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## Supplementary information

# An amygdala circuit that suppresses social engagement

In the format provided by the authors and unedited

**Figure 1b: Percent mice mounting to PBS or LPS-female**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =5.238, df=1
p=0.0221
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| PBS-female | 90.9 | 11 |
| LPS-female | 45.5 | 11 |

**Figure 1c: Mounting time to PBS- or LPS- female:** Unpaired two-tailed t-test (95% confidence interval)
t=4.826, df=20
p=0.0001
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS-female | 41.61 | 7.927 | 11 |
| LPS-female | 2.866 | 1.279 | 11 |

**Figure 1d: Number of mounts to PBS- or LPS-female:** Unpaired two-tailed t-test (95% confidence interval)
t=5.745, df=20
p<0.0001
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS-female | 10 | 1.495 | 11 |
| LPS-female | 1.0 | 0.4671 | 11 |

**Figure 1e: Latency to mount to PBS- or LPS-female:** Unpaired two-tailed t-test (95% confidence interval)
t=3.535, df=20
p=0.0021
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS-female | 254.0 | 39.31 | 11 |
| LPS-female | 475.4 | 48.76 | 11 |

**Figure 1g**: **FOS fold change in AOBmi, AOBgr, BST, MEApd, MEApv, and COApm after exposure to PBS or LPS-female:** Two-way ANOVA (95% confidence interval)

| Source of variation | F (DFn, DFd) | P value |
|---|---|---|
| Treatment (PBS or LPS) | F (1, 84) = 73.15 | P < 0.0001 |
| Brain Region (AOBmi, AOBgr, BST, MEApd, MEApv, COApm) | F (5, 84) = 20.69 | P < 0.0001 |
| Interaction | F (5, 84) = 20.69 | P < 0.0001 |

Post-hoc (Sidak's):

| PBS vs. LPS | P Value |
|---|---|
| AOBmi | >0.9999 |
| AOBgr | 0.0111 |
| BST | 0.9580 |
| MEApd | 0.9898 |
| MEApv | 0.0019 |
| COApm | <0.0001 |

Descriptive Statistics:

| | AOBmi | | | AOBgr | | | BST | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | n | Mean | SEM | n | Mean | SEM | n |
| PBS | 1 | 0.170 | 8 | 1 | 0,162 | 8 | 1 | 0.107 | 8 |
| LPS | 1.089 | 0.159 | 8 | 2.231 | 0.373 | 8 | 1.317 | 0/119 | 8 |
| | **MEApd** | | | **MEApv** | | | **COApm** | | |
| | Mean | SEM | n | Mean | SEM | n | Mean | SEM | n |
| PBS | 1 | 0.193 | 8 | 1 | 0.165 | 8 | 1 | 0.193 | 8 |
| LPS | 1.239 | 0.206 | 8 | 2.439 | 0.369 | 8 | 5.706 | 0.596 | 8 |

**Figure 1l: Mean z-score of COApm bulk fluorescence signal during investigation of PBS or LPS-female:**
Paired two-tailed t-test (95% confidence interval)
t=3.736, df=5
p=0.0135
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS-female | 2.606 | 0.307 | 6 |
| LPS-female | 5.984 | 1.058 | 6 |

**Figure 2b: Percent male mounting during COApm photoactivation**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =6.112, df=1
p=0.0134
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| EYFP | 88.9 | 9 |

| | | |
|---|---|---|
| ChR2 | 28.6 | 7 |

**Figure 2c: Mounting Time to healthy female during COApm photoactivation**: Two-way repeated measures ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (EYFP vs. ChR2) | F(1,14)=4.976 | 0.0426 |
| Light (On vs. Off) | F(1,14)=4.683 | 0.0482 |
| Interaction | F(1,14)=9.578 | 0.0079 |

Post-hoc (Bonferroni's):

| **EYFP vs. ChR2** | **P Value** |
|---|---|
| Light On | 0.0028 |
| Light Off | >0.9999 |

Descriptive Statistics:

| | **Light On** | | | **Light Off** | | |
|---|---|---|---|---|---|---|
| | Mean | SEM | **n** | Mean | SEM | **n** |
| EYFP | 52.862 | 11.764 | 9 | 45.498 | 9.516 | 9 |
| ChR2 | 0.834 | 0.647 | 7 | 42.446 | 13.097 | 7 |

**Figure 2d: Number of mounts to healthy female during COApm photoactivation**: Two-way repeated measures ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (EYFP vs. ChR2) | F(1,14) = 3.498 | 0.0825 |
| Light (On vs. Off) | F(1,14) = 10.60 | 0.0057 |
| Interaction | F(1,14) = 5.999 | 0.0281 |

Post-hoc (Bonferroni's):

| **EYFP vs. ChR2** | **P Value** |
|---|---|
| Light On | 0.0113 |
| Light Off | >0.9999 |

Descriptive Statistics:

| | **Light On** | | | **Light Off** | | |
|---|---|---|---|---|---|---|
| | Mean | SEM | **n** | Mean | SEM | **n** |

| | | | | | | |
|---|---|---|---|---|---|---|
| EYFP | 12.444 | 2.298 | 9 | 14.444 | 3.010 | 9 |
| ChR2 | 0.714 | 0.565 | 7 | 14.857 | 3.906 | 7 |

