Supplemental information

Neurexin1α differentially regulates synaptic efficacy within striatal circuits

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**Figure S1.** Nrx1α heterozygous and homozygous animals display altered spontaneous synaptic transmission without a change in overall number of excitatory synapses (related to Fig.1)

(A and C) Representative traces of mEPSCs WT, Nrx1α Het, and Nrx1α KO cells (top), cumulative distribution of mEPSC amplitude (lower left; inset shows average mEPSC amplitude), and cumulative distribution of inter-event intervals (lower right; inset shows average mEPSC frequency) in WT (n = 23; N = 7), Nrx1α Het (n = 18; N = 6) or Nrx1α KO (n = 24; N = 7) dSPNs (A) and WT (n = 20; N = 9), Nrx1α Het (n = 15; N = 6), and Nrx1α KO (n = 22; N = 7) iSPNs (C).

(B and D) Representative confocal images of Alexa 488 fluorescence of fixed SPNs (left) and secondary dendrites from WT (top), Nrx1α Het (middle), and Nrx1α KO (bottom). Summary of spine density of dSPN (B) and iSPN (D) spines. Mature spines were morphologically classified as stubby and mushroom (example mature spine denoted with blue arrow) while immature include filopodia (example immature spine denoted with red arrow). Each point represents a neuron. Z-stacks were acquired on a confocal at 40x magnification and quantified in Image J.

Data are means ± SEM; *significant difference between groups (ANOVA).
Figure S2. Inhibitory spontaneous synaptic transmission is unaltered in Nrxn1α mutants (related to Fig.1).

(A and B) Representative traces of mIPSCs WT, Nrxn1α Het, and Nrxn1α KO cells (top), cumulative distribution of mIPSC amplitude (lower left; inset shows average mIPSC amplitude), and cumulative distribution of inter-event intervals (lower right; inset shows average mIPSC frequency) in WT (n = 20; N = 5), Nrxn1α Het (n = 16; N = 4) or Nrxn1α KO (n = 18; N = 5) dSPNs (A) and WT (n = 17; N = 5), Nrxn1α Het (n = 16; N = 5), and Nrxn1α KO (n = 16; N = 5) iSPNs (C).

Summary data are mean ± SEM
Figure S3. Measurement of striatal field recordings (related to Figs. 1, 3, and 5)

(A) Field recording from Figure 2G showing placement of cursors (typically from ~3.5ms – 5ms after light stimulation) for analysis of fiber volley. (B) Field recording overlayed with recordings done at different LED intensities ranging from low LED (light gray) to higher LED intensities (black) and the change in fiber volley amplitude. (C) Measurement of field slope taken from 10-90% of the rising phase of the second negativity component of the field.
Figure S4. Optical EPSC amplitude reduced at dPFC-iSPN synapses in Nrnx1α heterozygotes and knockout (related to Fig1).

Maximal optical EPSC amplitudes from dPFC projection (taken from input-output measurements in Figure 1) were similar across genotypes onto (A) dSPNs and was significantly reduced onto (B) iSPNs, regardless of normalization to the field fiber volley.

Data are means ± SEM; *significant difference between groups (ANOVA)
Figure S5. Probability of release is unchanged at dPFC-dSPN synapses regardless of external calcium levels and spot illumination measurements (related to Fig.1).

(A) Plot of paired-pulse ratio across multiple ISIs for optical stimulation of dPFC-dSPN synapses using a 50μM spot (Mightex Optical Systems) and full field illumination. Further recordings were done using an ISI of 50ms (B).

(C) Graph of paired-pulse ratio, 50ms ISI, at external calcium levels of 1.3mM and 2.5mM employing 50μM spot illumination.

(D) Plot of paired-pulse ratio across multiple ISIs for 50μM optical stimulation of dPFC-dSPN at external calcium levels of 1.3mM in Nrxn1α WT and KO.

