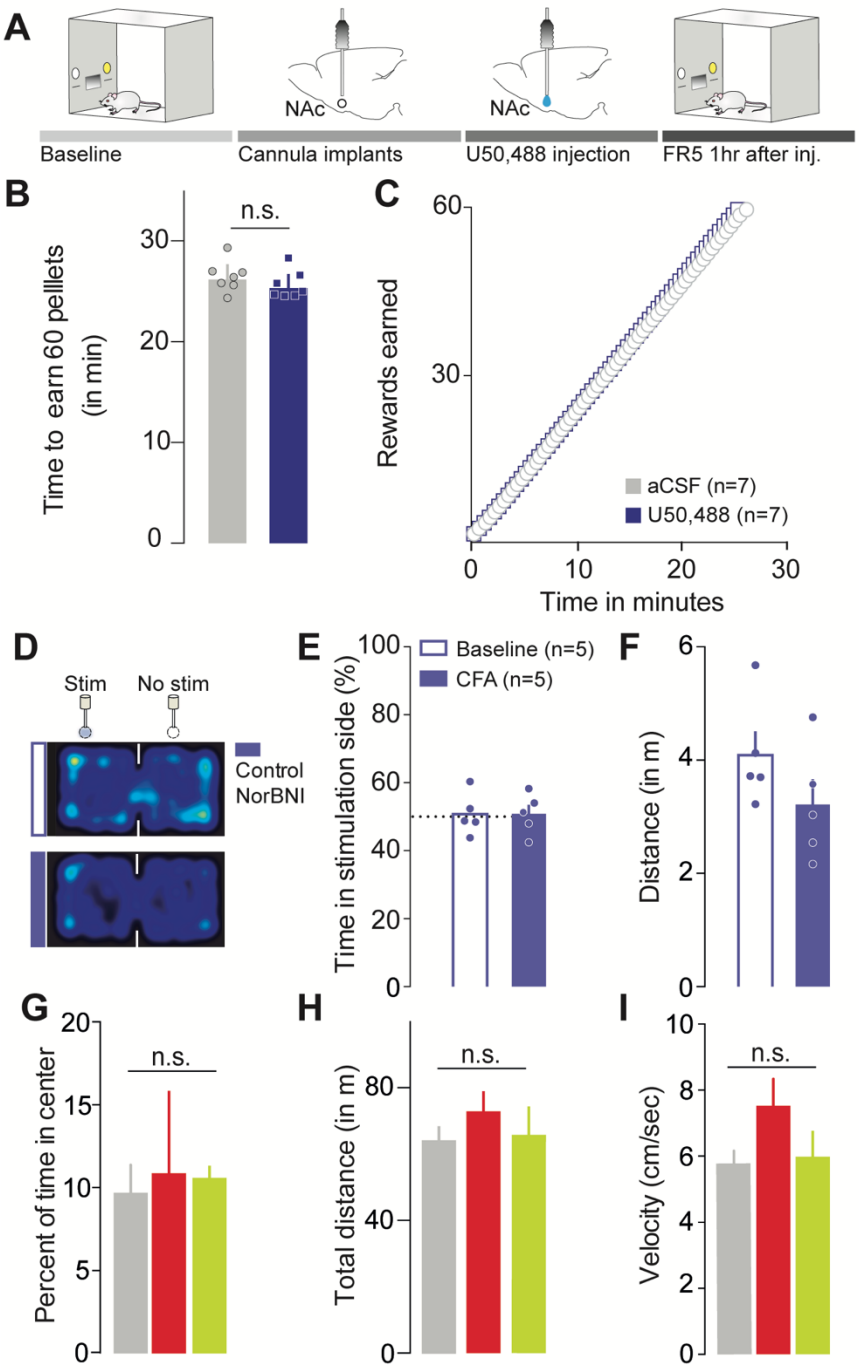


Figure S1: Pharmacological stimulation of KORs and norBNI pretreatment do not impact general behavior or pain-induced anxiety. Related to Figures 1 and 2.

