**Supplemental Table 1.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on *ΔF/F0* during 200 nM CNO treatment from AM | POA mouse brain slices (Fig. 2C).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | | | Proestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq |
| Brain slices (n) | 7 | 9 | 9 | | 8 | 9 | | 8 |
| Mice (n) | 3 | 3 | 3 | | 3 | 3 | | 3 |
| **Three-way mixed model ANOVA** | | | | | | | | |
| Effect | | | | Statistic | | | Partial *η*2 | |
| Treatment period | | | | *F*(2,88) = 67.924, ***p* < 0.001** | | | 0.607 | |
| Sex/cycle stage | | | | *F*(2,44) = 1.777, *p* = 0.181 | | | 0.075 | |
| AAV | | | | *F*(1,44) = 54.948, ***p* < 0.001** | | | 0.555 | |
| Treatment period \* Sex/cycle stage | | | | *F*(4,88) = 1.643, *p* = 0.204 | | | 0.069 | |
| Treatment period \* AAV | | | | *F*(2,88) = 64.226, ***p* < 0.001** | | | 0.593 | |
| Sex/cycle stage \* AAV | | | | *F*(2,44) = 1.734, *p* = 0.188 | | | 0.073 | |
| Treatment period \* Sex/cycle stage \* AAV | | | | *F*(4,88) = 1.560, *p* = 0.221 | | | 0.066 | |

**Supplemental Table 2.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on *ΔF/F0* during 200 nM CNO treatment from PM | POA mouse brain slices (Fig. 2D).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | | | Proestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq |
| Brain slices (n) | 10 | 11 | 8 | | 10 | 9 | | 7 |
| Mice (n) | 4 | 4 | 3 | | 4 | 3 | | 3 |
| **Three-way mixed model ANOVA** | | | | | | | | |
| Effect | | | | Statistic | | | Partial *η*2 | |
| Treatment period | | | | *F*(2,98) = 78.199, ***p* < 0.001** | | | 0.615 | |
| Sex/cycle stage | | | | *F*(2,49) = 2.135, *p* = 0.129 | | | 0.080 | |
| AAV | | | | *F*(1,49) = 92.628, ***p* < 0.001** | | | 0.654 | |
| Treatment period \* Sex/cycle stage | | | | *F*(4,98) = 4.564, ***p* = 0.013** | | | 0.157 | |
| Treatment period \* AAV | | | | *F*(2,98) = 74.861, ***p* < 0.001** | | | 0.604 | |
| Sex/cycle stage \* AAV | | | | *F*(2,49) = 1.766, *p* = 0.182 | | | 0.067 | |
| Treatment period \* Sex/cycle stage \* AAV | | | | *F*(4,98) = 4.000, ***p* = 0.021** | | | 0.140 | |

**Supplemental Table 3.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on *ΔF/F0* during 200 nM CNO treatment from AM | ARC mouse brain slices (Fig. 3C).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | | | Proestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq |
| Brain slices (n) | 7 | 9 | 6 | | 10 | 8 | | 9 |
| Mice (n) | 3 | 3 | 3 | | 4 | 3 | | 3 |
| **Three-way mixed model ANOVA** | | | | | | | | |
| Effect | | | | Statistic | | | Partial *η*2 | |
| Treatment period | | | | *F*(2,86) = 63.059, ***p* < 0.001** | | | 0.595 | |
| Sex/cycle stage | | | | *F*(2,43) = 0.194, *p* = 0.824 | | | 0.009 | |
| AAV | | | | *F*(2,43) = 58.678, ***p* < 0.001** | | | 0.577 | |
| Treatment period \* Sex/cycle stage | | | | *F*(4,86) = 0.099, *p* = 0.920 | | | 0.005 | |
| Treatment period \* AAV | | | | *F*(2,86) = 65.021, ***p* < 0.001** | | | 0.602 | |
| Sex/cycle stage \* AAV | | | | *F*(2,43) = 0.211, *p* = 0.811 | | | 0.010 | |
| Treatment period \* Sex/cycle stage \* AAV | | | | *F*(4,86) = 0.115, *p* = 0.907 | | | 0.005 | |

