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Supplemental information

Endocannabinoid release at ventral hippocampal-amygdala synapses regulates stress-induced behavioral adaptation

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SUPPLEMENTAL FIGURES

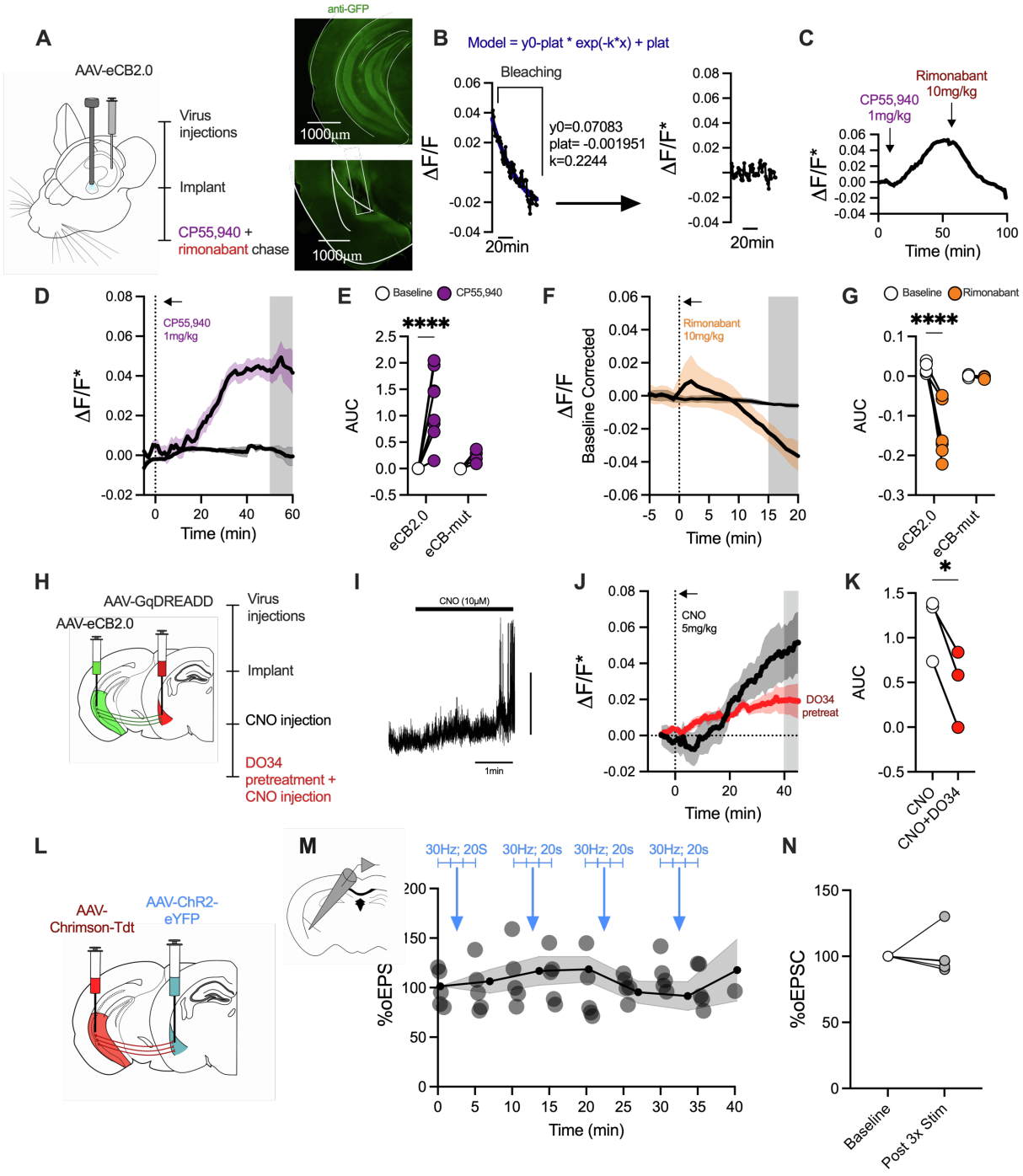

Figure S1: Validation of eCB2.0 biosensor at vHPC-BLA circuitry (See also Figure 1)