(A) Schematic representation of the behavioral methodology for fixed ratio sucrose self-administration following KORs stimulation in the NAcShCS. Briefly, animals were trained to self-administer sucrose pellets. Then, rats were injected with U50,488 (1µg/side) in the NAcShCS and exposed to a fixed-ratio 5 schedule of reinforcement an hour later. (B) No difference in time necessary to obtain 60 pellets in a FR5 schedule

was observed in between U50,488 and aCSF injected rats (two-tailed t-test: p=0.3089) or (C) in their pattern of self-administration indicating that KOR stimulation in the NAcShCS does not affect general behavior in rats. (D) Representative heat maps of the animal activity during the 20 minutes RTPT. (E) norBNI does not change the percentage of time spent in the photo-stimulated chamber in channelrhodopsin negative control animals. No aversion could be observed in either baseline (n=5, one sample t-test compared to 50%: p=0.7364) or in inflammatory pain conditions (n=5, one sample t-test compared to 50%: p=0.1636). (F) norBNI treatment does not impair general locomotion as compared to other groups tested in RTPT (two-way ANOVA for repeated measures: time: $F_{1, 35}$ = 36.74, p<0.0001; treatment: $F_{3, 35}$ = 0.3845, p= 0.7648; interaction: $F_{3, 35}$ = 0.1192, p=0.9482). (G) No changes in anxiety related behavior (one-way ANOVA: $F_{2, 22}$= 1.172, p=0.3284), (H) in the total distance ran (one-way ANOVA: $F_{2, 22}$= 0.2599, p=0.7735) or (I) in mice velocity (one-way ANOVA: $F_{2,22}$= 1.570, p=0.2314) were observed during the open field test performed three days after inflammatory pain induction.

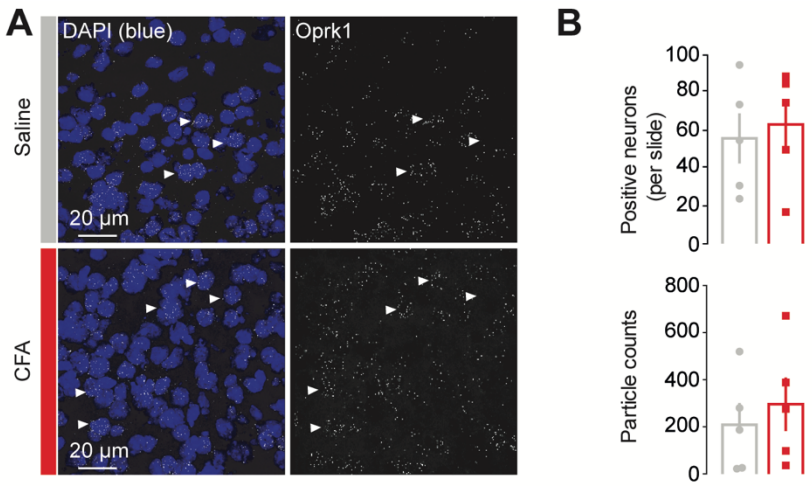


Figure S2: Inflammatory pain does not upregulate Oprk1 mRNA expression 48 hours after its induction. Related to Figure 3.

(A) Representative pictures of Oprk1 mRNA expression in the NAcShCS in either saline- or CFA- injected mice (Blue: DAPI, White: Oprk1 mRNA; pictures obtained using a 63X objective). (B) Oprk1 mRNA is not expressed in more neurons (upper panel: Mann-Whitney test for unpaired values; n=5, p=0.8016) and its overall content in the vNAcSh is not enhanced by the presence of inflammatory pain (lower panel: Mann-Whitney test for unpaired values; n=5, p=0.5317).