**Figure 2e: Latency to mount to healthy female during COApm photoactivation**: Two-way repeated measures ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (EYFP vs. ChR2) | F(1,14) = 6.636 | 0.0220 |
| Light (On vs. Off) | F(1,14) = 17.32 | 0.0010 |
| Interaction | F(1,14) = 12.40 | 0.0034 |

Post-hoc (Bonferroni's):

| **EYFP vs. ChR2** | **P Value** |
|---|---|
| Light On | 0.0005 |
| Light Off | >0.9999 |

Descriptive Statistics:

| | **Light On** | | | **Light Off** | | |
|---|---|---|---|---|---|---|
| | Mean | SEM | **n** | Mean | SEM | **n** |
| EYFP | 293.396 | 52.964 | 9 | 265.178 | 64.458 | 9 |
| ChR2 | 596.026 | 2.738 | 7 | 257.600 | 42.0101 | 7 |

**Figure 2f. Feeding during COApm photoactivation-Amount:** Unpaired two-tailed t-test (95% confidence interval)
t=0.05443, df=14
p=0.9574
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 0.3975 | 0.04003 | 8 |
| ChR2 | 0.3950 | 0.02252 | 8 |

**Figure 2f: Feeding during COApm photoactivation-Time spent:** Unpaired two-tailed t-test (95% confidence interval)
t=0.9208, df=14
p=0.3728
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 6.188 | 0.3140 | 8 |

| | | | |
|---|---|---|---|
| ChR2 | 5.742 | 0.3688 | 8 |

**Figure 2g: Sociability during COApm photoactivation-Social preference (%):** Unpaired two-tailed t-test (95% confidence interval)
t=0.2554, df=13
p=0.8024
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 72.95 | 3.891 | 9 |
| ChR2 | 71.46 | 4.113 | 6 |

**Figure 2g: Sociability during COApm photoactivation-Total investigation (min):** Unpaired two-tailed t-test (95% confidence interval)
t=1.088, df=13
p=0.2962
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 7.028 | 0.1994 | 9 |
| ChR2 | 6.461 | 0.5735 | 6 |

**Figure 2h: RTPP during COApm photoactivation-Stim.preference (%):** Unpaired two-tailed t-test (95% confidence interval)
t=1.749, df=15
p=0.1007
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 48.42 | 2.126 | 9 |
| ChR2 | 55.05 | 3.241 | 8 |

**Figure 2j: Percent male mounting to LPS-female during COApm hM4Di inhibition**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$=1.418, df=1
p=0.2337
Descriptive Statistics:

| | **Percent** | **N** |
|---|---|---|
| mCherry | 60 | 15 |
| hM4Di | 81.8 | 11 |

**Figure 2k: Mounting time to LPS-female during COApm hM4Di Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=3.158, df=24
p=0.0046

Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| mCherry | 9.142 | 2.491 | 15 |
| hM4Di | 27.11 | 5.743 | 11 |

**Figure 2l: Number of mounts to LPS-female during COApm DREADD Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=3.105, df=24
p=0.0048
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| mCherry | 3.933 | 1.209 | 15 |
| hM4Di | 11.45 | 2.310 | 11 |

**Figure 2m: Latency to mount to LPS-female during COApm DREADD Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=1.934, df=24
p=0.0650
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| mCherry | 420.9 | 45.44 | 15 |
| hM4Di | 276.8 | 61.26 | 11 |

**Figure 3d: Max amplitude of bulk fluorescence signal in MEA *Vglut2*(+)/*Vgat*(+) neurons evoked by photoactivation of COApm with 400 pulses of light:** Unpaired two-tailed t-test (95% confidence interval)
t=3.291, df=6
p=0.0166
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| *Vglut2*-Cre | 0.4981 | 0.1061 | 5 |
| *Vgat*-Cre | 0.03228 | 0.004260 | 3 |

**Figure 3e: Number of light pulses for COApm photoactivation to elicit max bulk fluorescence signal in MEA *Vglut2*(+)/*Vgat*(+) neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=4.056, df=6
p=0.0067
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|

| | | | |
|---|---|---|---|
| *Vglut2*-Cre | 10.18 | 1.553 | 5 |
| *Vgat*-Cre | 1.780 | 0.1060 | 3 |

**Figure 3f: Mounting time to healthy female during photoactivation of COApm-MEA projection:** Unpaired two-tailed t-test (95% confidence interval)
t=5.533, df=18
p<0.0001
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 41.74 | 5.358 | 10 |
| ChR2 | 5.824 | 3.663 | 10 |

**Figure 3g: Mounting time to healthy female during photoactivation of MEA-*Vglut2* neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=4.214, df=13
p=0.0010
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 60.86 | 12.54 | 7 |
| ChR2 | 7.420 | 4.635 | 8 |

**Figure 3h**: **Mounting time to LPS-female with DREADD inhibition of MEA-*Vglut2* neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=3.1952, df=18
p=0.0050
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| mCherry | 7.471 | 4.004 | 9 |
| hM4Di | 32.31 | 6.200 | 11 |

**Figure 3i: Mounting time to healthy female during concurrent photoactivation of COApm-MEA projections and hM4Di-inhibition of MEA-*Vglut2* neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=3.205, df=14
p=0.0064
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| mCherry | 10.86 | 3.968 | 8 |
| hM4Di | 48.13 | 10.93 | 8 |