**Supplemental Table 4.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on *ΔF/F0* during 200 nM CNO treatment from PM | ARC mouse brain slices (Fig. 3D).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | | | Proestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq |
| Brain slices (n) | 11 | 9 | 8 | | 9 | 7 | | 11 |
| Mice (n) | 4 | 3 | 3 | | 3 | 3 | | 4 |
| **Three-way mixed model ANOVA** | | | | | | | | |
| Effect | | | | Statistic | | | Partial *η*2 | |
| Treatment period | | | | *F*(2,98) = 48.263, ***p* < 0.001** | | | 0.496 | |
| Sex/cycle stage | | | | *F*(2,49) = 5.721, ***p* = 0.006** | | | 0.189 | |
| AAV | | | | *F*(1,49) = 62.064, ***p* < 0.001** | | | 0.559 | |
| Treatment period \* Sex/cycle stage | | | | *F*(4,98) = 2.561, *p* = 0.084 | | | 0.095 | |
| Treatment period \* AAV | | | | *F*(2,98) = 50.397, ***p* < 0.001** | | | 0.507 | |
| Sex/cycle stage \* AAV | | | | *F*(2,49) = 4.273, ***p* = 0.019** | | | 0.149 | |
| Treatment period \* Sex/cycle stage \* AAV | | | | *F*(4,98) = 3.145, ***p* = 0.048** | | | 0.114 | |

**Supplemental Table 5.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on GnRH neuron response to 200 nM CNO treatment from AM | POA mouse brain slices (Fig. 4C).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | | | Proestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq |
| Brain slices (n) | 12 | 9 | 12 | | 12 | 5 | | 9 |
| Mice (n) | 4 | 6 | 4 | | 6 | 2 | | 4 |
| **Three-way mixed model ANOVA** | | | | | | | | |
| Effect | | | | Statistic | | | Partial *η*2 | |
| Treatment period | | | | *F*(2,106) = 27.045, ***p* < 0.001** | | | 0.338 | |
| Sex/cycle stage | | | | *F*(2,53) = 0.240, *p* = 0.788 | | | 0.009 | |
| AAV | | | | *F*(1,53) = 33.802, ***p* < 0.001** | | | 0.389 | |
| Treatment period \* Sex/cycle stage | | | | *F*(4,106) = 0.710, *p* = 0.576 | | | 0.026 | |
| Treatment period \* AAV | | | | *F*(2,106) = 27.245, ***p* < 0.001** | | | 0.340 | |
| Sex/cycle stage \* AAV | | | | *F*(2,53) = 1.078, *p* = 0.348 | | | 0.039 | |
| Treatment period \* Sex/cycle stage \* AAV | | | | *F*(4,106) = 1.048, *p* = 0.383 | | | 0.038 | |

**Supplemental Table 6.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on GnRH neuron response to 200 nM CNO treatment from PM | POA mouse brain slices (Fig. 4D).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | | | Proestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq |
| Brain slices (n) | 12 | 10 | 10 | | 10 | 9 | | 10 |
| Mice (n) | 4 | 6 | 4 | | 5 | 3 | | 6 |
| **Three-way mixed model ANOVA** | | | | | | | | |
| Effect | | | | Statistic | | | Partial *η*2 | |
| Treatment period | | | | *F*(2,110) = 49.291, ***p* < 0.001** | | | 0.473 | |
| Sex/cycle stage | | | | *F*(2,55) = 4.485, ***p* = 0.016** | | | 0.140 | |
| AAV | | | | *F*(1,55) = 90.536, ***p* < 0.001** | | | 0.622 | |
| Treatment period \* Sex/cycle stage | | | | *F*(4,110) = 2.060, *p* = 0.104 | | | 0.070 | |
| Treatment period \* AAV | | | | *F*(2,110) = 47.776, ***p* < 0.001** | | | 0.465 | |
| Sex/cycle stage \* AAV | | | | *F*(2,55) = 0.679, *p* = 0.511 | | | 0.024 | |
| Treatment period \* Sex/cycle stage \* AAV | | | | *F*(4,110) = 2.465, *p* = 0.061 | | | 0.082 | |