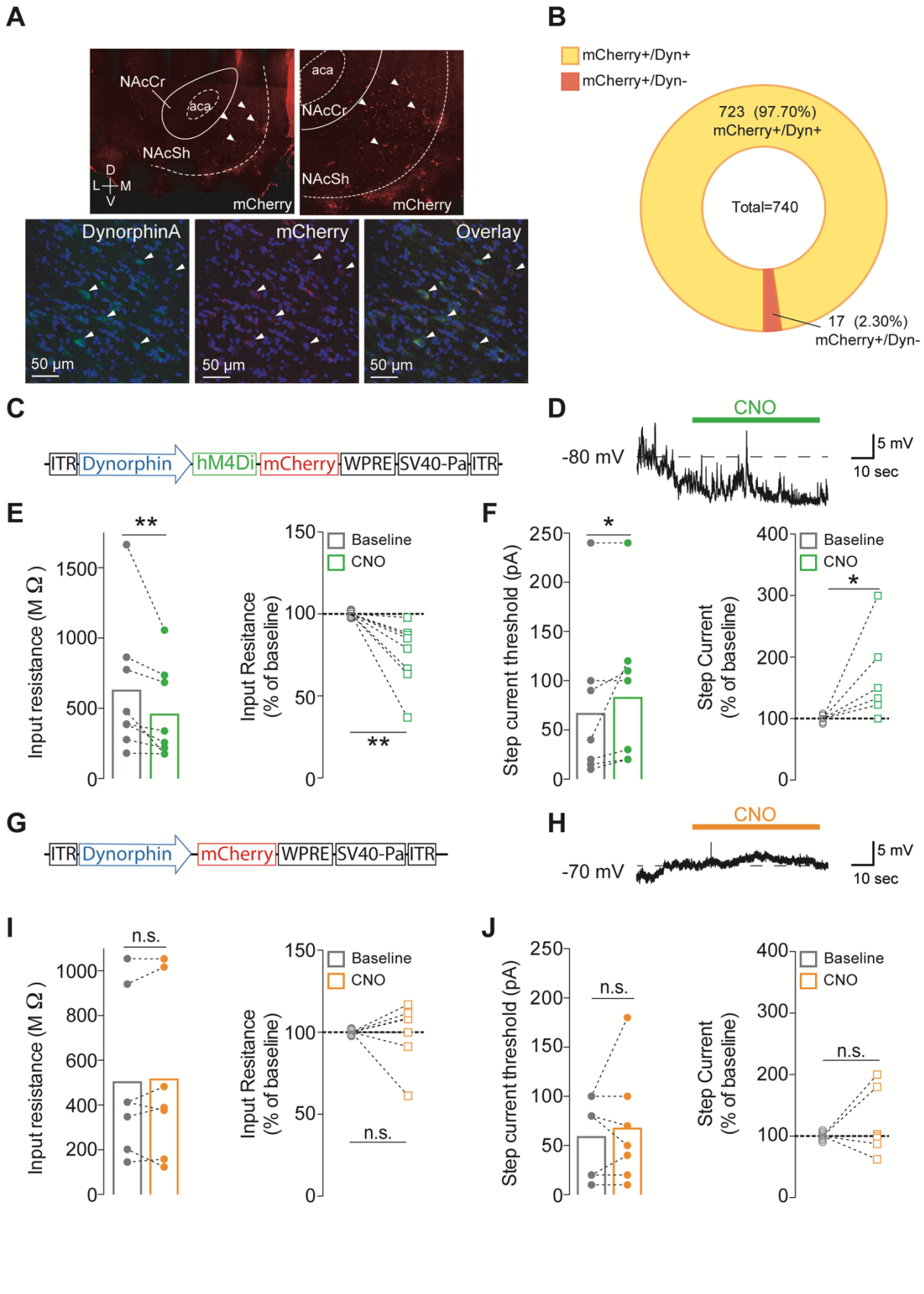
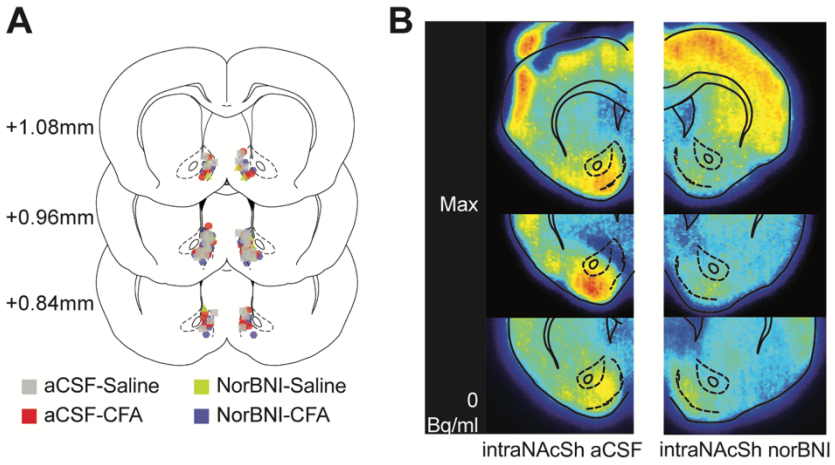