**Figure 4e: Percent male mounting to LPS-female during COApm hM4Di inhibition**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =8.306, df=1
p=0.0046
Descriptive Statistics:

| | **Percent** | **N** |
|---|---|---|
| EYFP | 100 | 13 |
| ChR2 | 50 | 10 |

**Figure 4f: Mounting time to healthy female during photoactivation COApm-TRH neurons:** Two-way repeated measures ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (EYFP vs. ChR2) | F(1,21) = 5.202 | 0.0331 |
| Light (On vs. Off) | F(1,21) = 11.37 | 0.0029 |
| Interaction | F(1,21) = 24.63 | <0.0001 |

Post-hoc (Bonferroni's):

| **EYFP vs. ChR2** | **P Value** |
|---|---|
| Light On | 0.0004 |
| Light Off | >0.9999 |

Descriptive Statistics:

| | **Light On** | | | **Light Off** | | |
|---|---|---|---|---|---|---|
| | Mean | SEM | **n** | Mean | SEM | **n** |
| EYFP | 57.012 | 9.085 | 13 | 49.846 | 6.510 | 13 |
| ChR2 | 11.476 | 6.633 | 10 | 49.000 | 8.101 | 10 |

**Figure 4g: Number of mounts to healthy female during photoactivation of COApm-TRH neurons:** Two-way repeated measures ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (EYFP vs. ChR2) | F(1,21) =4.515 | 0.0456 |
| Light (On vs. Off) | F(1,21) = 10.78 | 0.0035 |
| Interaction | F(1,21) = 12.87 | 0.0017 |

Post-hoc (Bonferroni's):

| **EYFP vs. ChR2** | **P Value** |
|---|---|

| | |
|---|---|
| Light On | 0.0042 |
| Light Off | 0.9886 |

Descriptive Statistics:

| | Light On | | | Light Off | | |
|---|---|---|---|---|---|---|
| | Mean | SEM | **n** | Mean | SEM | **n** |
| EYFP | 15.308 | 2.863 | 13 | 14.923 | 2.605 | 13 |
| ChR2 | 3.800 | 1.837 | 10 | 12.500 | 1.797 | 10 |

**Figure 4h: Latency to mount to healthy female during photoactivation of COApm-TRH neurons:** Two-way repeated measures ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (EYFP vs. ChR2) | F(1,21) =4.633 | 0.0431 |
| Light (On vs. Off) | F(1,21) = 15.32 | 0.0008 |
| Interaction | F(1,21) = 24.90 | <0.0001 |

Post-hoc (Bonferroni's):

| **EYFP vs. ChR2** | **P Value** |
|---|---|
| Light On | 0.0001 |
| Light Off | 0.8440 |

Descriptive Statistics:

| | Light On | | | Light Off | | |
|---|---|---|---|---|---|---|
| | Mean | SEM | **n** | Mean | SEM | **n** |
| EYFP | 181.071 | 19.675 | 13 | 213 | 33.939 | 13 |
| ChR2 | 431.896 | 66.741 | 10 | 167.448 | 34.661 | 10 |

**Figure 4j: Percent Co-expression of *Trhr* with *Vglut2*/*Vgat* in MEA:** Paired two-tailed t-test (95% confidence interval)
t=7.663, df=9
p<0.0001
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| Vglut2/Trhr | 58.58 | 3.614 | 10 |
| Vgat/Trhr | 28.28 | 1.921 | 10 |

**Figure 4l: Percent male mounting to healthy female following microinjection of TRH analog taltirelin into MEA**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$=10.15, df=1
p=0.0014
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| PBS | 92.85 | 14 |
| Taltirelin | 40 | 15 |

**Figure 4m: Mounting time to healthy female following microinjection of TRH analog taltirelin into MEA**: Unpaired two-tailed t-test (95% confidence interval)
t=4.987, df=27
p<0.0001
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS | 24.75 | 4.574 | 14 |
| Taltirelin | 2.227 | 0.9290 | 15 |

**Figure 4n: Number of mounts to healthy female following microinjection of TRH analog taltirelin into MEA**: Unpaired two-tailed t-test (95% confidence interval)
t=3.900, df=24
p=0.0006
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS | 10.21 | 2.307 | 14 |
| Taltirelin | 1.200 | 0.6032 | 15 |

**Figure 4o: Latency to mount to healthy female following microinjection of TRH analog taltirelin into MEA**: Unpaired two-tailed t-test (95% confidence interval)
t=3.696, df=27
p=0.0010
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS | 284.5 | 39.55 | 14 |
| Taltirelin | 501.4 | 43.05 | 15 |

**Figure 4q: Percent male mounting in Trhr conditional KO mice**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$=10.06, df=2
p=0.0065
Descriptive Statistics:

| | Percent | N |
|---|---|---|

| | | |
|---|---|---|
| WT:Cre | 27.3 | 11 |
| $Trhr^{fl/fl}$:GFP | 50 | 8 |
| $Trhr^{fl/fl}$:Cre | 91.7 | 12 |

**Figure 4r: Mounting time in Trhr conditional KO mice:** One-way ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group | F (2,28) = 3.686 | 0.0379 |

Post-hoc (Bonferroni's):

| **Comparison** | **Significant** |
|---|---|
| WT:Cre vs. $Trhr^{fl/fl}$:GFP | No |
| WT:Cre vs. $Trhr^{fl/fl}$:Cre | P < 0.01 |
| $Trhr^{fl/fl}$:GFP vs. $Trhr^{fl/fl}$:Cre | P <0.05 |

Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| WT:Cre | 5.280 | 2.733 | 11 |
| $Trhr^{fl/fl}$:GFP | 8.380 | 3.729 | 8 |
| $Trhr^{fl/fl}$:Cre | 43.22 | 11.45 | 12 |

**Figure 4s: Number of mounts in Trhr conditional KO mice:** One-way ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group | F (2,28) = 0.9628 | 0.3941 |

Post-hoc (Bonferroni's):

| **Comparison** | **Significant** |
|---|---|
| WT:Cre vs. $Trhr^{fl/fl}$:GFP | No |
| WT:Cre vs. $Trhr^{fl/fl}$:Cre | P < 0.01 |
| $Trhr^{fl/fl}$:GFP vs. $Trhr^{fl/fl}$:Cre | P <0.05 |

Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| WT:Cre | 2 | 1.070 | 11 |

| | | | |
|---|---|---|---|
| $Trhr^{fl/fl}$:GFP | 2.750 | 1.373 | 8 |
| $Trhr^{fl/fl}$:Cre | 8 | 1.409 | 12 |

**Figure 4t: Latency to mount in Trhr conditional KO mice:** One-way ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group | F (2,28) = 1.320 | 0.2832 |

Post-hoc (Bonferroni's):

| **Comparison** | **Significant** |
|---|---|
| WT:Cre vs. $Trhr^{fl/fl}$:GFP | No |
| WT:Cre vs. $Trhr^{fl/fl}$:Cre | P < 0.001 |
| $Trhr^{fl/fl}$:GFP vs. $Trhr^{fl/fl}$:Cre | P <0.05 |

Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| WT:Cre | 531.0 | 37.33 | 11 |
| $Trhr^{fl/fl}$:GFP | 447.9 | 62.38 | 8 |
| $Trhr^{fl/fl}$:Cre | 267.6 | 41.36 | 12 |

**Extended Data Figure 1b: Mounting time to PBS/LPS female after presentation with PBS/LPS female pair:**
Paired two-tailed t-test (95% confidence interval)
t=11.58, df=8
p<0.0001
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| PBS | 43.09 | 3.544 | 9 |
| LPS | 2.612 | 0.8804 | 9 |

**Extended Data Figure 1c: Mounting time to untreated healthy female:** Paired two-tailed t-test (95% confidence interval)
t=0.1176, df=7
p<0.9097
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| Female1 | 22.95 | 3.058 | 8 |

| | | | |
|---|---|---|---|
| Female2 | 22.56 | 2.341 | 8 |

**Extended Data Figure 1d: Investigation time of PBS- and LPS-females in 3ch assay:** Paired two-tailed t-test (95% confidence interval)
t=3.994, df=10
p=0.0025
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| Sham | 0.2000 | 0.2000 | 5 |
| VNOX | 13.45 | 2.760 | 7 |

**Extended Data Figure 1f: Duration of other typical male behaviors during direct interaction with a PBS- or LPS-female:** Two-way ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (PBS or LPS) | F (1, 60) = 4.004 | P = 0.0499 |
| Male behavior (Facial investigation, Anogenital investigation, Social grooming) | F (2, 60) = 41.36 | P < 0.0001 |
| Interaction | F (2, 60) = 11.86 | P < 0.0001 |

Post-hoc (Sidak's):

| **PBS vs. LPS** | **P Value** |
|---|---|
| Facial investigation | ns |
| Anogenital investigation | P < 0.0001 |
| Social grooming | ns |

Descriptive Statistics:

| | Facial investigation | | | Anogenital investigation | | | Social grooming | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | n | Mean | SEM | n | Mean | SEM | N |
| PBS | 48.09 | 5.578 | 11 | 149.0 | 22.6636 | 11 | 9.42 | 2.287 | 11 |
| LPS | 63.83 | 6.85 | 11 | 71.93 | 7.9402 | 11 | 18.62 | 4.276 | 11 |

**Extended Data Figure 1g: Percentage of individual female behaviors during males' mounting attempts:** Two-way ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|

| | | |
|---|---|---|
| Group (PBS or LPS) | F (1, 52) = 1.099e-014 | P > 0.9999 |
| Female responses (Stand/lordosis, Run, Rear, Sit) | F (3, 52) = 5.844 | P =0.0016 |
| Interaction | F (3, 52) = 6.461 | P =0.0008 |

Post-hoc (Sidak's):

| **PBS vs. LPS** | **P Value** |
|---|---|
| Stand/Lordosis | Ns |
| Run | Ns |
| Rear | Ns |
| Sit | P < 0.01 |

Descriptive Statistics:

| | Stan/Lordosis | | | Run | | | Rear | | | Sit | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | n | Mean | SEM | N | Mean | SEM | N | Mean | SEM | N |
| PBS | 14.83 | 9.5407 | 11 | 56.78 | 9.6639 | 11 | 15.02 | 4.7006 | 11 | 13.38 | 5.4607 | 11 |
| LPS | 3.750 | 2.5282 | 11 | 24.25 | 8.1429 | 11 | 7.5 | 5.0564 | 11 | 64.50 | `12.3444 | 11 |

**Extended Data Figure 1h: Number of crossings :**Unpaired two-tailed t-test (95% confidence interval)
t=4.821, df=20
p=0.0001
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| PBS | 44.82 | 5.331 | 11 |
| LPS | 17.73 | 1.779 | 11 |