(E) Plot of frequency trains across multiple frequencies (right; 10Hz; 20Hz; 50Hz from left to right) recorded onto dSPNs in Nrxn1α WT and KO.

Summary data are mean ± SEM
Figure S6. Altered NMDAR decay at thalamic projections onto iSPNs in Nrxn1α KO (related to Fig4).

(A, left) Representative traces across genotypes of NMDA currents measured at +40mV in the presence of 100μM picrotoxin at dPFC-dSPN and dPFC-iSPN synapses. Traces are rescaled to same amplitude for comparison of decay time constants. Summary graph of weighted NMDAR decay values calculated from a biexponential fit for (A) dSPNs (B) and iSPNs. (C, left) Representative NMDA currents for PFas-dSPN and PFas-iSPN synapses. Summary graph of weighted NMDAR decay values calculated from a biexponential fit for (C) dSPNs (D and iSPNs.

Fast and slow decay components are used to calculate the weighted decay time constant using the equation $\tau_w = \left[ \frac{A_f}{A_f + A_s} \right] \times \tau_f + \left[ \frac{A_s}{(A_f + A_s)} \times \tau_s \right]$, $A_f$ = fast component amplitude and $A_s$ = slow component amplitude.

Summary data are mean ± SEM, *significant difference between groups (ANOVA)
Figure S7. Initial EPSP amplitude and recording duration are similar in Nrxn1α WT and KO (related to Fig6 and Fig7).

(A) Frequencies represented in an in vivo modeled optical stimulus pattern represented as 500ms windows and their corresponding local frequencies.

(B, D) Plot of initial EPSP (averaged 10 traces) recorded at “down-state” membrane potential of -80mV on x-axis and overall spiking efficiency on y-axis for (B) dSPNs and (D) iSPNs across genotypes for dPFC projection.

(C, E) Plot of spiking efficiency across recording duration of 5 minutes consisting of 10 unique optical patterns, binned in 10-second intervals for (C) dSPNs and (E) iSPNs recorded in Nrxn1α WT, Het, and KO from dPFC projection.

(F, H) Plot of initial EPSP (averaged 10 traces) recorded at “down-state” membrane potential of -80mV on x-axis and overall spiking efficiency on y-axis for (F) dSPNs and (H) iSPNs across genotypes for PFas projection.

(G, I) Plot of spiking efficiency across recording duration of 5 minutes consisting of 10 unique optical patterns, binned in 10-second intervals for (G) dSPNs and (I) iSPNs recorded in Nrxn1α WT and KO from PFas projection.

Summary data are mean ± SEM.
Table S1. Student’s t-test values, related to Figures 3, 5, 6, and S7.

| Figure | Test | t and p values   |
|--------|------|-----------------|
| 3C     | Two-tailed Student’s t-test | t_{31} = 0.07521, p = 0.9405 |
| 3E     | Two-tailed Student’s t-test | t_{26} = 1.157, p = 0.2578 |
| 5B     | Two-tailed Student’s t-test | t_{29} = 1.173, p = 0.2504 |
| 5D, left | Two-tailed Student’s t-test (Paired) | t_{18} = 3.052, p = 0.0069 |
| 5D, right | Two-tailed Student’s t-test (Paired) | t_{21} = 0.1329, p = 0.8955 |
| 5E     | Two-tailed Student’s t-test | t_{39} = 2.193, p = 0.0343 |
| 5F     | Two-tailed Student’s t-test | t_{31} = 2.044, p = 0.0495 |
| 5G     | Two-tailed Student’s t-test | t_{31} = 2.159, p = 0.0387 |
| 6B     | Two-tailed Student’s t-test | t_{33} = 0.7286, p = 0.4714 |
| 6D     | Two-tailed Student’s t-test | t_{27} = 2.411, p = 0.0230 |
| 7A     | Two-tailed Student’s t-test | t_{34} = 1.597, p = 0.1196 |
| 7C     | Two-tailed Student’s t-test | t_{29} = 1.454, p = 0.1567 |
Table S2. One-way ANOVA values, related to Figures 1, 2, 4, 6, S1, S2, S4, and S6.