**Supplemental Table 7.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on KNDy neuron response to 200 nM CNO treatment from AM | ARC mouse brain slices (Fig. 5C).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | | | Proestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq |
| Brain slices (n) | 12 | 10 | 10 | | 11 | 6 | | 9 |
| Mice (n) | 6 | 6 | 6 | | 4 | 3 | | 4 |
| **Three-way mixed model ANOVA** | | | | | | | | |
| Effect | | | | Statistic | | | Partial *η*2 | |
| Treatment period | | | | *F*(2,104) = 3.097, *p* = 0.076 | | | 0.056 | |
| Sex/cycle stage | | | | *F*(2,52) = 1.384, *p* = 0.260 | | | 0.051 | |
| AAV | | | | *F*(1,52) = 1.028, *p* = 0.315 | | | 0.019 | |
| Treatment period \* Sex/cycle stage | | | | *F*(4,104) = 1.808, *p* = 0.165 | | | 0.065 | |
| Treatment period \* AAV | | | | *F*(2,104) = 0.529, *p* = 0.502 | | | 0.010 | |
| Sex/cycle stage \* AAV | | | | *F*(2,52) = 1.872, *p* = 0.164 | | | 0.067 | |
| Treatment period \* Sex/cycle stage \* AAV | | | | *F*(4,104) = 0.747, *p* = 0.501 | | | 0.028 | |

**Supplemental Table 8.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on KNDy neuron response to 200 nM CNO treatment from PM | ARC mouse brain slices (Fig. 5D).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | | | Proestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq | AAV-GFAP-mCherry | | AAV-GFAP-hM3Dq |
| Brain slices (n) | 7 | 10 | 9 | | 10 | 11 | | 7 |
| Mice (n) | 5 | 5 | 4 | | 6 | 5 | | 3 |
| **Three-way mixed model ANOVA** | | | | | | | | |
| Effect | | | | Statistic | | | Partial *η*2 | |
| Treatment period | | | | *F*(2,96) = 4.644, ***p* = 0.031** | | | 0.088 | |
| Sex/cycle stage | | | | *F*(2,48) = 0.461, *p* = 0.634 | | | 0.019 | |
| AAV | | | | *F*(1,48) = 0.280, *p* = 0.599 | | | 0.006 | |
| Treatment period \* Sex/cycle stage | | | | *F*(4,96) = 0.645, *p* = 0.550 | | | 0.026 | |
| Treatment period \* AAV | | | | *F*(2,96) = 1.425, *p* = 0.242 | | | 0.029 | |
| Sex/cycle stage \* AAV | | | | *F*(2,48) = 0.653, *p* = 0.525 | | | 0.026 | |
| Treatment period \* Sex/cycle stage \* AAV | | | | *F*(4,96) = 0.202, *p* = 0.846 | | | 0.008 | |

**Supplemental Table 9.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on LH levels during 0.3 mg/kg CNO treatment from AM | POA mice (Fig. 6C left).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | | AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | |
| Mice (n) | 5 | 6 | | 5 | 9 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Sample | | | *F*(12,252) = 40.870, ***p* < 0.001** | | | 0.661 |
| Sex | | | *F*(1,21) = 10.068, ***p* = 0.005** | | | 0.324 |
| AAV | | | *F*(1,21) = 71.863, ***p* < 0.001** | | | 0.774 |
| Sample \* Sex | | | *F*(12,252) = 5.688, ***p* = 0.012** | | | 0.213 |
| Sample \* AAV | | | *F*(12,252) = 45.073, ***p* < 0.001** | | | 0.682 |
| Sex \* AAV | | | *F*(1,21) = 6.156, ***p* = 0.022** | | | 0.227 |
| Sample \* Sex \* AAV | | | *F*(12,252) = 5.585, ***p* = 0.012** | | | 0.210 |