**Extended Data Figure 2a**: **Mounting time of VNO-removed or sham-surgery males to LPS-female:** Unpaired two-tailed t-test (95% confidence interval)
t=3.994, df=10
p=0.0025
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Sham | 0.2000 | 0.2000 | 5 |
| VNOX | 13.45 | 2.760 | 7 |

**Extended Data Figure 2d: Number of tdTomato(+) neurons in BST,MEA, and COApm after Cre injection Ai14 reporter mice:** One-way ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Brain Region (BST, MEA, COApm) | F (2,9) = 46.40 | 0.0001 |

Post-hoc (Bonferroni's):

| **Comparison** | **p Value** |
|---|---|
| BST vs. MEA | 0.0031 |
| BST vs. COApm | <0.0001 |
| MEA vs. COApm | 0.0027 |

Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| BST | 46.25 | 14.72 | 4 |
| MEA | 540.3 | 52.75 | 4 |
| COApm | 1045 | 114.5 | 4 |

**Extended Data Figure 2h**: **Number of FOS (+) cells in AOBmi, AOBgr, BST, MEApd, MEApv, and COApm after exposure to PBS or LPS-female:** Two-way ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Treatment (PBS or LPS) | F (1, 84) = 3.599 | P < 0.0001 |
| Brain Region (AOBmi, AOBgr, BST, MEApd, MEApv, COApm) | F (5, 84) = 51.44 | P < 0.0054 |
| Interaction | F (5, 84) = 6.670 | P < 0.0001 |

Post-hoc (Sidak's):

| **PBS vs. LPS** | **P Value** |
|---|---|
| AOBmi | 0.9992 |
| AOBgr | 0.0008 |
| BST | 0.5652 |
| MEApd | 0.9900 |
| MEApv | 0.0014 |

| | |
|---|---|
| COApm | <0.0001 |

Descriptive Statistics:

| | AOBmi | | | AOBgr | | | BST | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | n | Mean | SEM | n | Mean | SEM | n |
| PBS | 32.8229 | 5.5653 | 8 | 24.3021 | 3.9374 | 8 | 36.0938 | 3.8553 | 8 |
| LPS | 35.75 | 6.0112 | 8 | 54.2188 | 4.03 | 8 | 47.53 | 3.93 | 8 |
| | **MEApd** | | | **MEApv** | | | **COApm** | | |
| | Mean | SEM | n | Mean | SEM | n | Mean | SEM | n |
| PBS | 19.4 | 3.76 | 8 | 19.927 | 3.284 | 8 | 11.406 | 2.204 | 8 |
| LPS | 24.093 | 4.029 | 48.593 | 48.594 | 3.53 | 8 | 65.08 | 1.2 | 8 |

**Extended Data Figure 3e**: **Mean z-score of the fluorescence during direct investigation of the estrus or diestrus female:** Paired two-tailed t-test (95% confidence interval)
t=1.197, df=4
p=0.2975
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| Estrus | 3.307 | 0.2163 | 5 |
| Diesturs | 2.965 | 0.4058 | 5 |

**Extended Data Figure 3f: Percent mice mounting to Estrus or Diestrus female**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$=1.091, df=1
p=0.2963
Descriptive Statistics:

| | **Percent** | **N** |
|---|---|---|
| Estrus | 100 | 6 |
| Diesturs | 83.3 | 6 |

**Extended Data Figure 3g: Mounting time to Estrus or Diestrus female**: Unpaired two-tailed t-test (95% confidence interval)
t=0.6582, df=10
p=0.5252
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| Estrus | 43.02 | 11.22 | 6 |
| Diesturs | 33.98 | 7.913 | 6 |

**Extended Data Figure 3h: Number of mounts to Estrus or Diestrus female:** Unpaired two-tailed t-test (95% confidence interval)
t=0.5392, df=10
p<0.6016
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| Estrus | 9.667 | 1.926 | 6 |
| Diesturs | 11.50 | 2.802 | 6 |

**Extended Data Figure 3i: Latency to mount to Estrus or Diestrus female:** Unpaired two-tailed t-test (95% confidence interval)
t=0.2871, df=10
p=0.7799
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| Estrus | 226.7 | 32.72 | 6 |
| Diesturs | 249.8 | 73.44 | 6 |

**Extended Data Figure 3j: Percent mating plug at 24h of Estrus or Diestrus females**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =5.333, df=1
p=0.0209
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| Estrus | 83.3 | 6 |
| Diesturs | 16.7 | 6 |

**Extended Data Figure 4c: Number of FOS (+) cells in COApm of males after exposure to PBS- or LPS-odor:** Unpaired two-tailed t-test (95% confidence interval)
t=5.979, df=12
p<0.0001
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS-odor | 22.38 | 2.426 | 6 |
| LPS-odor | 43.61 | 2.473 | 8 |

**Extended Data Figure 4c: Mean z-score of the fluorescence in COApm evoked by the PBS- or LPS-odor investigation:** Paired two-tailed t-test (95% confidence interval)
t=4.424, df=5
p=0.0069
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS-odor | 4.295 | 0.8788 | 6 |
| LPS-odor | 7.498 | 1.161 | 6 |

**Extend Data Figure 4g: Percent mice mounting to PBS- or LPS-odor applied female**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =5.838, df=1
p=0.0157
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| PBS-odor | 80 | 10 |
| LPS-odor | 27.27 | 11 |