A) Schematic of experimental design. Mice were injected with the eCB2.0 (AAV9-hSyn-GRABeCB2.0) or the eCB-mut (AAV9-hSyn-GRABeCB2.0-mutant) into the vHPC and implanted with a fiber optic above the BLA. (Right) Representative image of eCB2.0 in the vHPC and fiber optic implant above the BLA; scale bar represents 1000μm
B) Exponential decay model used to correct for photobleaching in long-term recordings. Data that are corrected using model are represented as ΔF/F*
C) Representative trace of ΔF/F* following pharmacological manipulation of CB1R in eCB2.0 injected mouse
D) Time course of ΔF/F* following administration of CP-55,940 (1mg/kg; i.p.) in eCB2.0 (purple) or eCB-mut (gray)
E) Quantification of area under the curve (AUC) from (D) in eCB2.0 (n=8) or control (eCB-mut; n=4)
F) Time course of ΔF/F following administration of CB1R inverse agonist, rimonabant (10mg/kg; i.p.) in mice that express eCB2.0 (orange) or eCB-mut (gray)
G) Quantification of AUC from (F) in eCB2.0 (n=6) or eCB-mut mice (n=3)
H) Experimental design for chemogenetic BLA activation
I) Resulting depolarization and activation of BLA neurons following CNO wash-on using whole-cell patch clamp electrophysiology in acute brain slices
J) Time course of ΔF/F* following administration of CNO (5mg/kg; i.p.) in naïve mice (black) or mice pretreated with DAGL inhibitor, DO34 (red)
K) Quantification of AUC comparing effects of CNO in naïve or DO34-treated mice (n=3)
L) Methodology for assessing DSE *ex vivo* (n=6 cells from 2 mice)
M) oEPSC amplitude (%baseline) following 3 rounds of optogenetic stimulation of the BLA at 30Hz for 20s demonstrating individual points, as well as average across time
N) Average %oEPSC amplitude (%baseline) after 3 rounds of BLA stimulation (>30min)

Data were analyzed by 2-Way ANOVA (E,G) or paired student's t-test (K, N); n=number of mice unless otherwise stated.

