**Sample size and Statistics**

**Figure 2B – C**

Raster plots – (B) Each instance of head butting and shoving for both flies in the trial following the removal of the barrier.

Analysis – (C) Average fraction of time spent performing head butting and shoving behavior over each of five 2-minute periods: 11 – 13, 13 – 15, 15 – 17, 17 – 19, and 19 – 21 minutes after the stimulus.

Three biological repeats were performed, and the data was combined.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 2C | Cntrl>CsChrimson | 1 | 23 | Kruskal-Wallis | 11 – 13 min: |  |
|  | pC1d>CsChrimsonaIPg>CsChrimson | 23 | 2320 | (P = 0.0002) | 1 v 21 v 3 | >0.99990.0048 |
|  | pC1d+e>CsChrimson | 4 | 25 | Kruskal-Wallis | 1 v 413 – 15 min: | 0.0005 |
|  |  |  |  | (P = 0.0029)Kruskal-Wallis(P = 0.1368)Kruskal-Wallis(P = 0.2928)Kruskal-Wallis(P = 0.3120) | 1 v 21 v 31 v 415 – 17 min:1 v 21 v 31 v 417 – 19 min:1 v 21 v 31 v 419 – 21 min:1 v 2 1 v 3 1 v 4 | >0.99990.06360.0166>0.99990.83130.2141>0.99990.50710.19600.5090>0.9999>0.9999 |
|  |  |  |  |  |  |  |

**Figure 2 – Figure Supplement 1**

Raster plots – Each instance of head butting and shoving for only the tester flies in the trial.

Analysis – Average fraction of time the tester fly spent performing head butting and shoving behavior over each of three periods: during, immediately following (0 – 45 seconds), and 45 – 105 seconds after the stimulus.

Three biological repeats were performed, and the data was combined.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 2.1 | Cntrl>CsChrimson | 1 | 25 | Kruskal-Wallis | During: |  |
|  | pC1d>CsChrimsonaIPg>CsChrimson | 23 | 2641 | (P<0.0001) | 1 v 21 v 3 | <0.00010.0349 |
|  | pC1d+e>CsChrimson | 4 | 28 | Kruskal-Wallis | 1 v 40 – 45 sec: | <0.0001 |
|  |  |  |  | (P<0.0001)Kruskal-Wallis(P = 0.0003) | 1 v 21 v 31 v 445 – 105 min:1 v 21 v 31 v 4 | 0.0760<0.00010.00370.66750.00020.3495 |
|  |  |  |  |  |  |  |

**Figure 3 – Figure Supplement 1**

Analysis – Average fraction of time spent performing head butting and shoving behavior over each of four periods: prior, during, immediately following (0 – 30 seconds), and 30 – 60 seconds after the stimulus.

Three biological repeats were performed, and the data was combined.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 3.1 | Cntrl>Kir2.1 | 1 | 16 | Mann-Whitney | Prior: |  |
|  | pC1d>Kir2.1 | 2 | 15 | Mann-Whitney | 1 v 2During: | 0.3729 |
|  |  |  |  | Mann-WhitneyMann-Whitney | 1 v 20 – 30 s:1 v 230 – 60 s:1 v 2 | 0.28860.28740.2011 |
|  |  |  |  |  |  |  |

**Figure 4**

Analysis – Average area under curve value over the 10-second window in the baseline (5-15 seconds), stimulation (39-49 seconds), and post-stimulation (start at 3 decay time constant after stimulation).

Three to four biological repeats were performed, and the data was combined.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 4A | pC1d>Chrimson pC1d>GCaMP BaselinepC1d>Chrimson pC1d>GCaMP StimpC1d>Chrimson pC1d>GCaMP Post-Stim | 123 | 4 | Paired t-testPaired t-testPaired t-test | 1 v 22 v 31 v 3 | 0.00120.00250.0426 |
|  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 4B | aIPg>Chrimson aIPg>GCaMP BaselineaIPg>Chrimson aIPg>GCaMP StimaIPg>Chrimson aIPg>GCaMP Post-Stim | 123 | 3 | Paired t-testPaired t-testPaired t-test | 1 v 22 v 31 v 3 | 0.00030.00750.1352 |
|  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 4C | pC1d>Chrimson aIPg>GCaMP BaselinepC1d>Chrimson aIPg>GCaMP StimpC1d>Chrimson aIPg>GCaMP Post-Stim | 123 | 3 | Paired t-testPaired t-testPaired t-test | 1 v 22 v 31 v 3 | 0.03590.02120.1948 |
|  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 4D | aIPg>Chrimson pC1d>GCaMP BaselineaIPg>Chrimson pC1d>GCaMP StimaIPg>Chrimson pC1d>GCaMP Post-Stim | 123 | 3 | Paired t-testPaired t-testPaired t-test | 1 v 22 v 31 v 3 | 0.08930.43000.2089 |
|  |  |  |  |  |  |  |

**Figure 4 – Figure Supplement 1**

Analysis – Average area under curve value over the 10-second window in the baseline (5-15 seconds), stimulation (309-319 seconds), and post-stimulation (start at 3 decay time constant after stimulation).

Three biological repeats were performed, and the data was combined.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 4.1A | pC1d>Chrimson pC1d>GCaMP BaselinepC1d>Chrimson pC1d>GCaMP StimpC1d>Chrimson pC1d>GCaMP Post-Stim | 123 | 3 | Paired t-testPaired t-testPaired t-test | 1 v 22 v 31 v 3 | 0.00160.00140.0349 |
|  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 4.1B | aIPg>Chrimson aIPg>GCaMP BaselineaIPg>Chrimson aIPg>GCaMP StimaIPg>Chrimson aIPg>GCaMP Post-Stim | 123 | 3 | Paired t-testPaired t-testPaired t-test | 1 v 22 v 31 v 3 | 0.00040.02410.2100 |
|  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 4.1C | pC1d>Chrimson aIPg>GCaMP BaselinepC1d>Chrimson aIPg>GCaMP StimpC1d>Chrimson aIPg>GCaMP Post-Stim | 123 | 3 | Paired t-testPaired t-testPaired t-test | 1 v 22 v 31 v 3 | 0.01720.02120.2388 |
|  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Figure | Condition | # | Sample Size | Statistical Test | Comparison | P-value |
| 4.1D | aIPg>Chrimson pC1d>GCaMP BaselineaIPg>Chrimson pC1d>GCaMP StimaIPg>Chrimson pC1d>GCaMP Post-Stim | 123 | 3 | Paired t-testPaired t-testPaired t-test | 1 v 22 v 31 v 3 | 0.22940.34800.1675 |
|  |  |  |  |  |  |  |