**Extend Data Figure 4h: Mounting time to PBS- or LPS-odor applied female:** Unpaired two-tailed t-test (95% confidence interval)
t=2.371, df=19
p=0.0285
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS-odor | 29.18 | 10.89 | 10 |
| LPS-odor | 3.949 | 2.323 | 11 |

**Extend Data Figure 4i: Number of mounts to PBS- or LPS-odor applied female:** Unpaired two-tailed t-test (95% confidence interval)
t=2.828, df=19
p<0.0108
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS-odor | 8.1 | 2.297 | 10 |
| LPS-odor | 1.455 | 0.8242 | 11 |

**Extend Data Figure 4j: Latency to mount to PBS- or LPS-odor applied female:** Unpaired two-tailed t-test (95% confidence interval)
t=3.222, df=19
p=0.0045
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS-odor | 298.8 | 63.83 | 10 |
| LPS-odor | 531.6 | 37.31 | 11 |

**Extended Data Figure 4k: Duration of other typical male behaviors during direct interaction with a PBS- or LPS-odor applied female:** Two-way ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (PBS or LPS) | F (1, 57) = 4.172 | P = 0.0457 |
| Male behavior (Facial investigation, Anogenital investigation, Social grooming) | F (2, 57) = 94.47 | P < 0.0001 |
| Interaction | F (2, 57) = 3.909 | P = 0.0256 |

Post-hoc (Sidak's):

| **PBS vs. LPS** | **P Value** |
|---|---|
| Facial investigation | P = 0.9661 |
| Anogenital investigation | P = 0.0038 |
| Social grooming | P = 0.9219 |

Descriptive Statistics:

| | Facial investigation | | | Anogenital investigation | | | Social grooming | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | n | Mean | SEM | n | Mean | SEM | N |
| PBS | 46.5 | 7.935 | 10 | 131.0 | 8.825 | 10 | 7.108 | 3.755 | 10 |
| LPS | 51.19 | 6.066 | 11 | 93.09 | 13.232 | 11 | 0.7673 | 0.444 | 11 |

**Extended Data Figure 4l: Percentage of individual female behaviors during males' mounting attempts:** Two-way ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (PBS or LPS) | F (1, 36) = 1.137e-014 | P > 0.9999 |
| Female responses (Stand/lordosis, Run, Rear, Sit) | F (3, 36) = 10.12 | P < 0.0001 |
| Interaction | F (3, 36) = 0.2828 | P =0.8374 |

Post-hoc (Sidak's):

| **PBS vs. LPS** | **P Value** |
|---|---|
| Stand/Lordosis | 0.9751 |

| | |
|---|---|
| Run | 0.9271 |
| Rear | >0.9999 |
| Sit | 0.9986 |

Descriptive Statistics:

| | Stan/Lordosis | | | Run | | | Rear | | | Sit | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | n | Mean | SEM | N | Mean | SEM | N | Mean | SEM | N |
| PBS | 16.241 | 10.64 | 8 | 59.745 | 13.792 | 8 | 10.01 | 6.03 | 8 | 14.0 | 7.22 | 8 |
| LPS | 7.246 | 7.246 | 3 | 71.956 | 5.728 | 3 | 11.014 | 5.862 | 3 | 9.78 | 7.71 | 3 |

**Extended Data Figure 4m: Number of cage crossings:** Unpaired two-tailed t-test (95% confidence interval)
t=0.01627, df=19
p=0.9872
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| PBS | 46 | 4.650 | 10 |
| LPS | 45.91 | 3.251 | 11 |

**Extended Data Figure 5b: Investigation time to healthy female during COApm photoactivation**: Two-way repeated measures ANOVA (95% confidence interval)

| **Source of variation** | **F (DFn, DFd)** | **P value** |
|---|---|---|
| Group (EYFP vs. ChR2) | F(1,14)=0.6806 | 0.4232 |
| Light (On vs. Off) | F(1,14)=0.5351 | 0.4766 |
| Interaction | F(1,14)=0.3492 | 0.5640 |

Post-hoc (Bonferroni's):

| **EYFP vs. ChR2** | **P Value** |
|---|---|
| ON | 0.9988 |
| OFF | 0.5807 |

Descriptive Statistics:

| | **Light On** | | | **Light Off** | | |
|---|---|---|---|---|---|---|
| | Mean | SEM | **n** | Mean | SEM | **n** |

| | | | | | | |
|---|---|---|---|---|---|---|
| EYFP | 223.942 | 18.561 | 9 | 194.796 | 14.653 | 9 |
| ChR2 | 222.771 | 24.212 | 7 | 219.674 | 16.500 | 7 |

**Extended Data Figure 5c: Grooming time during COApm photoactivation**: Unpaired two-tailed t-test (95% confidence interval)
t=2.175, df=14
p=0.0473
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 8.543 | 1.910 | 9 |
| ChR2 | 16.68 | 3.48 | 7 |

**Extended Data Figure 5d: Percentage of photoactivation trials with self-grooming**: Unpaired two-tailed t-test (95% confidence interval)
t=1.219, df=14
p=0.2429
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 16.91 | 3.330 | 9 |
| ChR2 | 22.41 | 2.773 | 7 |

**Extended Data Figure 5f: Investigation time to LPS-female during COApm DREADD Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=0.8427, df=24
p=0.4077
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| mCherry | 203.6 | 15.28 | 15 |
| hM4Di | 226.8 | 24.48 | 11 |

