

Figure S1, related to figure 2: Stimulation of BLA terminals in the pIPFC elicits a monosynaptic excitatory and disynaptic inhibitory current

- Effect of the AMPAR antagonist CNQX (20 μ M) on oEPSC amplitude at BLA-pIPFC synapses. Bath application of CNQX abolished oEPSCs ($n=3$, $N=2$).
- Effect of the Voltage Gated Sodium Channel blocker Tetrodotoxin (TTX)(500nM) on oEPSC amplitude at BLA-pIPFC synapses. Bath application of TTX abolished oEPSCs ($n=3$, $N=2$).
- Effect of the GABA_AR channel blocker picrotoxin (PTX)(50 μ M) on oEPSC amplitude at BLA-pIPFC synapses. Bath application of PTX did not affect oEPSCs, while subsequent addition of CNQX abolished oEPSCs ($n=3$, $N=2$).
- Schematic for voltage-clamp recordings of oEPSCs from L2/3 rAAV-positive (rAAV+) and rAAV-negative (rAAV-) neurons at -70mV (left) and -40mV (right).
- Optically evoked input/output curve from L2/3 rAAV+ and rAAV- neurons at -70mV. The BLA sends stronger excitatory input to reciprocally projecting L2/3 neurons ($n=11$, $N=5$) than non-reciprocally projecting neurons L2/3 neurons ($n=12$, $N=5$)
- Optically evoked input/output curve from L2/3 rAAV+ and negative neurons at -40mV. Stimulation of BLA terminals in the pIPFC elicits a monosynaptic excitatory and disynaptic inhibitory current, presumably through feed-forward inhibition. Both

excitatory and inhibitory responses are larger in L2/3 rAAV+ neurons (n=10, N=5) compared to rAAV- neurons at -40mV (n=9, N=5).

- (g) Schematic for voltage-clamp recordings of oEPSCs from L2/3 rAAV+ and rAAV- neurons at -70mV (left) and -40mV (right).
- (h) Optically evoked input/output curve from L5 rAAV+ and rAAV- neurons at -70mV. No difference in excitatory input to L5 rAAV+ (n=11, N=5) compared to rAAV- neurons (n=11, N=5) was observed.
- (i) Optically evoked input/output curve from L5 rAAV+ and rAAV- neurons at -40mV. Stimulation of BLA terminals in the pIPFC elicited a monosynaptic excitatory and disynaptic inhibitory current. No difference in excitatory or inhibitory response in L5 rAAV+ (n=7, N=5) compared to rAAV- neurons at -40mV was observed (n=7, N=5).

All error bars represent \pm SEM. “n” represents number of neurons, “N” represents number of mice. F and P values from 2-way ANOVA shown in relevant panels.