**Supplemental Table 10.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on LH levels during 0.3 mg/kg CNO treatment from PM | POA mice (Fig. 6C right).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | | AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | |
| Mice (n) | 5 | 7 | | 5 | 8 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Sample | | | *F*(12,252) = 47.682, ***p* < 0.001** | | | 0.694 |
| Sex | | | *F*(1,21) = 8.472, ***p* = 0.008** | | | 0.287 |
| AAV | | | *F*(1,21) = 32.094, ***p* < 0.001** | | | 0.604 |
| Sample \* Sex | | | *F*(12,252) = 8.463, ***p* < 0.001** | | | 0.287 |
| Sample \* AAV | | | *F*(12,252) = 54.976, ***p* < 0.001** | | | 0.724 |
| Sex \* AAV | | | *F*(1,21) = 3.446, *p* = 0.077 | | | 0.141 |
| Sample \* Sex \* AAV | | | *F*(12,252) = 11.389, ***p* < 0.001** | | | 0.352 |

**Supplemental Table 11.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on LH levels during 0.3 mg/kg CNO treatment from AM | POA mice (Fig. 6D left).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | | AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | |
| Mice (n) | 5 | 6 | | 5 | 9 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Sample | | | *F*(2,42) = 66.820, ***p* < 0.001** | | | 0.761 |
| Sex | | | *F*(1,21) = 9.843, ***p* = 0.005** | | | 0.319 |
| AAV | | | *F*(1,21) = 62.819, ***p* < 0.001** | | | 0.749 |
| Sample \* Sex | | | *F*(2,42) = 6.706, ***p* = 0.015** | | | 0.242 |
| Sample \* AAV | | | *F*(2,42) = 74.792, ***p* < 0.001** | | | 0.781 |
| Sex \* AAV | | | *F*(1,21) = 5.442, ***p* = 0.030** | | | 0.206 |
| Sample \* Sex \* AAV | | | *F*(2,42) = 5.903, ***p* = 0.022** | | | 0.219 |

**Supplemental Table 12.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on LH levels during 0.3 mg/kg CNO treatment from PM | POA mice (Fig. 6D right).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | | AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | |
| Mice (n) | 5 | 7 | | 5 | 8 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Sample | | | *F*(2,42) = 74.714, ***p* < 0.001** | | | 0.781 |
| Sex | | | *F*(1,21) = 6.796, ***p* = 0.016** | | | 0.244 |
| AAV | | | *F*(1,21) = 19.604, ***p* < 0.001** | | | 0.483 |
| Sample \* Sex | | | *F*(2,42) = 11.531, ***p* = 0.002** | | | 0.354 |
| Sample \* AAV | | | *F*(2,42) = 89.697, ***p* < 0.001** | | | 0.810 |
| Sex \* AAV | | | *F*(1,21) = 1.512, *p* = 0.232 | | | 0.067 |
| Sample \* Sex \* AAV | | | *F*(2,42) = 17.242, ***p* < 0.001** | | | 0.451 |

**Supplemental Table 13.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on LH levels during 0.3 mg/kg CNO treatment from AM | ARC mice (Fig. 7C left).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | | AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | |
| Mice (n) | 7 | 6 | | 6 | 5 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Sample | | | *F*(12,240) = 4.252, ***p* = 0.029** | | | 0.175 |
| Sex | | | *F*(1,20) = 0.014, *p* = 0.907 | | | 0.001 |
| AAV | | | *F*(1,20) = 2.432, *p* = 0.135 | | | 0.108 |
| Sample \* Sex | | | *F*(12,240) = 3.031, *p* = 0.070 | | | 0.132 |
| Sample \* AAV | | | *F*(12,240) = 5.266, ***p* = 0.014** | | | 0.208 |
| Sex \* AAV | | | *F*(1,20) = 6.882, ***p* = 0.016** | | | 0.256 |
| Sample \* Sex \* AAV | | | *F*(12,240) = 3.590, ***p* = 0.046** | | | 0.152 |

