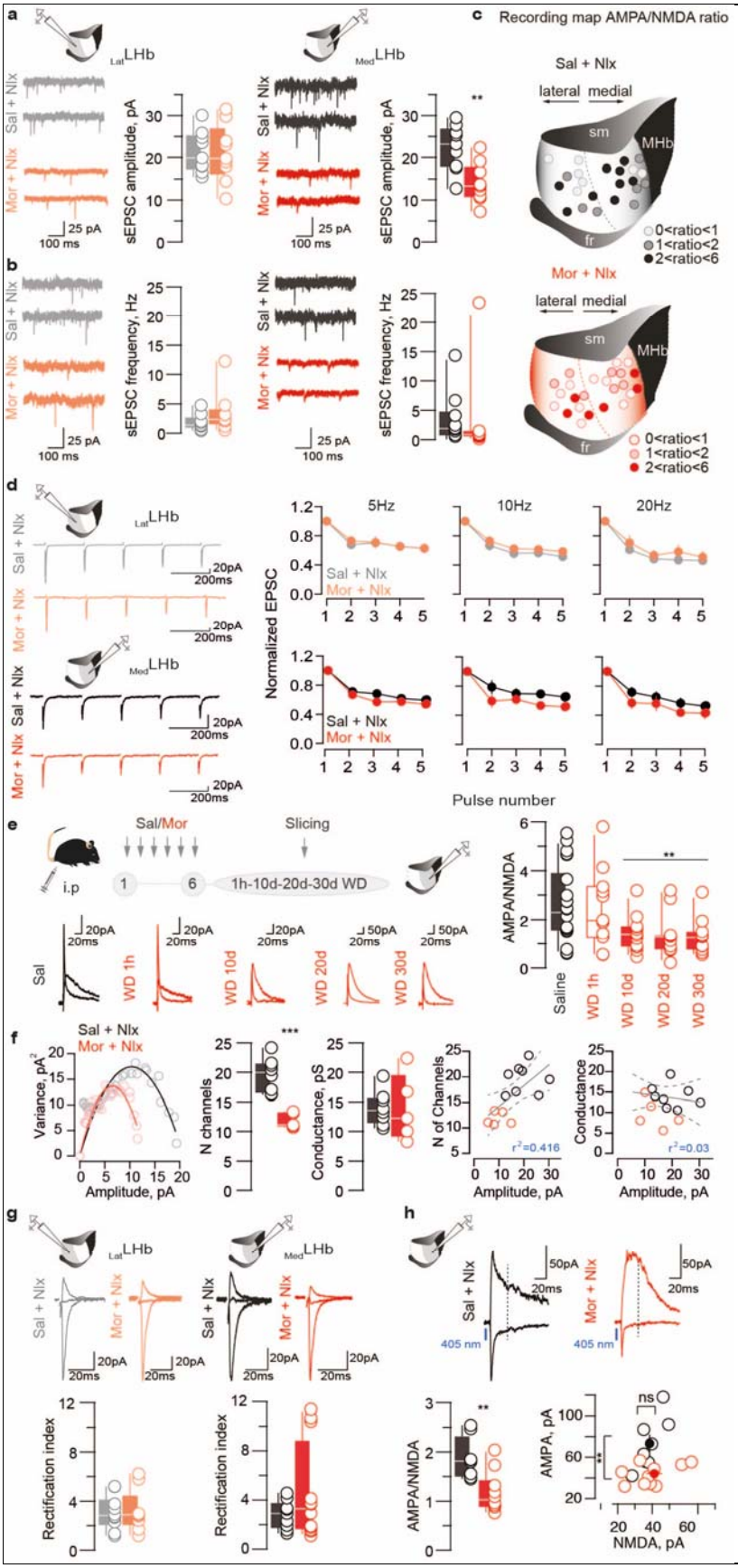
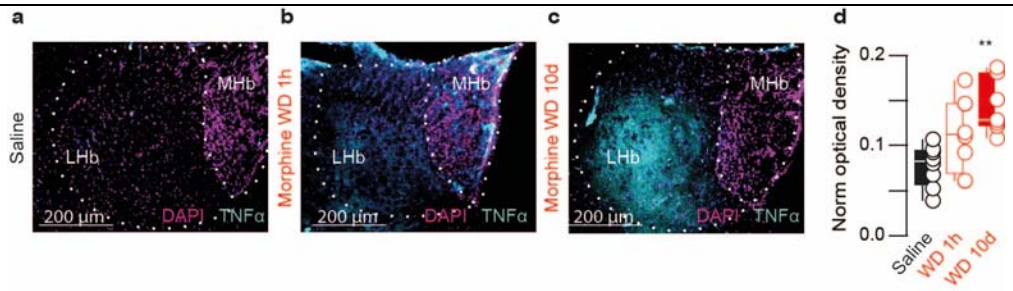

a
Lat LHb
Med LHb
Sal + Nlx
Mor + Nlx
sEPSC amplitude, pA
25 pA
100 ms
**
b
sEPSC frequency, Hz
c
Recording map AMPA/NMDA ratio
lateral medial
sm
MHb
fr
0<ratio<1
1<ratio<2
2<ratio<6
d
5Hz
10Hz
20Hz
Normalized EPSC
Pulse number
20pA
200ms
e
Sal/Mor
Slicing
i.p
1h-10d-20d-30d WD
Sal
WD 1h
WD 10d
WD 20d
WD 30d
AMPA/NMDA
Saline
f
Variance, pA²
Amplitude, pA
N channels
***
Conductance, pS
N of Channels
r²=0.416
Conductance
r²=0.03
g
Rectification index
h
405 nm
50pA
20ms
ns
AMPA, pA
NMDA, pA

Supplementary Figure 1

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

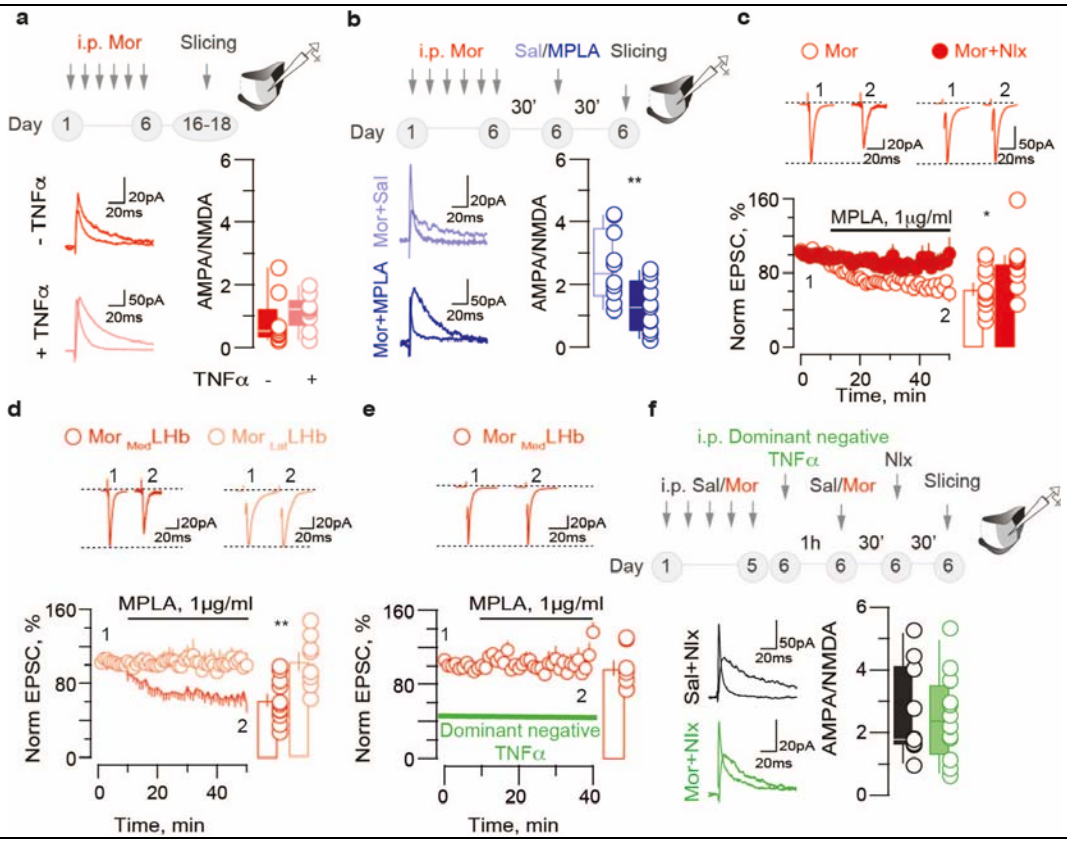
(a) Left: sample traces, box and scatter plots of sEPSCs amplitudes recorded in ^{Lat}LHb (saline+naloxone ($n_{cells/mice}$=9/3; gray) versus NP-MORwd ($n_{cells/mice}$=9/4; orange), two-sided t-test,, t_{16}=0.098, P=0.923). Right: same but sEPSCs were recorded in ^{Med}LHb (saline+naloxone ($n_{cells/mice}$=9/4; black) versus NP- MORwd ($n_{cells/mice}$=9/5; red), two-sided t-test, t_{16}=3.493, **P=0.003). (b) Left: sample traces, box and scatter plots of sEPSCs frequencies recorded in ^{Lat}LHb (saline+naloxone ($n_{cells/mice}$=9/4, gray) versus NP-MORwd ($n_{cells/mice}$=9/4, orange), two-sided t-test,, t_{16}=1.331, P=0.202). Right: same but sEPSCs were recorded in ^{Med}LHb (saline+naloxone ($n_{cells/mice}$=9/5, black) versus NP-MORwd ($n_{cells/mice}$=9/4,red), two-sided