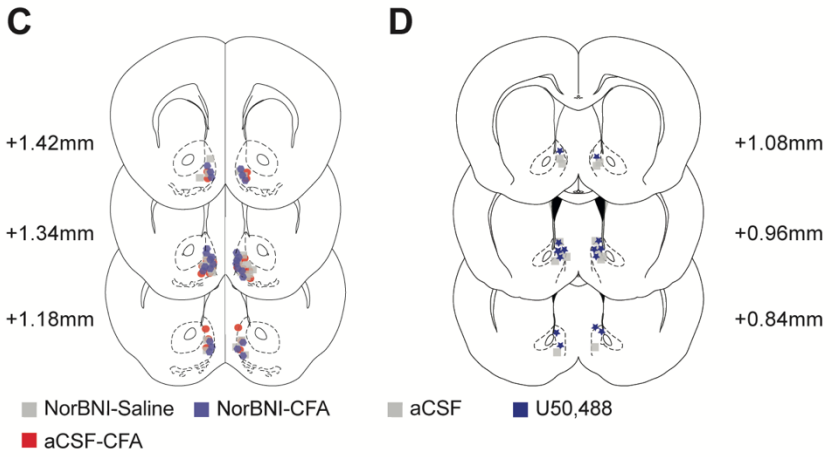
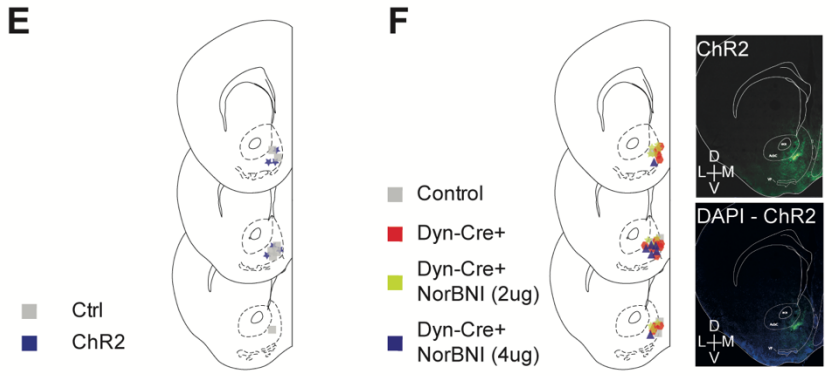