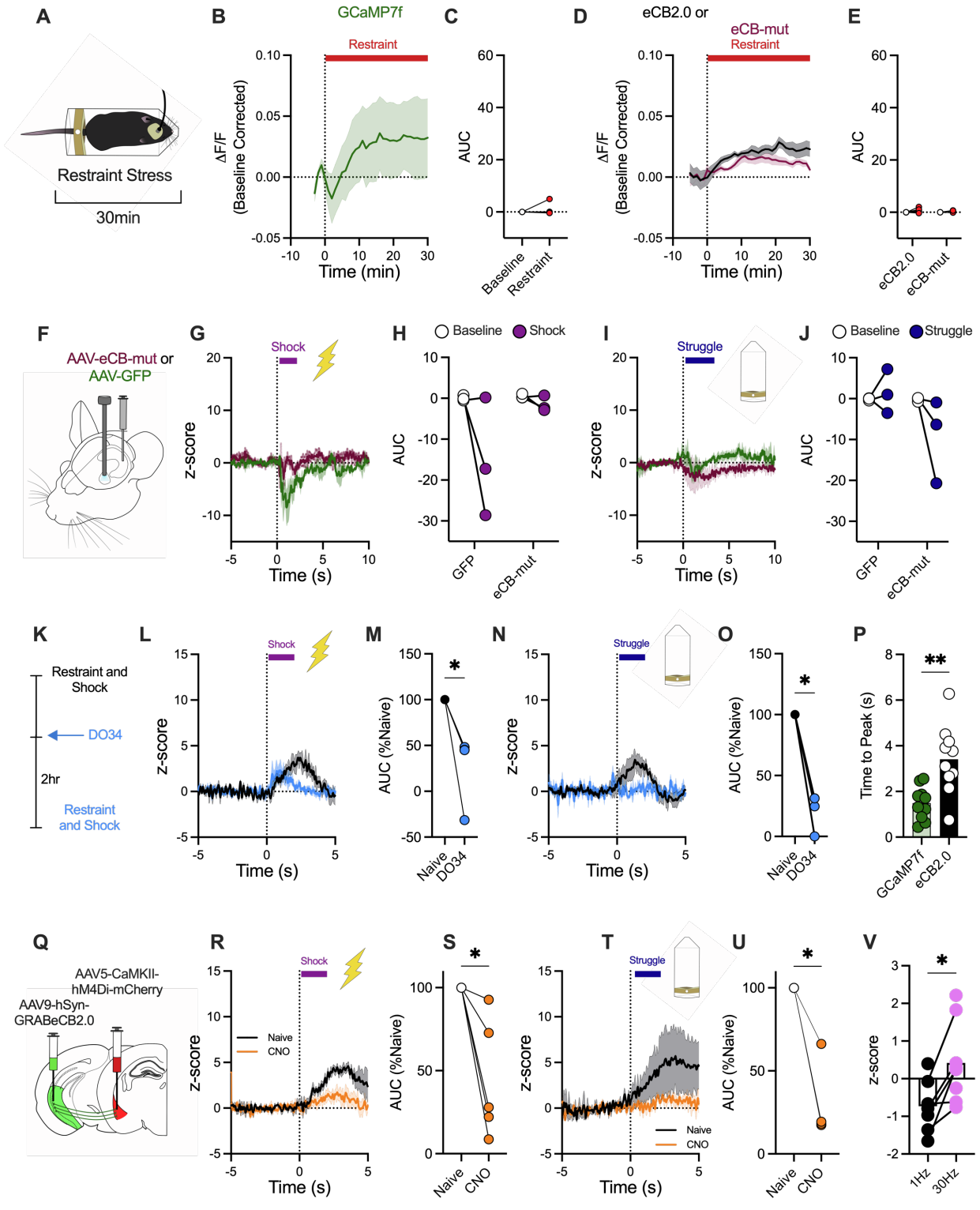
A
Restraint Stress
30min
B
GCaMP7f
Restraint
ΔF/F (Baseline Corrected)
Time (min)
C
AUC
Baseline
Restraint
D
eCB2.0 or
eCB-mut
Restraint
ΔF/F (Baseline Corrected)
Time (min)
E
AUC
eCB2.0
eCB-mut
F
AAV-eCB-mut or
AAV-GFP
G
Shock
z-score
Time (s)
H
Baseline
Shock
AUC
GFP
eCB-mut
I
Struggle
z-score
Time (s)
J
Baseline
Struggle
AUC
GFP
eCB-mut
K
Restraint and Shock
DO34
2hr
Restraint and Shock
L
Shock
z-score
Time (s)
M
AUC (%Naive)
Naive
DO34
N
Struggle
z-score
Time (s)
O
AUC (%Naive)
Naive
DO34
P
Time to Peak (s)
GCaMP7f
eCB2.0
Q
AAV5-CaMKII-hM4Di-mCherry
AAV9-hSyn-GRABeCB2.0
R
Shock
Naive
CNO
z-score
Time (s)
S
AUC (%Naive)
Naive
CNO
T
Struggle
Naive
CNO
z-score
Time (s)
U
AUC (%Naive)
Naive
CNO
V
z-score
1Hz
30Hz

Figure S2: eCB production and vHPC$^{eCB2.0}$ signal in response to stress requires active sensor expression, is dependent on 2-AG synthesis, and requires BLA activity (See also Figure 2)

A) Mice were injected with GCaMP7f in the BLA, or eCB2.0 or eCB-mut in the vHPC. All mice were implanted with a fiber optic in the BLA. Mice were exposed to restraint stress for 30 minutes.
B) Time course of ΔF/F in GCaMP7f mice following onset of restraint stress (n=4)
C) Average AUC following restraint session onset
D) Time course of ΔF/F in eCB2.0 (black) or eCB-mut (purple) mice following onset of restraint stress session (n=8) and eCB-mut mice (n=5)
E) AUC following restraint onset in eCB2.0
F) Schematic of experimental design. Mice were injected with a virus expressing GFP or eCB-mut in the vHPC and implanted with a fiber optic above the BLA
G) Time average of z-score ΔF/F in GFP (green; n=3) or eCB-mut (purple; n=3) mice following 2s footshock exposure
H) Average AUC following footshock
I) Time course of z-score in GFP or eCB-mut mice following struggle onset
J) AUC following struggle behavior
K) Experimental design for DO34 pretreatment experiments
L) Time course of z-score in naïve (black) and DO34-pretreated (green) mice exposed to footshock
M) AUC following footshock stress, presented as %naive AUC (n=4)
N) Time course of z-score in naïve (black) and DO34-pretreated (green) mice after struggle behavior onset
O) Quantification of AUC (%naive) following struggle onset (n=3)
P) Quantification of the time for the z-score to reach its peak following onset of footshock or struggle behavior during restraint (GCaMP: n=6; eCB2.0: n=5)
Q) Experimental design of chemogenetic BLA inhibition
R) Time course of z-score of ΔF/F following footshock in naïve mice or mice pretreated with CNO
S) Quantification of AUC (%Naive) following footshock comparing naïve mice to CNO-pretreated mice (n=5)
T) Time course of z-score of ΔF/F following whole body struggle behavior in naïve or CNO-pretreated mice
U) AUC (%Naive) following struggle behavior after CNO pretreatment (n=4)
V) Average z-score following 5 seconds of BLA stimulation at 1Hz or 30Hz (n=7)

Data were analyzed by paired student's t-test (C,E,M,O,S,U-V) or 2-Way ANOVA (H,J), or unpaired student's t-test (P). n=number of mice unless otherwise stated.