**Supplemental Table 14.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on LH levels during 0.3 mg/kg CNO treatment from PM | ARC mice (Fig. 7C right).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | | AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | |
| Mice (n) | 7 | 6 | | 5 | 4 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Sample | | | *F*(12,216) = 5.499, ***p* = 0.015** | | | 0.234 |
| Sex | | | *F*(1,18) = 1.226, *p* = 0.283 | | | 0.064 |
| AAV | | | *F*(1,18) = 5.780, ***p* = 0.027** | | | 0.243 |
| Sample \* Sex | | | *F*(12,216) = 4.422, ***p* = 0.030** | | | 0.197 |
| Sample \* AAV | | | *F*(12,216) = 5.727, ***p* = 0.013** | | | 0.241 |
| Sex \* AAV | | | *F*(1,18) = 1.033, *p* = 0.323 | | | 0.054 |
| Sample \* Sex \* AAV | | | *F*(12,216) = 5.543, ***p* = 0.015** | | | 0.235 |

**Supplemental Table 15.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on LH levels during 0.3 mg/kg CNO treatment from AM | ARC mice (Fig. 7D left).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | | AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | |
| Mice (n) | 7 | 6 | | 6 | 5 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Sample | | | *F*(2,40) = 5.554, ***p* = 0.020** | | | 0.217 |
| Sex | | | *F*(1,20) = 0.478, *p* = 0.497 | | | 0.023 |
| AAV | | | *F*(1,20) = 1.033, *p* = 0.322 | | | 0.049 |
| Sample \* Sex | | | *F*(2,40) = 3.462, *p* = 0.066 | | | 0.148 |
| Sample \* AAV | | | *F*(2,40) = 8.029, ***p* = 0.006** | | | 0.286 |
| Sex \* AAV | | | *F*(1,20) = 6.647, ***p* = 0.018** | | | 0.249 |
| Sample \* Sex \* AAV | | | *F*(2,40) = 4.224, ***p* = 0.043** | | | 0.174 |

**Supplemental Table 16.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex/cycle stage and AAV on LH levels during 0.3 mg/kg CNO treatment from PM | ARC mice (Fig. 7D right).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | | AAV-GFAP-mCherry | AAV-GFAP-hM3Dq | |
| Mice (n) | 7 | 6 | | 5 | 4 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Sample | | | *F*(2,36) = 5.550, ***p* = 0.026** | | | 0.236 |
| Sex | | | *F*(1,18) = 0.629, *p* = 0.438 | | | 0.034 |
| AAV | | | *F*(1,18) = 5.015, ***p* = 0.038** | | | 0.218 |
| Sample \* Sex | | | *F*(2,36) = 4.507, ***p* = 0.044** | | | 0.200 |
| Sample \* AAV | | | *F*(2,36) = 5.499, ***p* = 0.027** | | | 0.234 |
| Sex \* AAV | | | *F*(1,18) = 0.375, *p* = 0.548 | | | 0.020 |
| Sample \* Sex \* AAV | | | *F*(2,36) = 6.413, ***p* = 0.018** | | | 0.263 |

**Supplemental Table 17.** Statistical parameters assessing the viral ablation efficiency of KNDy neuron in male mice (Fig. 8F).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Control | | Ablation | |
| Mice (n) | 6 | | 12 | |
| **Two-tailed independent samples Welch’s t-test** | | | | |
| Property | | Statistic | | *η*2 |
| KNDy neuron count | | t(10.67), df = 5.04, ***p* < 0.001** | | 0.958 |