t-test, t_{16}=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 ^{Lat}LHb (saline+naloxone ($n_{cells/mice}$=10/2, gray) versus NP-MORwd ($n_{cells/mice}$=10/3, orange), 5Hz interaction factor $F_{(4, 36)}$=0.227, P=0.921; 10Hz interaction factor $F_{(4,36)}$=0.251, P=0.907; 20Hz interaction factor $F_{(4,36)}$=0.573, P=0.683 two-way ANOVA Repeated Measures). Bottom: same but in ^{Med}LHb ($n_{cells/mice}$=10/3, saline+naloxone (black) versus NP- MORwd (red), 5Hz interaction factor $F_{(4,36)}$=0.1.183, P=0.334; 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) Spontaneous withdrawal timeline. AMPAR:NMDAR ratios from ^{Med}LHb 1 hour, 10, 20 or 30 days post-saline or MOR (saline 1 hour and 10 days pooled ($n_{mice/cells}$=6/22; black) versus MORwd 1 hour ($n_{mice/cells}$=5/11; open red) and MOR 10 ($n_{mice/cells}$=3/12), 20 ($n_{mice/cells}$=3/11) and 30 days withdrawal ($n_{mice/cells}$=3/11; red), $F_{(4, 62)}$=3.90 one-way ANOVA, **P=0.007). (f) Example of peak-scaled NSFA of ^{Med}LHb 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, $n_{cells/mice}$=5/4; MORwd, $n_{cells/mice}$=8/5; N of channels, two-sided t-test, t_{11}=5.67, ***P=0.0001, $r^2_{(N\text{-}Channels)}$ = 0.416; *P=0.017; Conductance, t_{11}=0.006, P=0.99, $r^2_{(Conductance)}$ = 0.03, P=0.55). (g) Left: sample traces, box and scatter plots for rectification index calculated from AMPAR EPSCs recorded at -70, 0 and 40 mV in ^{Lat}LH (saline+naloxone ($n_{cells/mice}$=9/7, gray) versus