/ <0.001                                  |
| ED 10f | 6 mice                                                      | Paired t-test                                                       | <i>P</i> =0.011                                                  |
|        |                                                             | Vehicle                                                             | -0.023 ± 0.005                                                   |
|        |                                                             | CNO                                                                 | 0.214 ± 0.061                                                    |
|        |                                                             | Difference                                                          | 0.237 ± 0.059                                                    |
| ED 10g | 6 mice                                                      | Paired t-test                                                       | <i>P</i> =0.002                                                  |
|        |                                                             | Vehicle                                                             | -0.037 ± 0.0054                                                  |
|        |                                                             | CNO                                                                 | 0.208 ± 0.044                                                    |
|        |                                                             | Difference                                                          | 0.245 ± 0.043                                                    |
| ED 10h | 6 mice                                                      | Paired t-test                                                       | <i>P</i> =0.242                                                  |

|        |                                                    |                                   |                                                          |
|--------|----------------------------------------------------|-----------------------------------|----------------------------------------------------------|
| ED 10i | 6 mice                                             | Vehicle                           | -0.02213 ± 0.011                                         |
|        |                                                    | CNO                               | -0.0389 ± 0.009                                          |
|        |                                                    | Difference                        | -0.0162 ± 0.012                                          |
| ED 10j | 13 mice                                            | Paired t-test                     | <i>P</i> =0.418                                          |
|        |                                                    | Vehicle                           | -0.0435 ± 0.009                                          |
|        |                                                    | CNO                               | -0.0532 ± 0.011                                          |
| ED 10l | 7 mice                                             | Difference                        | -0.00967 ± 0.011                                         |
|        |                                                    | Pearson correlation               | <i>r</i> =-0.85<br><i>P</i> <0.001                       |
|        |                                                    | Repeated measures two-way ANOVA   |                                                          |
| ED 10m | 7 mice                                             | Interaction                       | <i>F</i> (10, 120) = 1.19, <i>P</i> =0.301               |
|        |                                                    | Factor 1: time                    | <i>F</i> (2.59, 31.1) = 15.1, <i>P</i> <0.001            |
|        |                                                    | Factor 2: groups                  | <i>F</i> (1, 12) = 0.448, <i>P</i> =0.516                |
| ED 10n | 7 mice                                             | Paired t-test                     | <i>P</i> =0.410                                          |
|        |                                                    | Vehicle                           | -0.0284 ± 0.00927                                        |
|        |                                                    | CNO                               | -0.0181 ± 0.0163                                         |
| ED 11g | vGluT2 aDCN-LAT: 7 mice<br>vGluT2 aDCN-INT: 6 mice | Difference                        | 0.0104 ± 0.0117                                          |
|        |                                                    | Paired t-test                     | <i>P</i> =0.367                                          |
|        |                                                    | Vehicle                           | -0.047 ± 0.0069                                          |
| ED 11h | 7 mice                                             | CNO                               | -0.038 ± 0.0127                                          |
|        |                                                    | Difference                        | 0.00873 ± 0.0132                                         |
|        |                                                    | Two-way ANOVA                     |                                                          |
| ED 11i | 7 mice                                             | Interaction                       | <i>F</i> (30, 242) = 8.50, <i>P</i> <0.001               |
|        |                                                    | Factor 1: time                    | <i>F</i> (10, 242) = 4.73, <i>P</i> <0.001               |
|        |                                                    | Factor 2: groups                  | <i>F</i> (3, 242) = 171, <i>P</i> <0.001                 |
| ED 11j | 6 mice                                             | Holm-Sidak's multiple comparisons | aDCN-LAT CNO to vehicle/ aDCN-INT CNO/ aDCN-INT vehicle: |