**Extended Data Figure 5h: Percent male mounting to LPS-female during COApm hM4Di inhibition**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$=3.616, df=1
p=0.0572
Descriptive Statistics:

| | **Percent** | **N** |
|---|---|---|
| Saline | 44.4 | 8 |
| CNO | 85.7 | 7 |

**Extended Data Figure 5i: Mounting time to LPS female during COApm hM4Di Inhibition**: Unpaired two-tailed t-test (95% confidence interval)

t=3.044, df=13
p=0.0094
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| Saline | 1.010 | 0.5569 | 8 |
| CNO | 20.19 | 6.745 | 7 |

**Extended Data Figure 5j: Number of mounts to LPS female during COApm hM4Di Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=2.723, df=13
p=0.0174
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| Saline | 0.7500 | 0.4119 | 8 |
| CNO | 6.143 | 2.075 | 7 |

**Extended Data Figure 5k: Latency to mount to LPS female during COApm hM4Di Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=1.821, df=13
p=0.0917
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| Saline | 511.4 | 45.45 | 8 |
| CNO | 361.5 | 71.24 | 7 |

**Extended Data Figure 5m: Percent male mounting to LPS-female during COApm hM4Di inhibition**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =N/A, df=NA
p=NA
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| mCherry | 100 | 8 |
| hM4Di | 100 | 8 |

**Extended Data Figure 5n: Mounting time to healthy female during COApm hM4Di Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=0.7289, df=14
p=0.4781
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|

| | | | |
|---|---|---|---|
| mCherry | 41.66 | 8.492 | 8 |
| hM4Di | 50.96 | 9.523 | 8 |

**Extended Data Figure 5o: Number of mounts to healthy female during COApm DREADD Inhibition**:
Unpaired two-tailed t-test (95% confidence interval)
t=0.08068, df=14
p=0.9368
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| mCherry | 12.38 | 2.375 | 8 |
| hM4Di | 12.63 | 1.990 | 8 |

**Extended Data Figure 5p: Latency to mount to healthy female during COApm DREADD Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=0.9176, df=14
p=0.3743
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| mCherry | 166.6 | 26.84 | 8 |
| hM4Di | 201.3 | 26.65 | 8 |

**Extended Data Figure 5r: Percent male mounting to diestrus female during COApm hM4Di inhibition**: Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =NA, df=NA
p=NA
Descriptive Statistics:

| | **Percent** | **N** |
|---|---|---|
| Saline | 100 | 6 |
| CNO | 100 | 6 |

**Extended Data Figure 5s: Mounting time to diestrus female during COApm hM4Di Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=0.1304, df=10
p=0.8988
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| Saline | 32.88 | 5.485 | 6 |
| CNO | 33.91 | 5.719 | 6 |

**Extended Data Figure 5t: Number of mounts to diestrus female during COApm hM4Di Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=0.7984, df=10

p=0.4432
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| Saline | 10.17 | 1.990 | 6 |
| CNO | 8.333 | 1.1145 | 6 |

**Extended Data Figure 5u: Latency to mount to diestrus female during COApm hM4Di Inhibition**: Unpaired two-tailed t-test (95% confidence interval)
t=0.3211, df=10
p=0.7548
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| Saline | 248.8 | 27.02 | 6 |
| CNO | 263.9 | 38.62 | 6 |

**Extended Data Figure 6i: Mean z-score of the MEA fluorescence during direct investigation of the PBS- or LPS-female**: Paired two-tailed t-test (95% confidence interval)
t=4.512, df=2
p=0.0458
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS | 2.044 | 0.9928 | 3 |
| LPS | 6.247 | 1.321 | 3 |

**Extended Data Figure 7a: Percent male mounting to healthy female during photoactivation of COApm-MEA projection:** Chi-Squared Test of Independence (95% confidence interval)
$X^2$=8.571, df=1
p=0.0034
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| EYFP | 100 | 10 |
| ChR2 | 40 | 10 |

**Extended Data Figure 7b: Percent male mounting to healthy female during photoactivation of MEA-*Vglut2* neurons:** Chi-Squared Test of Independence (95% confidence interval)
$X^2$=4.773, df=1
p=0.0289
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| EYFP | 100 | 7 |
| ChR2 | 50 | 8 |

**Extended Data Figure 7c: Percent male mounting to LPS-female with DREADD inhibition of MEA-*Vglut2* neurons:** Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =12.18, df=1
p=0.0005
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| mCherry | 44.4 | 9 |
| hM4Di | 100 | 11 |

**Extended Data Figure 7d: Percent male mounting to healthy female during concurrent photoactivation of COApm-MEA projections and hM4Di-inhibition of MEA-*Vglut2* neurons:** Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =2.286, df=1
p=0.1306
Descriptive Statistics:

| | Percent | N |
|---|---|---|
| mCherry | 75 | 8 |
| hM4Di | 100 | 8 |

**Extended Data Figure 7e: Number of mounts to healthy female during photoactivation of COApm-MEA projection:** Unpaired two-tailed t-test (95% confidence interval)
t=5.686, df=18
p<0.0001
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| EYFP | 14.60 | 1.408 | 10 |
| ChR2 | 2.600 | 1.572 | 10 |

**Extended Data Figure 7f: Number of mounts to healthy female during photoactivation of MEA-*Vglut2* neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=3.427, df=13
p=0.0045
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| EYFP | 14 | 2.862 | 7 |

| | | | |
|---|---|---|---|
| ChR2 | 3 | 1.669 | 8 |

**Extended Data Figure 7g: Number of mounts to LPS-female with DREADD inhibition of MEA-*Vglut2* neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=2.250, df=18
p=0.0372
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| mCherry | 3.333 | 1.818 | 9 |
| hM4Di | 8.818 | 1.628 | 11 |