**Supplemental Table 18.** Statistical parameters from two-way mixed model ANOVA assessing the effect of KNDy neuron ablation on LH levels during 0.3 mg/kg CNO treatment from AM | ARC male mice (Fig. 8D left).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Males | | | |
| AAV-GFAP-mCherry + AAV-flex-taCasp3 | | AAV-GFAP-hM3Dq + AAV-GFAP-hM3Dq | |
| Mice (n) | 4 | | 6 | |
| **Two-way mixed model ANOVA** | | | | |
| Effect | | Statistic | | Partial *η*2 |
| Sample | | *F*(12,96) = 7.682, ***p* = 0.007** | | 0.490 |
| AAV | | *F*(1,8) = 11.074, ***p* = 0.010** | | 0.581 |
| Sample \* AAV | | *F*(12,96) = 4.466, ***p* = 0.037** | | 0.358 |

**Supplemental Table 19.** Statistical parameters from two-way mixed model ANOVA assessing the effect of KNDy neuron ablation on LH levels during 0.3 mg/kg CNO treatment from PM | ARC male mice (Fig. 8D right).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Males | | | |
| AAV-GFAP-mCherry + AAV-flex-taCasp3 | | AAV-GFAP-hM3Dq + AAV-GFAP-hM3Dq | |
| Mice (n) | 4 | | 5 | |
| **Two-way mixed model ANOVA** | | | | |
| Effect | | Statistic | | Partial *η*2 |
| Sample | | *F*(12,84) = 3.624, *p* = 0.078 | | 0.341 |
| AAV | | *F*(1,7) = 7.321, ***p* = 0.030** | | 0.511 |
| Sample \* AAV | | *F*(12,84) = 5.024, ***p* = 0.041** | | 0.418 |

**Supplemental Table 20.** Statistical parameters from two-way mixed model ANOVA assessing the effect of KNDy neuron ablation on LH levels during 0.3 mg/kg CNO treatment from AM | ARC male mice (Fig. 8E left).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Males | | | |
| AAV-GFAP-mCherry + AAV-flex-taCasp3 | | AAV-GFAP-hM3Dq + AAV-GFAP-hM3Dq | |
| Mice (n) | 4 | | 6 | |
| **Two-way mixed model ANOVA** | | | | |
| Effect | | Statistic | | Partial *η*2 |
| Sample | | *F*(2,16) = 9.625, ***p* = 0.014** | | 0.546 |
| AAV | | *F*(1,8) = 12.009, ***p* = 0.009** | | 0.600 |
| Sample \* AAV | | *F*(2,16) = 5.220, ***p* = 0.050** | | 0.395 |

**Supplemental Table 21.** Statistical parameters from two-way mixed model ANOVA assessing the effect of KNDy neuron ablation on LH levels during 0.3 mg/kg CNO treatment from PM | ARC male mice (Fig. 8E right).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Males | | | |
| AAV-GFAP-mCherry + AAV-flex-taCasp3 | | AAV-GFAP-hM3Dq + AAV-GFAP-hM3Dq | |
| Mice (n) | 4 | | 5 | |
| **Two-way mixed model ANOVA** | | | | |
| Effect | | Statistic | | Partial *η*2 |
| Sample | | *F*(2,14) = 3.043, *p* = 0.116 | | 0.303 |
| AAV | | *F*(1,7) = 6.830, ***p* = 0.035** | | 0.494 |
| Sample \* AAV | | *F*(2,14) = 5.399, ***p* = 0.045** | | 0.435 |

**Supplemental Table 22.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex and antagonists to receptors EP1 and EP2 on GnRH neuron firing during 200 nM dmPGE2 treatment from AM | POA mice (Fig. 9A).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| Vehicle | EP1 & E2 antagonists | | Vehicle | EP1 & EP2 antagonists | |
| Brain slices (n) | 7 | 8 | | 9 | 7 | |
| Mice (n) | 5 | 4 | | 4 | 3 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Treatment period | | | *F*(2,54) = 12.681, ***p* < 0.001** | | | 0.320 |
| Sex | | | *F*(1,27) = 1.102, *p* = 0.303 | | | 0.039 |
| Pretreatment | | | *F*(1,27) = 3.792, *p* = 0.062 | | | 0.123 |
| Treatment period \* Sex | | | *F*(2,54) = 5.457, ***p* = 0.018** | | | 0.168 |
| Treatment period \* Pretreatment | | | *F*(2,54) = 6.640, ***p* = 0.009** | | | 0.197 |
| Sex \* Pretreatment | | | *F*(1,27) = 0.546, *p* = 0.466 | | | 0.020 |
| Treatment period \* Sex \* Pretreatment | | | *F*(2,54) = 6.959, ***p* = 0.008** | | | 0.205 |