NP- MORwd ($n_{cells/mice}$=10/7, orange), two-sided t-test, t_{17}=0.210, P=0.836). Right same but recordings in ^{Med}LHb (saline+naloxone ($n_{cells/mice}$=12/8, black) versus NP- MORwd ($n_{cells/mice}$=9/5, red), two-sided t-test, t_{19}=1.292, P=0.212). (h) Sample traces, box and scatter plots of AMPAR:NMDAR ratios recorded in ^{Med}LHb via 405 nm laser-assisted uncaging of MNI-glutamate, 500μM (saline+naloxone ($n_{cells/mice}$=8/2, black) versus NP-MORwd ($n_{cells/mice}$=10/3, red), two-sided t-test, t_{16}=3.521, **P=0.003). Bottom right: Absolute AMPAR versus absolute NMDAR 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_{16}=3.536, **P=0.003; NMDA, two-sided t-test, t_{16}=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

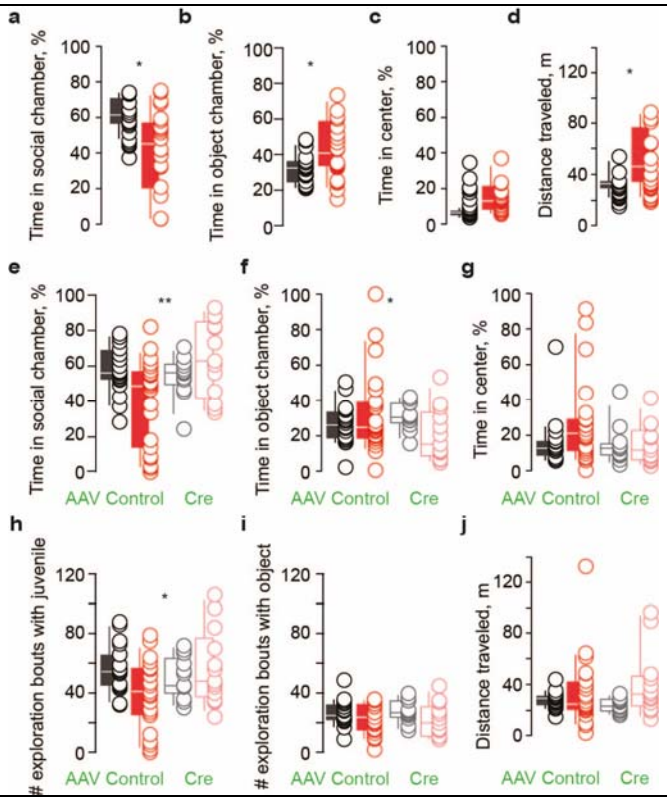
(a) TNFα (cyan) and DAPI (magenta) immunostaining in slices from saline-treated, (b) MOR-treated (sacrificed 1 hour after the last MOR injection) and (c) animals in spontaneous MORwd (10-13 days post last MOR injection). (d) Normalized LHb TNFα optical density in saline (black), MOR (open red) and spontaneous 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 ^{Med}LHb slices incubated with (+) or without (–) exogenous TNFα from spontaneous MORwd mice (10 days). (MORwd -TNFα (red) versus +TNFα (pink), $n_{cells/mice}$=9/4, two-sided t-test, t_{16}=0.986, P=0.339). (b) ^{Med}LHb AMPAR:NMDAR ratios from saline or MPLA-injected MOR-treated mice (MOR/saline ($n_{mice/cells}$=3/10; shaded