| Figure      | F value | DF | P value | Post-hoc test | Comparison          | Adjusted P value |
|-------------|---------|----|---------|---------------|---------------------|------------------|
| 1F          | 1.352   | 50 | 0.2683  | Dunnett’s     | WT v. Het           | 0.0059           |
|             |         |    |         |               | WT v. KO           | 0.0166           |
| 1H          | 5.816   | 43 | 0.0060  | Dunnett’s     | WT v. Het           | 0.1639           |
|             |         |    |         |               | WT v. KO           | 0.0080           |
| 2C          | 2.433   | 59 | 0.0968  | Dunnett’s     | WT v. Het           | 0.3180           |
|             |         |    |         |               | WT v. KO           | 0.0029           |
| 2F          | 1.234   | 61 | 0.2986  | Dunnett’s     | WT v. Het           | 0.0232           |
|             |         |    |         |               | WT v. KO           | 0.0349           |
| 4C          | 4.532   | 59 | 0.0149  | Dunnett’s     | WT v. Het           | 0.2039           |
|             |         |    |         |               | WT v. KO           | < 0.0001         |
| 4F          | 5.767   | 51 | 0.0056  | Dunnett’s     | WT v. Het           | 0.0213           |
|             |         |    |         |               | WT v. KO           | 0.0349           |
| 6B          | 0.4497  | 48 | 0.6406  | Dunnett’s     | WT v. Het           | 0.0029           |
| 6D          | 4.233   | 43 | 0.0213  | Dunnett’s     | WT v. Het           | 0.0213           |
|             |         |    |         |               | WT v. KO           | 0.0349           |
| S1A, Left   | 0.4396  | 64 | 0.6463  | Dunnett’s     | WT v. Het           | 0.2039           |
| S1A, Right  | 10.31   | 64 | 0.0001  | Dunnett’s     | WT v. KO           | < 0.0001         |
| S1B, Left   | 0.06115 | 44 | 0.9408  |               |                     |                  |
| S1B, Right  | 0.146   | 44 | 0.8646  |               |                     |                  |
| S1C, Left   | 0.8491  | 56 | 0.4334  |               |                     |                  |
| S1C, Right  | 2.896   | 56 | 0.0639  |               |                     |                  |
| S1D, Left   | 2.69    | 32 | 0.0843  |               |                     |                  |
| S1D, Right  | 0.6891  | 32 | 0.5098  |               |                     |                  |
| S2A, Left   | 0.5944  | 54 | 0.5556  |               |                     |                  |
| S2A, Right  | 1.698   | 54 | 0.1930  |               |                     |                  |
| S2B, Left   | 1.713   | 48 | 0.1916  |               |                     |                  |
| S2B, Right  | 0.2970  | 48 | 0.7445  |               |                     |                  |
| S4A         | 0.6478  | 50 | 0.5277  |               |                     |                  |
| S4B         | 6.793   | 43 | 0.0028  | Dunnett’s     | WT v. Het           | 0.0075           |
|             |         |    |         |               | WT v. KO           | 0.0035           |
| S6A         | 0.7301  | 59 | 0.4863  |               |                     |                  |
| S6B         | 2.634   | 66 | 0.0796  |               |                     |                  |
| S6C         | 3.871   | 54 | 0.0271  | Dunnett’s     | WT v. Het           | 0.0634           |
|             |         |    |         |               | WT v. KO           | 0.7600           |
| S6D         | 6.542   | 49 | 0.0031  | Dunnett’s     | WT v. Het           | 0.8297           |
|             |         |    |         |               | WT v. KO           | 0.0118           |
Table S3. Two-way ANOVA values, related to Figures 2, 4, 6, and 7.