|        |                                                    | Bin 1 (0-179 s)                   | <i>P</i> >0.999 / >0.999/ >0.999                         |
|        |                                                    | Bin 2 (180-359 s)                 | <i>P</i> >0.999 / >0.999/ >0.999                         |
| ED 11k | 6 mice                                             | Bin 3 (360-539 s)                 | <i>P</i> >0.999 / >0.999/ >0.999                         |
|        |                                                    | Bin 4 (540-719 s)                 | <i>P</i> >0.999 / >0.999/ >0.999                         |
|        |                                                    | Bin 5 (720-899 s)                 | <i>P</i> =0.001/ =0.001/ <0.001                          |
| ED 11l | aDCN-LAT: 6 mice<br>vGluT2 aDCN-LAT: 7 mice        | Bin 6 (900-1079 s)                | <i>P</i> <0.001/ <0.001/ <0.001                          |
|        |                                                    | Bin 7 (1080-1259 s)               | <i>P</i> <0.001/ <0.001/ <0.001                          |
|        |                                                    | Bin 8 (1260-1439 s)               | <i>P</i> <0.001/ <0.001/ <0.001                          |
| ED 11m | aDCN-LAT: 6 mice<br>vGluT2 aDCN-LAT: 7 mice        | Bin 9 (1440-1619 s)               | <i>P</i> <0.001/ <0.001/ <0.001                          |
|        |                                                    | Bin 10 (1620-1799 s)              | <i>P</i> <0.001/ <0.001/ <0.001                          |
|        |                                                    | Bin 11 (1800-1959 s)              | <i>P</i> <0.001/ <0.001/ <0.001                          |
| ED 11n | 13 mice                                            | Paired t-test                     | <i>P</i> <0.001                                          |
|        |                                                    | Vehicle                           | -0.0117 ± 0.0151                                         |
|        |                                                    | CNO                               | 0.326 ± 0.062                                            |
| ED 12d | 8 mice                                             | Difference                        | 0.338 ± 0.0544                                           |
|        |                                                    | Paired t-test                     | <i>P</i> =0.001                                          |
|        |                                                    | Vehicle                           | -0.0358 ± 0.0105                                         |
| ED 12e | 6 mice                                             | CNO                               | 0.286 ± 0.0585                                           |
|        |                                                    | Difference                        | 0.322 ± 0.0542                                           |
| ED 12f | 6 mice                                             | Paired t-test                     | <i>P</i> =0.644                                          |
|        |                                                    | Vehicle                           | -0.00729 ± 0.0142                                        |
|        |                                                    | CNO                               | -0.0173 ± 0.00902                                        |
| ED 12g | 6 mice                                             | Difference                        | -0.0100 ± 0.0203                                         |
|        |                                                    | Paired t-test                     | <i>P</i> =0.367                                          |
|        |                                                    | Vehicle                           | -0.0338 ± 0.0152                                         |
| ED 12h | aDCN-LAT: 6 mice<br>vGluT2 aDCN-LAT: 7 mice        | CNO                               | -0.0509 ± 0.0110                                         |
|        |                                                    | Difference                        | -0.0171 ± 0.0173                                         |
|        |                                                    | Unpaired t-test                   | <i>P</i> =0.250                                          |
| ED 12i | aDCN-LAT: 6 mice<br>vGluT2 aDCN-LAT: 7 mice        | aDCN-LAT                          | 0.229 ± 0.0478                                           |
|        |                                                    | vGluT2 aDCN-LAT                   | 0.326 ± 0.062                                            |
|        |                                                    | Difference                        | 0.0977 ± 0.081                                           |
| ED 12j | aDCN-LAT: 6 mice<br>vGluT2 aDCN-LAT: 7 mice        | Unpaired t-test                   | <i>P</i> =0.323                                          |
|        |                                                    | aDCN-LAT                          | 0.208 ± 0.044                                            |
|        |                                                    | vGluT2 aDCN-LAT                   | 0.286 ± 0.058                                            |
| ED 12k | 13 mice                                            | Difference                        | 0.078 ± 0.075                                            |
|        |                                                    | Pearson correlation               | <i>r</i> =-0.832, <i>P</i> <0.001                        |
|        |                                                    | Repeated measures two-way ANOVA   |                                                          |
| ED 12l | 8 mice                                             | Interaction                       | <i>F</i> (40, 350)=6.514, <i>P</i> <0.001                |
|        |                                                    | Factor 1: treatment               | <i>F</i> (4,35)=10.72, <i>P</i> <0.001                   |