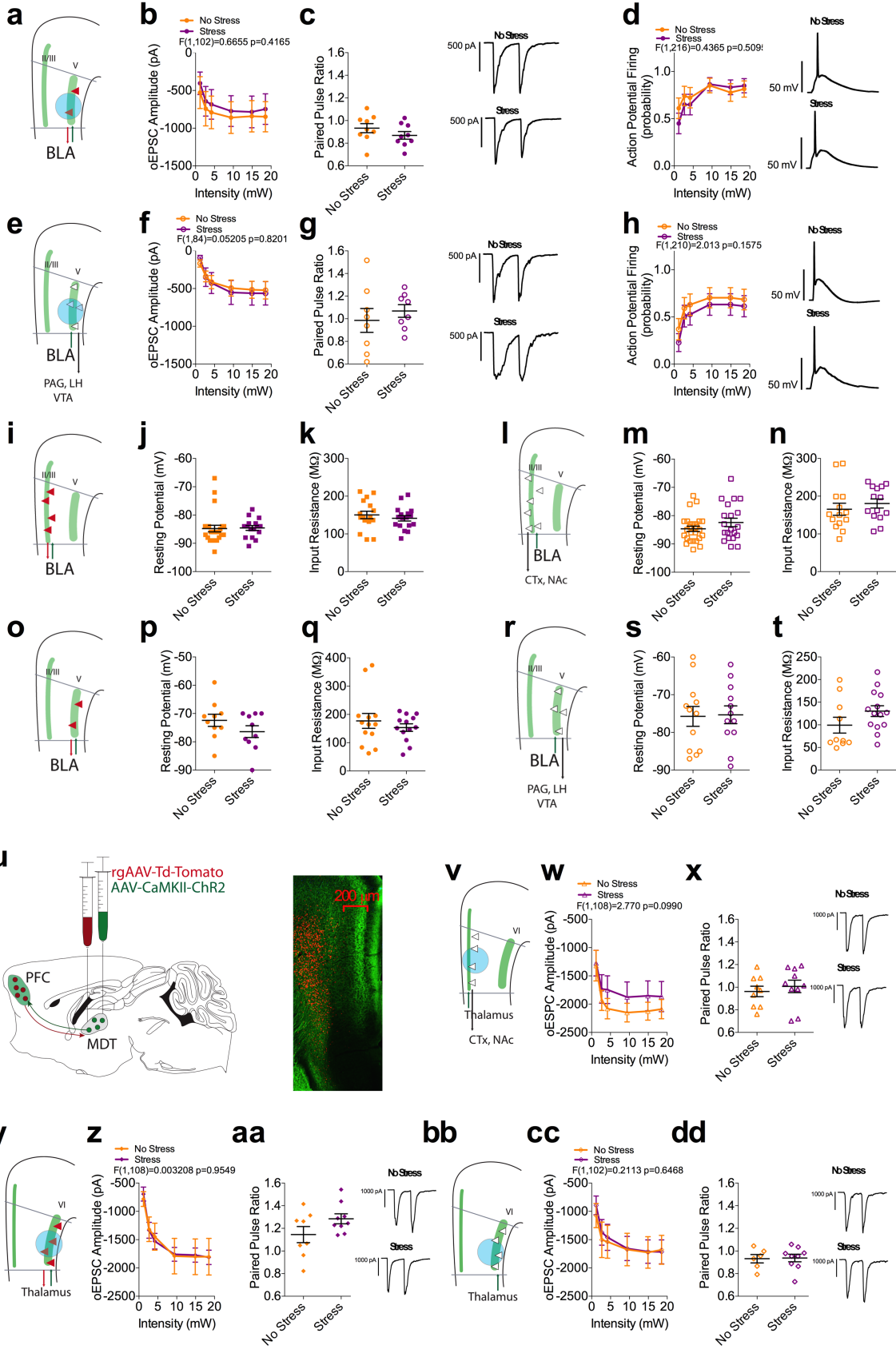


Figure S2, related to figure 2: Stress does not alter excitatory input from the BLA to pLPFC L5 neurons