| Figure | Test | Source of Variation | F value | Comparison | DF  | P value |
|--------|------|---------------------|---------|------------|-----|---------|
| 2A     | ANOVA| Interaction         | 1.614   |            | 8   | 0.1215  |
|        |      | ISI                 | 144.3   |            | 4   | < 0.0001|
|        |      | Genotype            | 1.46    |            | 2   | 0.2405  |
| 2B (10Hz) | ANOVA| Interaction         | 0.8324  |            | 6   | 0.5466  |
|        |      | Pulse #             | 17.56   |            | 3   | < 0.0001|
|        |      | Genotype            | 1.57    |            | 2   | 0.2182  |
| 2B (20Hz) | ANOVA| Interaction         | 2.026   |            | 6   | 0.0655  |
|        |      | Pulse #             | 36.11   |            | 3   | < 0.0001|
|        |      | Genotype            | 0.5166  |            | 2   | 0.5997  |
| 2B (50Hz) | ANOVA| Interaction         | 4.479   |            | 6   | 0.0003  |
|        |      | Pulse #             | 325     |            | 3   | < 0.0001|
|        |      | Genotype            | 1.465   |            | 2   | 0.2407  |
| 2D     | ANOVA| Interaction         | 1.594   |            | 8   | 0.1286  |
|        |      | ISI                 | 175.1   |            | 4   | < 0.0001|
|        |      | Genotype            | 4.372   |            | 2   | 0.0178  |
|        | Dunnett's| ISI 20, WT v. Het | 28.13   |            |     | 0.2012  |
|        |      | ISI 20, WT v. KO    | 26.08   |            |     | 0.0187  |
|        |      | ISI 50, WT v. Het   | 29.84   |            |     | 0.0160  |
|        |      | ISI 50, WT v. KO    | 28.88   |            |     | 0.0298  |
|        |      | ISI 100, WT v. Het  | 28.83   |            |     | 0.0811  |
|        |      | ISI 100, WT v. KO   | 28.39   |            |     | 0.2015  |
|        |      | ISI 200, WT v. Het  | 30.49   |            |     | 0.2436  |
|        |      | ISI 200, WT v. KO   | 30.7    |            |     | 0.2598  |
|        |      | ISI 500, WT v. Het  | 31      |            |     | 0.0771  |
|        |      | ISI 500, WT v. KO   | 32.46   |            |     | 0.0354  |
| 2E (10Hz) | ANOVA| Interaction         | 0.8491  |            | 6   | 0.5342  |
|        |      | Pulse #             | 87.51   |            | 3   | < 0.0001|
|        |      | Genotype            | 5.553   |            | 2   | 0.0068  |