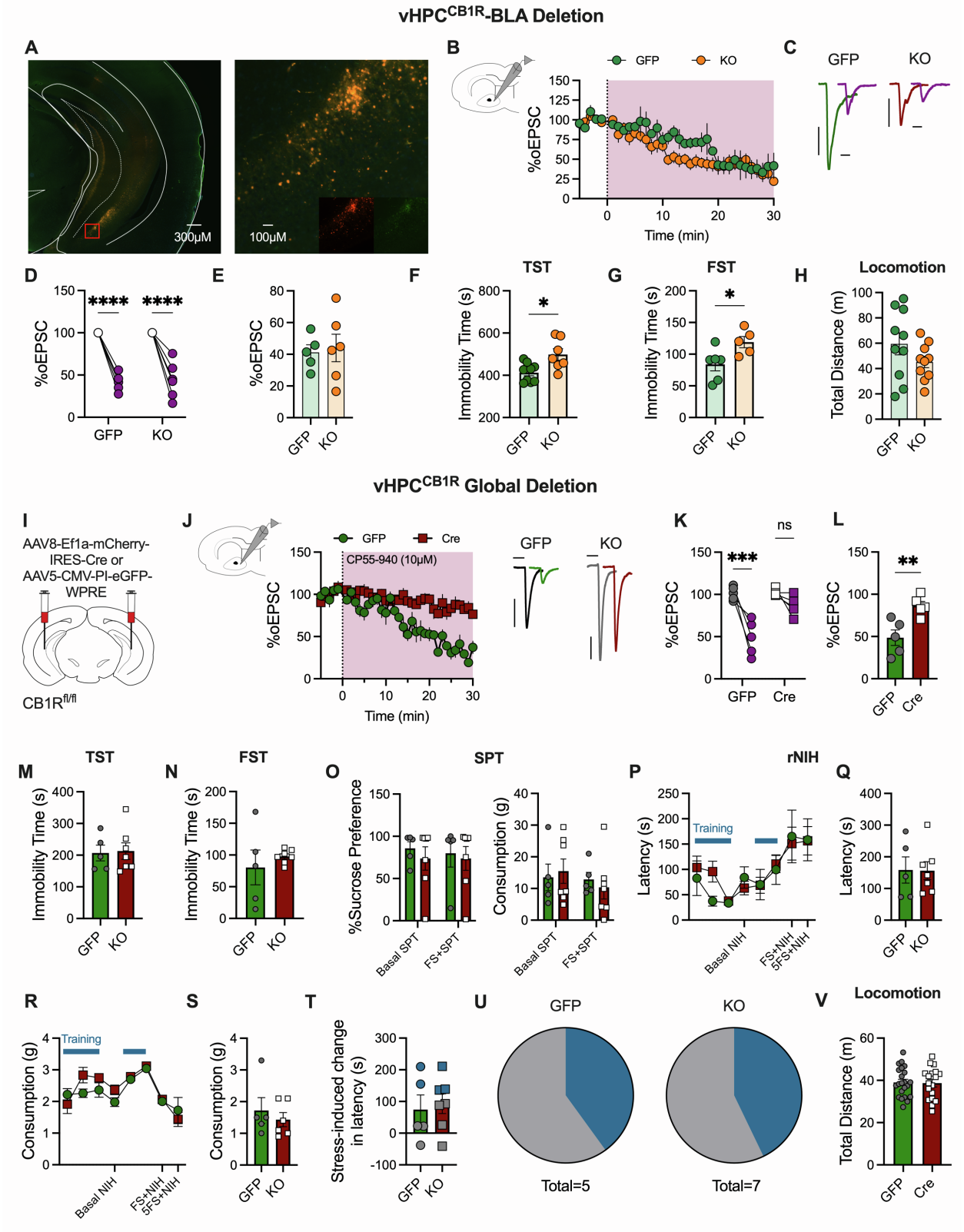
vHPC^CB1R-BLA Deletion
A
300μM
100μM
B
GFP
KO
%oEPSC
Time (min)
C
GFP
KO
D
%oEPSC
****
****
GFP
KO
E
%oEPSC
GFP
KO
F
TST
Immobility Time (s)
*
GFP
KO
G
FST
Immobility Time (s)
*
GFP
KO
H
Locomotion
Total Distance (m)
GFP
KO
vHPC^CB1R Global Deletion
I
AAV8-Ef1a-mCherry-IRES-Cre or AAV5-CMV-PI-eGFP-WPRE
CB1R^fl/fl
J
GFP
Cre
CP55-940 (10μM)
%oEPSC
Time (min)
GFP
KO
K
ns
***
%oEPSC
GFP
Cre
L
**
%oEPSC
GFP
Cre
M
TST
Immobility Time (s)
GFP
KO
N
FST
Immobility Time (s)
GFP
KO
O
SPT
%Sucrose Preference
Basal SPT
FS+SPT
Consumption (g)
Basal SPT
FS+SPT
P
rNIH
Training
Latency (s)
Basal NIH
FS+NIH 5FS+NIH
Q
Latency (s)
GFP
KO
R
Training
Consumption (g)
Basal NIH
FS+NIH 5FS+NIH
S
Consumption (g)
GFP
KO
T
Stress-induced change in latency (s)
GFP
KO
U
GFP
KO
Total=5
Total=7
V
Locomotion
Total Distance (m)
GFP
Cre