|        |                                                    | Factor 2: time                    | <i>F</i> (2, 66)=18.62, <i>P</i> <0.001                  |
| ED 12m | 8 mice                                             | Holm-Sidak's multiple comparisons |                                                          |
|        |                                                    | 6-9 min (0.025 vs. 2.5 mg/Kg)     | <i>P</i> =0.0289                                         |
|        |                                                    | 9-12 min (vehicle vs. 1 mg/Kg)    | <i>P</i> =0.0454                                         |
| ED 12n | 8 mice                                             | 9-12 min (vehicle vs. 2.5 mg/Kg)  | <i>P</i> =0.0115                                         |
|        |                                                    | 12-15 min (vehicle vs. 1 mg/Kg)   | <i>P</i> =0.0269                                         |
|        |                                                    | 12-15 min (vehicle vs. 2.5 mg/Kg) | <i>P</i> =0.0191                                         |
| ED 12o | 8 mice                                             | 15-18 min (vehicle vs. 2.5 mg/Kg) | <i>P</i> =0.0303                                         |
|        |                                                    | 15-18 min (0.025 vs. 2.5 mg/Kg)   | <i>P</i> =0.0342                                         |
|        |                                                    |                                   |                                                          |

|        |                                                                             |                                       |                                 |
|--------|-----------------------------------------------------------------------------|---------------------------------------|---------------------------------|
|        |                                                                             | 18-21 min (vehicle vs. 0.25 mg/Kg)    | $P<0.001$                       |
|        |                                                                             | 18-21 min (vehicle vs. 1 mg/Kg)       | $P=0.0067$                      |
|        |                                                                             | 18-21 min (vehicle vs. 2.5 mg/Kg)     | $P=0.0127$                      |
|        |                                                                             | 18-21 min (0.025 vs. 0.25 mg/Kg)      | $P=0.0363$                      |
|        |                                                                             | 18-21 min (0.025 vs. 1 mg/Kg)         | $P=0.0392$                      |
|        |                                                                             | 18-21 min (0.025 vs. 2.5 mg/Kg)       | $P=0.0415$                      |
|        |                                                                             | 21-24 min (vehicle vs. 0.25 mg/Kg)    | $P<0.001$                       |
|        |                                                                             | 21-24 min (vehicle vs. 1 mg/Kg)       | $P=0.0043$                      |
|        |                                                                             | 21-24 min (vehicle vs. 2.5 mg/Kg)     | $P=0.0056$                      |
|        |                                                                             | 21-24 min (0.025 vs. 0.25 mg/Kg)      | $P=0.0104$                      |
|        |                                                                             | 21-24 min (0.025 vs. 1 mg/Kg)         | $P=0.0222$                      |
|        |                                                                             | 21-24 min (0.025 vs. 2.5 mg/Kg)       | $P=0.0207$                      |
|        |                                                                             | 24-27 min (vehicle vs. 0.25 mg/Kg)    | $P<0.001$                       |
|        |                                                                             | 24-27 min (vehicle vs. 1 mg/Kg)       | $P=0.0046$                      |
|        |                                                                             | 24-27 min (vehicle vs. 2.5 mg/Kg)     | $P<0.001$                       |
|        |                                                                             | 24-27 min (0.025 vs. 0.25 mg/Kg)      | $P=0.0132$                      |
|        |                                                                             | 24-27 min (0.025 vs. 1 mg/Kg)         | $P=0.0306$                      |
|        |                                                                             | 24-27 min (0.025 vs. 2.5 mg/Kg)       | $P=0.0067$                      |
|        |                                                                             | 27-30 min (vehicle vs. 0.25 mg/Kg)    | $P=0.0024$                      |
|        |                                                                             | 27-30 min (vehicle vs. 1 mg/Kg)       | $P=0.0081$                      |
|        |                                                                             | 27-30 min (vehicle vs. 2.5 mg/Kg)     | $P=0.0020$                      |
|        |                                                                             | 27-30 min (0.025 vs. 0.25 mg/Kg)      | $P=0.0378$                      |
|        |                                                                             | 27-30 min (0.025 vs. 1 mg/Kg)         | $P=0.0486$                      |
|        |                                                                             | 27-30 min (0.025 vs. 2.5 mg/Kg)       | $P=0.0142$                      |
| ED 12e | 8 mice                                                                      | Repeated measures one-way ANOVA       | $F(2.22, 15.6) = 13.3, P<0.001$ |
|        |                                                                             | Holm-Sidak's multiple comparisons     |                                 |