|        | Dunnett’s| Pulse 2/1, WT v. Het| 29.22   |            |     | 0.0113  |
|        |      | Pulse 2/1, WT v. KO | 23.27   |            |     | 0.0838  |
|        |      | Pulse 3/1, WT v. Het| 27.95   |            |     | 0.0086  |
|        |      | Pulse 3/1 WT v. KO  | 23.99   |            |     | 0.1225  |
|        |      | Pulse 4/1, WT v. Het| 28.81   |            |     | 0.0255  |
|        |      | Pulse 4/1, WT v. KO | 26.65   |            |     | 0.0901  |
|        |      | Pulse 5/1, WT v. Het| 26.01   |            |     | 0.0124  |
|        |      | Pulse 5/1, WT v. KO | 21.33   |            |     | 0.1328  |
| 2E (20Hz) | ANOVA| Interaction         | 2.605   |            | 6   | 0.0200  |
|        |      | Pulse #             | 133.4   |            | 3   | < 0.0001|
|        |      | Genotype            | 5.845   |            | 2   | 0.0054  |
|        | Dunnett’s| Pulse 2/1, WT v. Het| 29.36   |            |     | 0.0234  |
|        |      | Pulse 2/1, WT v. KO | 21.91   |            |     | 0.0161  |
|        |      | Pulse 3/1, WT v. Het| 29.99   |            |     | 0.0197  |
|        |      | Pulse 3/1 WT v. KO  | 27.02   |            |     | 0.1101  |
|        |      | Pulse 4/1, WT v. Het| 30      |            |     | 0.0149  |
|        |      | Pulse 4/1, WT v. KO | 24.76   |            |     | 0.1149  |
|        |      | Pulse 5/1, WT v. Het| 28.97   |            |     | 0.0053  |
| 2E (50Hz) | Dunnett’s |  |  |  |  |  |
|---|---|---|---|---|---|---|
| ANOVA | Interaction 1.221 | Pulse 2/1, WT v. KO 24.63 |  | Genotype 8.012 |  | < 0.0001 |
| ANOVA | Pulse 427 | Pulse 2/1, WT v. KO 28.16 |  | Genotype 0.1525 |  | 0.2991 |
| ANOVA | Genotype 2.00 | Pulse 2/1, WT v. KO 29.49 |  | Genotype 0.0010 |  | 0.0016 |
| ANOVA | Pulse 427 | Pulse 2/1, WT v. KO 29.49 |  | Pulse 2/1, WT v. KO 29.77 |  | 0.0041 |
| ANOVA | Genotype 0.1525 | Pulse 3/1, WT v. KO 21.99 |  | Genotype 0.0297 |  | 0.0057 |
| ANOVA | Pulse 427 | Pulse 3/1, WT v. KO 29.77 |  | Genotype 0.0028 |  | 0.0057 |
| ANOVA | Genotype 2.00 | Pulse 4/1, WT v. KO 25.25 |  | Pulse 4/1, WT v. KO 25.25 |  | 0.0512 |
| ANOVA | Pulse 427 | Pulse 4/1, WT v. KO 25.25 |  | Pulse 4/1, WT v. KO 25.25 |  | 0.0512 |
| ANOVA | Genotype 0.1525 | Pulse 5/1, WT v. KO 27.98 |  | Pulse 5/1, WT v. KO 27.98 |  | 0.1281 |
| ANOVA | Pulse 427 | Pulse 5/1, WT v. KO 27.98 |  | Pulse 5/1, WT v. KO 27.98 |  | 0.1281 |