Figure S3: vHPC^{CB1R}-BLA KO has circuit-specific effects on stress adaptation (See Figure 3)

A) Representative merged images of INTRSCT viral strategy. Insets: red is Flpo-mCherry and green is fDIO-Cre; scale bar represents 300µm (left) and 100µm (right)
B) Ex vivo electrophysiological recordings in the nucleus accumbens (NAc) of vHPC^{CB1R}-BLA KO or control mice. vHPC-mediated oEPSC were stimulated and CP,55-940 (10µM) was bath applied. Time course demonstrated reduction in %oEPSC amplitude over time in both groups (5 cells from 3 mice GFP; 6 cells from 4 mice)
C) Representative traces before and after (purple) CP,55-940 wash-on in GFP and KO mice
D) Average %oEPSC amplitude reduction after CP,55940
E) Average maximal %oEPSC depression from 3B
F) Quantification of immobility time in tail suspension test (TST) (GFP: n=9; Cre: n=7)
G) Average immobility time in forced swim test (FST) (GFP: n=6; Cre: n=5)
H) Quantification of total distance travelled during open field test (n=10/group)
I) Experimental design for global vHPC^{CB1R} KO. A virus expressing Cre or GFP was injected bilaterally into the vHPC
J) Ex vivo electrophysiological recordings in the NAc. vHPC-mediated oEPSCs were stimulated. Time course of %oEPSC amplitude over time course of CP-55,940 wash-on. (Right) Representative traces before and after CP-55,940 application in GFP (top) and KO (bottom) mice (n= 5 cells per group from n=3 mice/group)
K) Average %oEPSC amplitude reduction after CP,55940 in Cre- and GFP-injected mice
L) Average maximal %oEPSC depression from 3J
M) Average immobility time during tail suspension test (TST) (GFP: n=5; Cre: n=7)
N) Average immobility time during forced swim test (FST) (GFP: n=5; Cre: n=7)
O) Average sucrose preference (left) and quantification of total consumption of sucrose solution and water (right) during sucrose preference test (SPT) (GFP: n=5; Cre: n=7)
P) Feeding latency of Ensure over several days of rNIH (GFP: n=5; Cre: n=7)
Q) Quantification of Ensure feeding latency following 5 days of footshock exposure
R) Total Ensure consumption during NIH and training
S) Average consumption of Ensure following 5 days of footshock
T) Average stress-induced change in latency comparing latency after 5 days of footshock exposure to naïve NIH latency
U) Proportion of stress susceptible and stress resilient mice based on stress-induced change in latency
V) Total distance travelled during open field test (GFP: n=22; Cre: n=18)

Data were analyzed by 2-Way ANOVA (D, K, O-P, R), unpaired student's t-test (E-H, L-N, Q, S-T, V), or Chi-squared (U) performed for analysis. n=number of mice unless otherwise stated.