A
NAcCr
aca
NAcSh
D
L
M
V
mCherry
aca
NAcCr
NAcSh
mCherry
DynorphinA
mCherry
Overlay
50 μm
50 μm
50 μm
B
mCherry+/Dyn+
mCherry+/Dyn-
723 (97.70%)
mCherry+/Dyn+
Total=740
17 (2.30%)
mCherry+/Dyn-
C
ITR
Dynorphin
hM4Di
mCherry
WPRE
SV40-Pa
ITR
D
CNO
-80 mV
5 mV
10 sec
E
Input resistance (M Ω)
**
Baseline
CNO
Input Resitance (% of baseline)
**
F
Step current threshold (pA)
*
Step Current (% of baseline)
*
G
ITR
Dynorphin
mCherry
WPRE
SV40-Pa
ITR
H
CNO
-70 mV
5 mV
10 sec
I
Input resistance (M Ω)
n.s.
Input Resitance (% of baseline)
n.s.
J
Step current threshold (pA)
n.s.
Step Current (% of baseline)
n.s.

Figure S3: HSV-Dyn2.0-hM4Di-IRES-mCherry specifically expresses in dynorphin containing neurons and CNO-induced stimulation reduces infected neurons excitability. Related to Figure 4.

(A) Representative pictures the area of viral expression and a zoomed-in confirmation of HSV-Dyn2.0-hM4Di-IRES-mcherry (red) and dynorphin A (green) co expression. (B) Representative chart of mCherry-dynorphinA co-localization. Out of 740 mCherrry positive neurons ($n_{cells/animals}$=740/4), a proxy for GiDREADDs expression, 727 neurons (97.7%) co-express dynorphinA. (C) Schematic of viral HSV-DynA-hM4Di-IRES-mCherry construct. (D) Representative trace of membrane potential hyperpolarization after CNO bath application. (E) Input resistance is significantly decreased ($n_{cells/animals}$=8/ 6 animals, two-tailed Wilcoxon match-paired test; p=0.0078) while (F) step current threshold is significantly increased ($n_{cells/animals}$=8/6, two-tailed Wilcoxon match-paired test; p= 0.0313) after CNO bath application (10 µM) in hM4Di-expressing neurons, a measure for decreased neuronal excitability potential. Both left panels represent raw obtained values while the right panels show the corrected percent change after CNO bath application in input resistance and step current threshold to each single cell baseline. (G) Schematic of viral HSV-DynA-IRES-mCherry construct. (H) Representative trace of membrane potential in fluorophore-only expressing control neurons after CNO bath application. (I) Neither input resistance ($n_{cells/animals}$=7/3, two-tailed Wilcoxon match-paired test; p=0.8125) nor (J) step current threshold ($n_{cells/animals}$=7/3, two-tailed Wilcoxon match-paired test; p= 0.6875) is impacted by CNO bath application (10 µM) in control construct infected neurons. Both left panels represent raw obtained values while the right panels show the corrected percent change after CNO bath application in input resistance and step current threshold to each single cell baseline.


A
+1.08mm
+0.96mm
+0.84mm
aCSF-Saline
NorBNI-Saline
aCSF-CFA
NorBNI-CFA
B
Max
0
Bq/ml
intraNAcSh aCSF
intraNAcSh norBNI


C
+1.42mm
+1.34mm
+1.18mm
NorBNI-Saline
NorBNI-CFA
aCSF-CFA
D
+1.08mm
+0.96mm
+0.84mm
aCSF
U50,488


E
Ctrl
ChR2
F
Control
Dyn-Cre+
Dyn-Cre+
NorBNI (2ug)
Dyn-Cre+
NorBNI (4ug)
ChR2
DAPI - ChR2
D
L
M
V

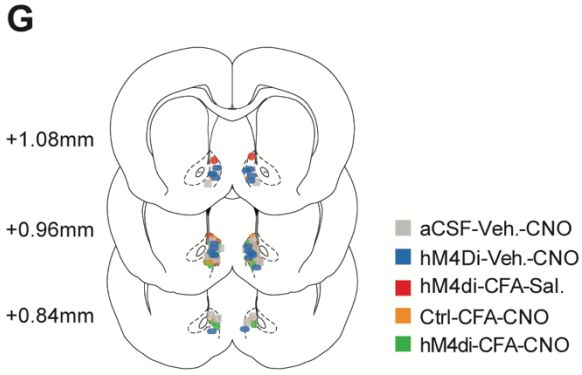

G
+1.08mm
+0.96mm
+0.84mm
aCSF-Veh.-CNO
hM4Di-Veh.-CNO
hM4di-CFA-Sal.
Ctrl-CFA-CNO
hM4di-CFA-CNO

Figure S4: Histology. Related to Figures 1, 2 and 4.

(A) Schematic representation of histological verification for rats used in Figure 1A-D. (B) Coronal section representing [^{11}C]-LY2795050 incorporation on slices from animals pretreated with either aCSF or norBNI in the NAcShCS. (C) Schematic representation of histological verification for rats used in Figure 1I-L. (D) Schematic representation of histological verification for mice used in Figure 1E-H. (E) Schematic representation of histological verification for mice used in Figure 2A-D. (F) Schematic representation of histological verification for mice used in Figure 2E-H with representative coronal section pictures of channelrhodopsin expression. (G) Schematic representation of histological verification for DREADDs-injected rats used in Figure 4.