Supplementary Figure 1

MORwd induces postsynaptic depression of AMPAR-mediated neurotransmission in medial LHb.

(a) Left: sample traces, box and scatter plots of sEPSCs amplitudes recorded in LatLHb (saline+naloxone (n=9/3, gray) versus NP-MORwd (n=9/4, orange), two-sided t-test, t=0.098, P=0.923). Right: same but sEPSCs were recorded in MedLHb (saline+naloxone (n=9/4, black) versus NP- MORwd (n=9/5, red), two-sided t-test, t=3.493, **P=0.003). (b) Left: sample traces, box and scatter plots of sEPSCs frequencies recorded in LatLHb (saline+naloxone (n=9/4, gray) versus NP-MORwd (n=9/4, orange), two-sided t-test, t=1.331, P=0.202). Right: same but sEPSCs were recorded in MedLHb (saline+naloxone (n=9/5, black) versus NP-MORwd (n=9/4, red), two-sided t-test, t=0.161, P=0.874). (c) Recording map color-coded for the value of AMPAR:NMDAR ratios recorded throughout the LHb. Lighter colors indicate smaller AMPAR:NMDAR ratios, while darker colors represent high AMPAR:NMDAR ratio. (d) Top: Sample traces and normalized EPSC versus pulse number plots recorded at 5, 10 and 20 Hz in LatLHb (saline+naloxone (n=10/2, gray) versus NP-MORwd (n=10/3, orange), 5Hz interaction factor F(4, 36)=0.227, P=0.921; 10Hz interaction factor F(4, 36)=1.71, P=0.2; 20Hz interaction factor F(4, 36)=0.573, P=0.683 two-way ANOVA Repeated Measures). Bottom: same but in MedLHb (n=10/3, saline+naloxone (black) versus NP- MORwd (red), 5Hz interaction factor F(4, 36)=0.1.183, P=0.34; 10Hz interaction factor F(4, 36)=1.171, P=0.34; 20Hz interaction factor F(4, 36)=0.88, P=0.485 two-way ANOVA Repeated Measures). (e) Example of peak-scaled NSFA of MedLHb neurons in the saline- and NP-MORwd group. Pooled data for conductance (γ) and number of channels (N) open at the peak together with amplitude versus N of channels and conductance plots (Saline+naloxone, n=5/4; MORwd, n=8/5; N of channels, two-sided t-test, t=5.67, ***P=0.0001, r^2 (N-Channels) = 0.416; *P=0.017; Conductance, t=0.099, P=0.555). (g) Left: sample traces, box and scatter plots for rectification index calculated from AMPAR EPSCs recorded at -70, 0 and 40 mV in LatLHb (saline+naloxone (n=9/7, gray) versus NP- MORwd (n=10/7, orange), two-sided t-test, t=0.210, P=0.836). Right: Same but recordings in MedLHb (saline+naloxone (n=12/8, black) versus NP- MORwd (n=9/5, red), two-sided t-test, t=0.192, P=0.848). (h) Sample traces, box and scatter plots of AMPAR:NMDAR ratios recorded in MedLHb via 405 nm laser-assisted uncaging of MNI-glutamate, 500μM (saline+naloxone (n=8/2, black) versus NP-MORwd (n=10/3, red), two-sided t-test, t=3.521, **P=0.003). Bottom right: Absolute AMPAR versus absolute NMDA uncaging-evoked current plots from saline+naloxone (open black circles) or NP-MORwd mice (open red circles). The mean with S.E.M. AMPA and NMDA currents are shown with black and red filled circles for saline versus MORwd respectively (saline+naloxone versus NP-MORwd: AMPA, two-sided t-test, t=3.536, **P=0.003; NMDA, two-sided t-test, t=0.195, P=0.848). Data are presented as box plots 10-90 percentiles and scatter.
Supplementary Figure 2

TNFα levels in the LHb increase following spontaneous MORwd

(a) TNFα (cyan) and DAPI (magenta) immunostaining in slices from saline-treated, (b) MOR-treated (sacrificed 1 hour after the last MOR injection) and (c) animals in spontaneous MORwd (10-13 days post last MOR injection). (d) Normalized LHb TNFα optical density in saline (black), MOR (open red) and spontaneous MORwd (red) (n_{mice}=8, saline (black) versus MOR (open red) versus spontaneous MORwd (red), F(2,20)=7.7 one-way ANOVA, **P=0.003). Data are presented as box plots 10-90 percentiles with median and scatter.
Supplementary Figure 3