**Extended Data Figure 7h: Number of mounts to healthy female during concurrent photoactivation of COApm-MEA projections and hM4Di-inhibition of MEA-*Vglut2* neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=2.167, df=14
p=0.0479
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| mCherry | 5.750 | 1.906 | 8 |
| hM4Di | 13.75 | 3.161 | 8 |

**Extended Data Figure 7i: Latency to mounts to healthy female during photoactivation of COApm-MEA projection:** Unpaired two-tailed t-test (95% confidence interval)
t=4.596, df=18
p=0.0002
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 201.7 | 38.34 | 10 |
| ChR2 | 507.6 | 53.68 | 10 |

**Extended Data Figure 7j: Latency to mounts to healthy female during photoactivation of MEA-*Vglut2* neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=3.669, df=13
p=0.0028
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| EYFP | 167.3 | 32.08 | 7 |
| ChR2 | 454.4 | 67.35 | 8 |

**Extended Data Figure 7k: Latency to mounts to LPS-female with DREADD inhibition of MEA-*Vglut2* neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=3.692, df=18

p=0.0017
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| mCherry | 446.2 | 68.71 | 9 |
| hM4Di | 155.8 | 43.85 | 11 |

**Extended Data Figure 7l: Latency to mounts to healthy female during concurrent photoactivation of COApm-MEA projections and hM4Di-inhibition of MEA-*Vglut2* neurons:** Unpaired two-tailed t-test (95% confidence interval)
t=1.854, df=14
p=0.0849
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| mCherry | 370.3 | 73.61 | 8 |
| hM4Di | 204.4 | 50.89 | 8 |

**Extended Data Figure 9c: Number of FOS(+) cells in COApm-TRH (-) cells photoactivation:** Unpaired two-tailed t-test (95% confidence interval)
t=5.533, df=4
p=0.0052
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| Control | 10.67 | 1.764 | 3 |
| ChR2 | 67.33 | 10.09 | 3 |

**Extended Data Figure 9d: Percent male mounting to healthy female with photoactivation of COApm-TRH (-) cells:** Chi-Squared Test of Independence (95% confidence interval)
$X^2$ =NA, df=NA
p=NA
Descriptive Statistics:

| | Mean | N |
|---|---|---|
| Control | 100 | 5 |
| ChR2 | 100 | 5 |

**Extended Data Figure 9e: Mounting time to healthy female with photoactivation of COApm-TRH(-) cells:** Unpaired two-tailed t-test (95% confidence interval)
t=1.329, df=8
p=0.2204
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Control | 35.58 | 5.929 | 5 |
| ChR2 | 61.72 | 17.96 | 5 |

**Extended Data Figure 9f: Number of mounts to healthy female with photoactivation of COApm-TRH(-) cells:**
Unpaired two-tailed t-test (95% confidence interval)
t=0.08528, df=8
p=0.9341
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| Control | 10.60 | 1.288 | 5 |
| ChR2 | 10.80 | 1.960 | 5 |

**Extended Data Figure 9g: Latency to mount to healthy female with photoactivation of COApm-TRH(-) cells:**
Unpaired two-tailed t-test (95% confidence interval)
t=0.9694, df=8
p=0.3608
Descriptive Statistics:

| | **Mean** | **SEM** | **N** |
|---|---|---|---|
| Control | 174.1 | 44.41 | 5 |
| ChR2 | 241 | 52.82 | 5 |

**Extended Data Figure 9l: ACU of the average fluorescence signal in MEA-Vglut2 neurons calcium imaging:**
Friedman test (95% confidence interval)

| **Source of variation** | **Friedman statistic** | **P value** |
|---|---|---|
| Time | 23.24 | 0.0003 |

Post-hoc (Bonferroni's):

| **Comparison** | **Significant** |
|---|---|
| Baseline vs. 0-30s | P > 0.9999 |
| Baseline vs. 0-60s | P = 0.2243 |
| Baseline vs. 0-90s | P = 0.0101 |
| Baseline vs. 0-120s | P = 0.0019 |
| Baseline vs. 0-150s | P = 0.0101 |

**Extended Data Figure 9m: Investigation time following microinjection of taltirelin into MEA:** Unpaired two-tailed t-test (95% confidence interval)
t=4.171, df=27

p=0.0003
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| PBS | 262.8 | 17.13 | 14 |
| Taltirelin | 150.8 | 20.43 | 15 |

**Extended Data Figure 10c: Percent of Trhr (+) cells following Cre expression in MEA:** Unpaired two-tailed t-test (95% confidence interval)
t=18.59, df=4
p<0.0001
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| $Trhr^{fl/fl}$ | 84.07 | 3.225 | 3 |
| $Trhr^{fl/fl}$ with Cre | 14.76 | 1.873 | 3 |

**Extended Data Figure 10g: Amplitudes of MEApv responses evoked by photoactivation of COApm inputs:** Unpaired two-tailed t-test (95% confidence interval)
t=4.663, df=10
p=0.0009
Descriptive Statistics:

| | Mean | SEM | N |
|---|---|---|---|
| GFP | 3.535 | 0.3301 | 6 |
| Cre | 1.570 | 0.2619 | 6 |