**Supplemental Table 23.** Statistical parameters from one-way repeated measures ANOVA assessing the effect of 1 μM dmPGE2 treatment on GnRH neuron firing from AM | POA diestrous female mice (Fig. 9B).

|  |  |  |
| --- | --- | --- |
| Property | Diestrous females | |
| Vehicle | |
| Brain slices (n) | 9 | |
| Mice (n) | 4 | |
| **One-way repeated measures ANOVA** | | |
| Effect | Statistic | Partial *η*2 |
| Treatment period | *F*(2,16) = 14.694, ***p* = 0.001** | 0.647 |

**Supplemental Table 24.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex and antagonists to receptors EP1 and EP2 on GnRH neuron firing during 200 nM dmPGE2 treatment from PM | POA mice (Fig. 9C).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| Vehicle | EP1 & E2 antagonists | | Vehicle | EP1 & EP2 antagonists | |
| Brain slices (n) | 9 | 9 | | 8 | 7 | |
| Mice (n) | 6 | 4 | | 5 | 5 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Treatment period | | | *F*(2,58) = 7.583, ***p* = 0.004** | | | 0.207 |
| Sex | | | *F*(1,29) = 0.902, *p* = 0.350 | | | 0.030 |
| Pretreatment | | | *F*(1,29) = 7.770, ***p* = 0.009** | | | 0.211 |
| Treatment period \* Sex | | | *F*(2,58) = 0.384, *p* = 0.618 | | | 0.013 |
| Treatment period \* Pretreatment | | | *F*(2,58) = 6.925, ***p* = 0.006** | | | 0.193 |
| Sex \* Pretreatment | | | *F*(1,29) = 0.007, *p* = 0.933 | | | 0.000 |
| Treatment period \* Sex \* Pretreatment | | | *F*(2,58) = 0.383, *p* = 0.618 | | | 0.013 |

**Supplemental Table 25.** Statistical parameters from two-way mixed model ANOVA assessing the effect of sex on KNDy neuron firing during 200 nM dmPGE2 treatment from AM | ARC mice (Fig. 10A).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | |
| Brain slices (n) | 6 | | 6 | |
| Mice (n) | 4 | | 3 | |
| **Two-way mixed model ANOVA** | | | | |
| Effect | | Statistic | | Partial *η*2 |
| Treatment period | | *F*(2,20) = 1.409, *p* = 0.268 | | 0.124 |
| Sex | | *F*(1,10) = 0.049, *p* = 0.829 | | 0.005 |
| Treatment period \* Sex | | *F*(2,20) = 0.806, *p* = 0.458 | | 0.075 |

**Supplemental Table 26.** Statistical parameters from two-way mixed model ANOVA assessing the effect of sex on KNDy neuron firing during 1 μM dmPGE2 treatment from AM | ARC mice (Fig. 10B).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | |
| Brain slices (n) | 5 | | 5 | |
| Mice (n) | 3 | | 3 | |
| **Two-way mixed model ANOVA** | | | | |
| Effect | | Statistic | | Partial *η*2 |
| Treatment period | | *F*(2,16) = 1.166, *p* = 0.312 | | 0.127 |
| Sex | | *F*(1,8) = 0.933, *p* = 0.362 | | 0.104 |
| Treatment period \* Sex | | *F*(2,16) = 0.834, *p* = 0.388 | | 0.094 |