| 4A | ANOVA | Interaction 1.373 |  | Genotype 0.4059 |  | 0.2456 |
|---|---|---|---|---|---|---|
| ANOVA | ISI 11.36 |  | Genotype 0.4059 |  | 0.2456 |
| ANOVA | Genotype 0.4059 |  | 0.5276 |  | 0.5276 |
| 4B (10Hz) | ANOVA | Interaction 0.632 |  | Pulse # 120.9 |  | 0.5950 |
|---|---|---|---|---|---|---|
| ANOVA | Genotype 0.2746 |  | 0.6032 |  | 0.6032 |
| 4B (20Hz) | ANOVA | Interaction 0.8874 |  | Genotype 0.4443 |  | 0.4499 |
|---|---|---|---|---|---|---|
| ANOVA | Pulse # 200 |  | 0.4499 |  | 0.4499 |
| 4B (50Hz) | ANOVA | Interaction 1.962 |  | Genotype 1.891 |  | 0.1769 |
|---|---|---|---|---|---|---|
| ANOVA | Pulse # 258.7 |  | 0.1235 |  | 0.1235 |
| 4D | ANOVA | Interaction 0.543 |  | Genotype 1.098 |  | 0.3026 |
|---|---|---|---|---|---|---|
| ANOVA | ISI 13.53 |  | 0.5090 |  | 0.5090 |
| ANOVA | Genotype 0.9886 |  | 0.3267 |  | 0.3267 |
| 4E (10Hz) | ANOVA | Interaction 0.632 |  | Genotype 1.098 |  | 0.3026 |
|---|---|---|---|---|---|---|
| ANOVA | Pulse # 119.3 |  | 0.3026 |  | 0.3026 |
| 4E (20Hz) | ANOVA | Interaction 1.953 |  | Genotype 2.062 |  | 0.1607 |
|---|---|---|---|---|---|---|
| ANOVA | Pulse # 201.2 |  | 0.1262 |  | 0.1262 |
| 4E (50Hz) | ANOVA | Interaction 2.032 |  | Genotype 0.5889 |  | 0.4485 |
|---|---|---|---|---|---|---|
| ANOVA | Pulse # 249.9 |  | 0.1146 |  | 0.1146 |
| 5C | ANOVA | Interaction 0.7454 |  | Genotype 0.5422 |  | 0.4659 |
|---|---|---|---|---|---|---|
| ANOVA | Time 149.9 |  | 0.4659 |  | 0.4659 |
| ANOVA | Genotype 0.5422 |  | 0.4659 |  | 0.4659 |
| 6C | ANOVA | Interaction 0.6695 |  | Genotype 0.6145 |  | 0.4387 |
|---|---|---|---|---|---|---|
| ANOVA | Frequency 27.32 |  | 0.4387 |  | 0.4387 |
| ANOVA | Genotype 0.6145 |  | 0.4387 |  | 0.4387 |
| 6E | ANOVA | Interaction 1.074 |  | Genotype 6.328 |  | 0.0181 |
|---|---|---|---|---|---|---|
| ANOVA | Frequency 32.09 |  | 0.0181 |  | 0.0181 |
| ANOVA | Genotype 6.328 |  | 0.0181 |  | 0.0181 |
| 7B | ANOVA | Interaction 0.9941 |  | Genotype 2.545 |  | 0.1199 |
|---|---|---|---|---|---|---|
| ANOVA | Frequency 4.055 |  | 0.1199 |  | 0.1199 |
| ANOVA | Genotype 2.545 |  | 0.1199 |  | 0.1199 |
| 7D | ANOVA | Interaction 0.6737 |  | Genotype 5.226 |  | 0.0013 |
|---|---|---|---|---|---|---|
| ANOVA | Frequency 5.226 |  | 0.0013 |  | 0.0013 |
|        | Genotype | Interaction | Time | Genotype |
|--------|----------|-------------|------|----------|
| S7C    | 1.980    | 0.8359      | 22.05| 0.8708   |
|        | 1        | 58          | 29   | 2        |
| S7E    | 0.8052   | 0.8359      | 58   | 0.4254   |
|        |          | 29          |      |          |
| S7G    | 0.0130   | 4.836       | 29   | 0.0966   |
|        |          | 29          |      |          |
| S7I    | 0.0938   | 0.7191      | 29   | 0.08618  |
|        |          | 29          |      |          |
|        | 1.395    | 29          |      | 1        |
|        | 1        | 29          |      | 0.2472   |
Table S4. Pearson correlation values, related to Figure S7.

| Figure             | Test                  | Pearson r | P value |
|--------------------|-----------------------|-----------|---------|
| S7B (WT D1; dPFC)  | Pearson; Two-tailed   | -0.0096   | 0.9730  |
| S7B (Het D1; dPFC) | Pearson; Two-tailed   | 0.3841    | 0.1751  |
| S7B (KO D1; dPFC)  | Pearson; Two-tailed   | -0.3056   | 0.1901  |
| S7D (WT D2; dPFC)  | Pearson; Two-tailed   | 0.2893    | 0.3158  |
| S7D (Het D2; dPFC) | Pearson; Two-tailed   | -0.0052   | 0.9855  |
| S7D (KO D2; dPFC)  | Pearson; Two-tailed   | -0.4258   | 0.1135  |
| S7F (WT D1; PFas)  | Pearson; Two-tailed   | -0.1150   | 0.6604  |
| S7F (KO D1; PFas)  | Pearson; Two-tailed   | -0.0518   | 0.8329  |
| S7H (WT D2; PFas)  | Pearson; Two-tailed   | -0.1517   | 0.6379  |
| S7H (KO D2; PFas)  | Pearson; Two-tailed   | 0.3607    | 0.1293  |