|        |                                                                             | Vehicle vs. 0.025 mg/Kg               | $P=0.012$                       |
|        |                                                                             | Vehicle vs. 0.25 mg/Kg                | $P=0.010$                       |
|        |                                                                             | Vehicle vs. 1 mg/Kg                   | $P=0.023$                       |
|        |                                                                             | Vehicle vs. 2.5 mg/Kg                 | $P=0.010$                       |
| ED 12f | 8 mice                                                                      | Repeated measures one-way ANOVA       | $F(2.27, 15.9) = 66.7, P<0.001$ |
|        |                                                                             | Holm-Sidak's multiple comparisons     |                                 |
|        |                                                                             | Vehicle vs. 0.025 mg/Kg               | $P<0.001$                       |
|        |                                                                             | Vehicle vs. 0.25 mg/Kg                | $P<0.001$                       |
|        |                                                                             | Vehicle vs. 1 mg/Kg                   | $P<0.001$                       |
|        |                                                                             | Vehicle vs. 2.5 mg/Kg                 | $P<0.001$                       |
| ED 12g | 8 mice                                                                      | Pearson correlation                   | $r = 0.89, P=0.0456$            |
| ED 12h | 8 mice                                                                      | Repeated measures one-way ANOVA       | $F(2.20, 15.4) = 13.9, P<0.001$ |
|        |                                                                             | Holm-Sidak's multiple comparison test |                                 |
|        |                                                                             | Vehicle versus 0.025 mg/Kg            | $P=0.004$                       |
|        |                                                                             | Vehicle versus 0.25 mg/Kg             | $P=0.004$                       |
|        |                                                                             | Vehicle versus 1 mg/Kg                | $P=0.004$                       |
|        |                                                                             | Vehicle versus 2.5 mg/Kg              | $P=0.004$                       |
| ED 12i | 8 mice                                                                      | Repeated measures one-way ANOVA       | $F(1.97, 13.8) = 5.06, P=0.023$ |
|        |                                                                             | Holm-Sidak's multiple comparison test | $P=0.034$                       |
|        |                                                                             | Vehicle versus. 2.5 mg/Kg             |                                 |
| ED 12l | 8 mice                                                                      | Ordinary one-way ANOVA                | $F(4,35)=3.781, P=0.0117$       |
|        |                                                                             | Holm-Sidak's multiple comparison test |                                 |
|        |                                                                             | Vehicle versus 0.25 mg/Kg             | $P=0.0389$                      |
|        |                                                                             | Vehicle versus 1 mg/Kg                | $P=0.0056$                      |
|        |                                                                             | Vehicle versus 2.5 mg/Kg              | $P=0.0121$                      |
| ED 12m | 8 mice                                                                      | Pearson correlation                   | $r = 0.98, P=0.0027$            |
| ED 12q | 3 mice, 8 brain slices each, 1047, 2745, 2710 neurons counted in each mouse | Unpaired t-test                       | $P=0.02$                        |
|        |                                                                             | VTA                                   | $1990 \pm 472$                  |
|        |                                                                             | SNC                                   | $190 \pm 77.0$                  |
|        |                                                                             | Difference                            | $-1800 \pm 478$                 |
| ED 12r | aDCN-LAT hM3D(Gq): 6 mice<br>aDCN-LAT hM3D(Gq) VTA hM4D(Gi): 6 mice         | Unpaired t-test                       | $P=0.003$                       |
|        |                                                                             | aDCN-LAT hM3D(Gq)                     | $0.208 \pm 0.0443$              |
|        |                                                                             | aDCN-LAT hM3D(Gq) VTA hM4D(Gi)        | $0.0219 \pm 0.0156$             |
|        |                                                                             | Difference                            | $-0.186 \pm 0.0469$             |
| ED 12s | aDCN-LAT hM3D(Gq): 9 mice<br>aDCN-LAT hM3D(Gq) VTA hM4D(Gi): 7 mice         | Unpaired t-test                       | $P=0.382$                       |
|        |                                                                             | aDCN-LAT hM3D(Gq)                     | $8.75 \pm 2.29$                 |
|        |                                                                             | aDCN-LAT hM3D(Gq) VTA hM4D(Gi)        | $5.86 \pm 2.11$                 |
|        |                                                                             | Difference                            | $-2.89 \pm 3.20$                |

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