**Supplemental Table 27.** Statistical parameters from two-way mixed model ANOVA assessing the effect of sex on KNDy neuron firing during 200 nM dmPGE2 treatment from PM | ARC mice (Fig. 10C).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | |
| Brain slices (n) | 5 | | 5 | |
| Mice (n) | 3 | | 4 | |
| **Two-way mixed model ANOVA** | | | | |
| Effect | | Statistic | | Partial *η*2 |
| Treatment period | | *F*(2,16) = 0.767, *p* = 0.413 | | 0.088 |
| Sex | | *F*(1,8) = 3.637, *p* = 0.093 | | 0.313 |
| Treatment period \* Sex | | *F*(2,16) = 0.615, *p* = 0.464 | | 0.071 |

**Supplemental Table 28.** Statistical parameters from two-way mixed model ANOVA assessing the effect of sex on KNDy neuron firing during 1 μM dmPGE2 treatment from PM | ARC mice (Fig. 10D).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Males | | Diestrous females | |
| Brain slices (n) | 6 | | 6 | |
| Mice (n) | 3 | | 3 | |
| **Two-way mixed model ANOVA** | | | | |
| Effect | | Statistic | | Partial *η*2 |
| Treatment period | | *F*(2,20) = 0.987, *p* = 0.356 | | 0.090 |
| Sex | | *F*(1,10) = 0.481, *p* = 0.504 | | 0.046 |
| Treatment period \* Sex | | *F*(2,20) = 0.795, *p* = 0.411 | | 0.074 |

**Supplemental Table 29.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex and antagonists to receptors EP1 and EP2 on GnRH neuron firing during 200 nM CNO treatment from AM | POA mice (Fig. 11B).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| Vehicle | EP1 & E2 antagonists | | Vehicle | EP1 & EP2 antagonists | |
| Brain slices (n) | 8 | 9 | | 10 | 9 | |
| Mice (n) | 4 | 5 | | 6 | 6 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Treatment period | | | *F*(2,64) = 30.563, ***p* < 0.001** | | | 0.489 |
| Sex | | | *F*(1,32) = 0.873, *p* = 0.357 | | | 0.027 |
| Pretreatment | | | *F*(1,32) = 7.951, ***p* = 0.008** | | | 0.199 |
| Treatment period \* Sex | | | *F*(2,64) = 1.525, *p* = 0.226 | | | 0.046 |
| Treatment period \* Pretreatment | | | *F*(2,64) = 8.101, ***p* < 0.001** | | | 0.202 |
| Sex \* Pretreatment | | | *F*(1,32) = 0.413, *p* = 0.525 | | | 0.013 |
| Treatment period \* Sex \* Pretreatment | | | *F*(2,64) = 0.547, *p* = 0.572 | | | 0.017 |

**Supplemental Table 30.** Statistical parameters from three-way mixed model ANOVA assessing the effect of sex and antagonists to receptors EP1 and EP2 on GnRH neuron firing during 200 nM CNO treatment from PM | POA mice (Fig. 11D).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Property | Males | | | Diestrous females | | |
| Vehicle | EP1 & E2 antagonists | | Vehicle | EP1 & EP2 antagonists | |
| Brain slices (n) | 11 | 10 | | 9 | 10 | |
| Mice (n) | 6 | 6 | | 5 | 4 | |
| **Three-way mixed model ANOVA** | | | | | | |
| Effect | | | Statistic | | | Partial *η*2 |
| Treatment period | | | *F*(2,72) = 37.452, ***p* < 0.001** | | | 0.510 |
| Sex | | | *F*(1,36) = 0.693, *p* = 0.411 | | | 0.019 |
| Pretreatment | | | *F*(1,36) = 0.292, *p* = 0.592 | | | 0.008 |
| Treatment period \* Sex | | | *F*(2,72) = 0.911, *p* = 0.383 | | | 0.025 |
| Treatment period \* Pretreatment | | | *F*(2,72) = 0.513, *p* = 0.550 | | | 0.014 |
| Sex \* Pretreatment | | | *F*(1,36) = 0.007, *p* = 0.936 | | | 0.000 |
| Treatment period \* Sex \* Pretreatment | | | *F*(2,72) = 0.041, *p* = 0.921 | | | 0.001 |