blue) versus MOR/MPLA ($n_{mice/cells}$=4/11; dark blue), two-sided t-test, t_{19}=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 ($n_{mice/cells}$=4/11 ; 63.93 ± 7.06%; open red), NP-MORwd ($n_{mice/cells}$=3/10 cells; 91.63 ± 8.86%; filled red), MOR versus NP-MORwd, two-sided t-test, t_{19}=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 (1μg/ml) on evoked AMPAR-EPSCs (baseline (1) vs 30 min post-MPLA (2)) recorded in ^{Lat}LHb (open orange) or ^{Med}LHb (open red) in slices obtained from morphine-treated animals ($n_{cells/mice}$=8/4, morphine ^{Lat}LHb 103.98 ± 10.13; $n_{cells/mice}$=11/5, morphine ^{Med}LHb 63.31 ± 7.06%; morphine ^{Lat}LHb versus morphine ^{Med}LHb, two-sided t-test, t_{17}=3.406, **P=0.003). Note that the data set for ^{Med}LHb 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 (1μg/ml) on evoked AMPAR-EPSCs (baseline (1) vs 30 min post-MPLA (2)) in the presence of TNFα dominant negative peptide (XENP1595, 6mg/1ml) recorded in ^{Med}LHb in slices obtained from morphine-treated animals ($n_{cells/mice}$=7/2, 98.88 ± 8.23 %, two-sided t-test, t-test, t_6=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 ^{Med}LHb (saline+naloxone ($n_{cells/mice}$=10/3, black) versus NP-MORwd ($n_{cells/mice}$=12/3, green), two-sided t-test, t-test, t_{20}=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 pink), 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 21.69 ± 3.16 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.