TNFα signaling is necessary and sufficient for MORwd-induced plasticity

(a) Spontaneous MORwd protocol, sample traces, box and scatter plots for AMPAR:NMDAR ratios recorded in MedLHb slices incubated with (+) or without (−) exogenous TNFα from spontaneous MORwd mice (10 days). (MORwd-TNFα (red) versus +TNFα (pink), ncells/mice=9/4, two-sided t-test, t16=0.986, P=0.339). (b) MedLHb AMPAR:NMDAR ratios from saline or MPLA-injected MOR-treated mice (MOR/saline (ncells/mice=4/11; shaded blue) versus MOR/MPLA (ncells/mice=4/11; dark blue), two-sided t-test, t19=3.070, **P=0.006). (c) Sample traces, time versus amplitude plot and bar graphs showing the effect of MPLA (1 g/ml) on AMPAR-EPSCs (MOR (ncells/mice=4/11; 63.93 ± 7.06%; open red), NP-MORwd (ncells/mice=3/10 cells; 91.63 ± 8.86%; filled red), MOR versus NP-MORwd, two-sided t-test, t19=2.419, *P=0.026). Data of this panel are represented as mean and sem. (d) Sample traces, time versus amplitude plot and bar graphs showing the effect of MPLA (1 μg/ml) on evoked AMPAR-EPSCs (baseline (1) vs 30 min post-MPLA (2)) recorded in LatLHb (open orange) or MedLHb (open red) in slices obtained from morphine-treated animals (ncells/mice=4/4, morphine LatLHb 103.98 ± 10.13; ncells/mice=11/5, morphine MedLHb 63.31 ± 7.06%; morphine LatLHb versus morphine MedLHb, two-sided t-test, t17=3.406, **P=0.003). Note that the data set for MedLHb is the same as in c and is used for comparison. Data are presented as mean and SEM. (e) Sample traces, time versus amplitude plot and bar graph showing the effect of MPLA (1 μg/ml) on evoked AMPAR-EPSCs (baseline (1) vs 30 min post-MPLA (2) in the presence of TNFα dominant negative peptide (XENP1595, 30mg/kg) recorded in MedLHb in slices obtained from morphine-treated animals (ncells/mice=7/2, 98.88 ± 8.23 %, two-sided t-test, t9=0.073, P=0.944). Data are presented with mean and SEM. (f) NP-MORwd protocol with dominant-negative TNFα (XENP1595, 30mg/kg) pretreatment, sample traces, box and scatter plots for AMPAR:NMDAR ratios recorded in MedLHb (saline+naloxone (ncells/mice=10/3, black) versus NP-MORwd (ncells/mice=12/3, green), two-sided t-test, t20=0.165, P=0.871). Data are presented as box plots 10-90 percentiles and scatter.
Supplementary Figure 4

Behavioral assessment of MORwd

(a) Box and scatter plot showing the percent time spent in the compartment containing the social stimulus for C57Bl6 mice (N=22 mice/group, saline+naloxone (black) versus NP-MORwd (red), two-sided t-test, t_{42}=2.401, *P=0.021). (b) Box and scatter plot showing the percent time spent in the compartment containing the object stimulus for C57Bl6 mice (N=22 mice/group, saline+naloxone (black) versus NP-MORwd (red), two-sided t-test, t_{42}=2.465, *P=0.02). (c) Box and scatter plot showing the percent time spent in the central compartment for C57Bl6 mice (N=22 mice/group, saline+naloxone (black) versus NP-MORwd (red), two-sided t-test, t_{42}=1.186, P=0.242). (d) Box and scatter plot showing locomotor activity during social preference test for C57Bl6 mice (N=22 mice/group, saline+naloxone (black) versus NP-MORwd (red), two-sided t-test, t_{42}=2.621, *P=0.012). (e) Box and scatter plot showing the percent time spent in the compartment containing the social stimulus for TNF-R1fl/fl mice (AAV-Control: 58.02 ± 2.96% saline+naloxone (N_{mice}=20, black) versus 40.45 ± 5.08% NP-MORwd (N_{mice}=23, red); AAV-Cre: 54.41 ± 3.15% saline (N_{mice}=13, open gray) versus 64.52 ± 5.94 % NP-MORwd (N_{mice}=13, open pink), interaction factor F_{(1,65)}=8.591 two-way ANOVA, **P=0.005). (f) Box and scatter plot showing the percent time spent in the compartment containing the object stimulus for TNF-R1fl/fl mice (N of mice same as panel e. AAV-Control: 26.42 ± 2.47 % saline+naloxone (black) versus 30.9 ± 4.79% NP-MORwd (red); AAV-Cre: 31.19 ± 2.07% saline+naloxone (open gray) versus 20.7 ± 4.13 % NP-MORwd (open gray), interaction factor F_{(1,65)}=4.136 two-way ANOVA, *P=0.046). (g) Box and scatter plot showing the percent time spent in the central compartment for TNF-R1fl/fl mice (N of mice same as panel e. AAV-Control: 15.38 ± 3.08% saline+naloxone (black) versus 27.82 ± 4.98% NP-MORwd (red); AAV-Cre: 14.23 ± 2.9 % saline+naloxone (open gray) versus 14.62 ± 3.08% NP-MORwd (open pink), interaction factor F_{(1,65)}=1.748 two-way ANOVA, P=0.191). (h) Box and scatter plot showing number of exploration bouts of TNF-R1fl/fl with the juvenile (N of mice same as panel e. AAV-Control: 56 ± 3.51 saline+naloxone (black) versus 39.30 ± 4.8 NP-MORwd (red); AAV-Cre: 50.38 ± 3.78 saline+naloxone (open gray) versus 57.15 ± 7.01 NP-MORwd (open pink), interaction factor F_{(1,65)}=5.519 two-way ANOVA, *P=0.022). (i) Box and scatter plot showing number of exploration bouts of TNF-R1fl/fl with the object (N of mice is the same as panel e. AAV-Control: 26.9 ± 1.88 saline+naloxone (black) versus 23.35 ± 2.13 NP-MORwd (red); AAV-Cre: 28.08 ± 2.01 saline+naloxone (open gray) versus 23.35 ± 2.13 NP-MORwd (open pink), interaction factor F_{(1,65)}=0.361, P=0.55). (j) Box and scatter plot showing locomotor activity during social preference test for TNF-R1fl/fl mice (N of mice is the same as panel e. AAV-Control: 28.13 ± 1.35m saline+naloxone (black) versus 33.8 ± 5.59m NP-MORwd (red); AAV-Cre: 23.78 ± 1.47m saline+naloxone (open gray) versus 40.17 ± 7.11m NP-MORwd (open pink), interaction factor F_{(1,65)}=1.224 two-way ANOVA, P=0.273). Data are presented as box plots 10-90 percentiles with median and scatter.