- (a) Schematic for voltage clamp-recordings of oEPSCs from L5 rAAV+ neurons at -70mV.
- (b) Optically evoked input/output curve from L5 rAAV+ neurons from non-stressed (n=9, N=4) and stressed mice (n=10, N=4). Stress does not alter excitatory input from the BLA to pLPFC L5 rAAV+ neurons.
- (c) Effect of stress on paired pulse ratio (PPR) at BLA-L5 rAAV+ synapses (No Stress n=10, N=4; Stress n=9, N=4). Stress exposure does not alter PPR at BLA-L5 rAAV+ synapses (p=0.2413).
- (d) Optically evoked spiking in L5 rAAV+ neurons in non-stressed (n=18, N=8) and stressed mice (n=20, N=8). Stress exposure does not alter optically evoked spiking in L5 rAAV+ neurons.
- (e) Schematic for voltage clamp-recordings of oEPSCs from L5 rAAV- neurons at -70mV.
- (f) Optically evoked input/output curve from L5 rAAV- neurons from non-stressed (n=8, N=4) and stressed (n=8, N=5) mice. Stress does not alter excitatory input onto L5 rAAV- neurons.
- (g) Effect of stress on PPR at BLA-L5 rAAV- synapses (No Stress: n=8, N=5; Stress: n=8, N=4). Stress exposure does not alter PPR at BLA-L5 rAAV- synapses (p=0.4958).
- (h) Optically evoked spiking in L5 rAAV- neurons in non-stressed (n=18, N=8) and stressed mice (n=19, N=7). Stress exposure does not alter optically evoked spiking in L5 rAAV- neurons.
- (i) Schematic for current-clamp recordings from L2/3 rAAV+ neurons
- (j) Resting membrane potential (RMP) of L2/3 rAAV+ neurons from non-stressed (n=24, N=10) and stressed (n=20, N=8) mice. Stress does not alter RMP in L2 rAAV positive neurons (p=0.8866).
- (k) Input resistance (IR) of L2/e rAAV+ neurons from non-stressed (n=16, N=6) and stressed (n=17, N=5) mice. Stress does not alter IR in L2/3 rAAV+ neurons (p=0.4725).
- (l) Schematic for current-clamp recordings from L2/3 rAAV- neurons
- (m) RMP of L2/3 rAAV- neurons from non-stressed (n=26, N=8) and stressed (n=23, n=7) mice. Stress does not alter RMP in L2/3 rAAV- neurons (p=0.1852)
- (n) IR of L2/3 rAAV- neurons from non-stressed (n=14, N=6) and stressed (n=15, N=6) mice. Stress does not alter IR in L2/3 rAAV- neurons (p=0.4494).
- (o) Schematic for current clamp-recordings from L5 rAAV+ neurons at -70mV.
- (p) RMP of L5 rAAV+ neurons from non-stressed (n=10, N=5) and stressed (n=10, N=3) mice. Stress does not alter RMP in L5 rAAV+ neurons (p=0.2014).
- (q) IR of L5 rAAV+ neurons from non-stressed (n=12, N=6) and stressed (n=13, N=4) mice. Stress does not alter IR in L5 rAAV+ neurons (p=0.4312).
- (r) Schematic for current-clamp recordings of oEPSCs from L5 rAAV- neurons
- (s) RMP of L5 rAAV+ neurons from non-stressed (n=12, N=5) and stressed (n=12, N=5). Stress does not alter RMP in L5 rAAV- neurons (p=0.9073).
- (t) IR of L5 rAAV- neurons from non-stressed (n=10, N=6) and stressed (n=14, N=5) mice. Stress does not alter IR in L5 rAAV- neurons (p=0.1415).
- (u) Schematic for stereotaxic delivery of AAV5-CaMKII-ChR2(H147R)-eYFP and rAAV2-CAG-tdTomato into the MDT
- (v) Schematic for voltage clamp-recordings of oEPSCs from L6 rAAV+ neurons at -70mV.

- (w) Optically evoked input/output curve from L6 rAAV+ neurons from non-stressed (n=10, N=3) and stressed mice (n=10, N=3). Stress does not alter excitatory input to L6 rAAV+ neurons.
- (x) Effect of stress on PPR at MDT-L6 rAAV- positive synapses (No Stress: n=8, N=3; Stress: n=9 N=3). Stress exposure does not alter PPR at MDT-L6 rAAV+ synapses (p=0.1075).
- (y) Schematic for voltage clamp recordings of oEPSCs from L6 rAAV- neurons at -70mV.
- (z) Optically evoked input output curve from L6 rAAV- neurons from non-stressed (n=9, N=3) and stressed mice (n=10, N=3). Stress does not alter excitatory input from the MDT to L6 rAAV- neurons.
- (aa) Effect of stress on PPR at MDT-L6 rAAV- synapses (No Stress n=6, N=3; Stress: n=9, N=3). Stress exposure does not alter PPR at MDT-L6 rAAV- synapses (p=0.9048).
- (bb) Schematic for voltage clamp recordings of oEPSCs from L2/3 rAAV- neurons at -70mV.
- (cc) Optically evoked input output curve from L2/3 rAAV- neurons from non-stressed (n=9, N=3) and stressed (n=11, N=3) mice. Stress does not alter excitatory input to L2/3 rAAV- neurons.
- (dd) Effect of stress on PPR at MDT-L2/3 rAAV- synapses (No Stress: n=9, N=3; Stress: n=10 N=3). Stress exposure does not alter PPR at MDT-L2/3 rAAV- synapses (p=0.5496).

All error bars represent \pm SEM. “n” represents number of neurons, “N” represents number of mice. P values reported from two-tailed unpaired t-test (c,g,j,k,m,n,p,q,s,t,x,aa,dd). F and P values from ANOVA shown in relevant panels.

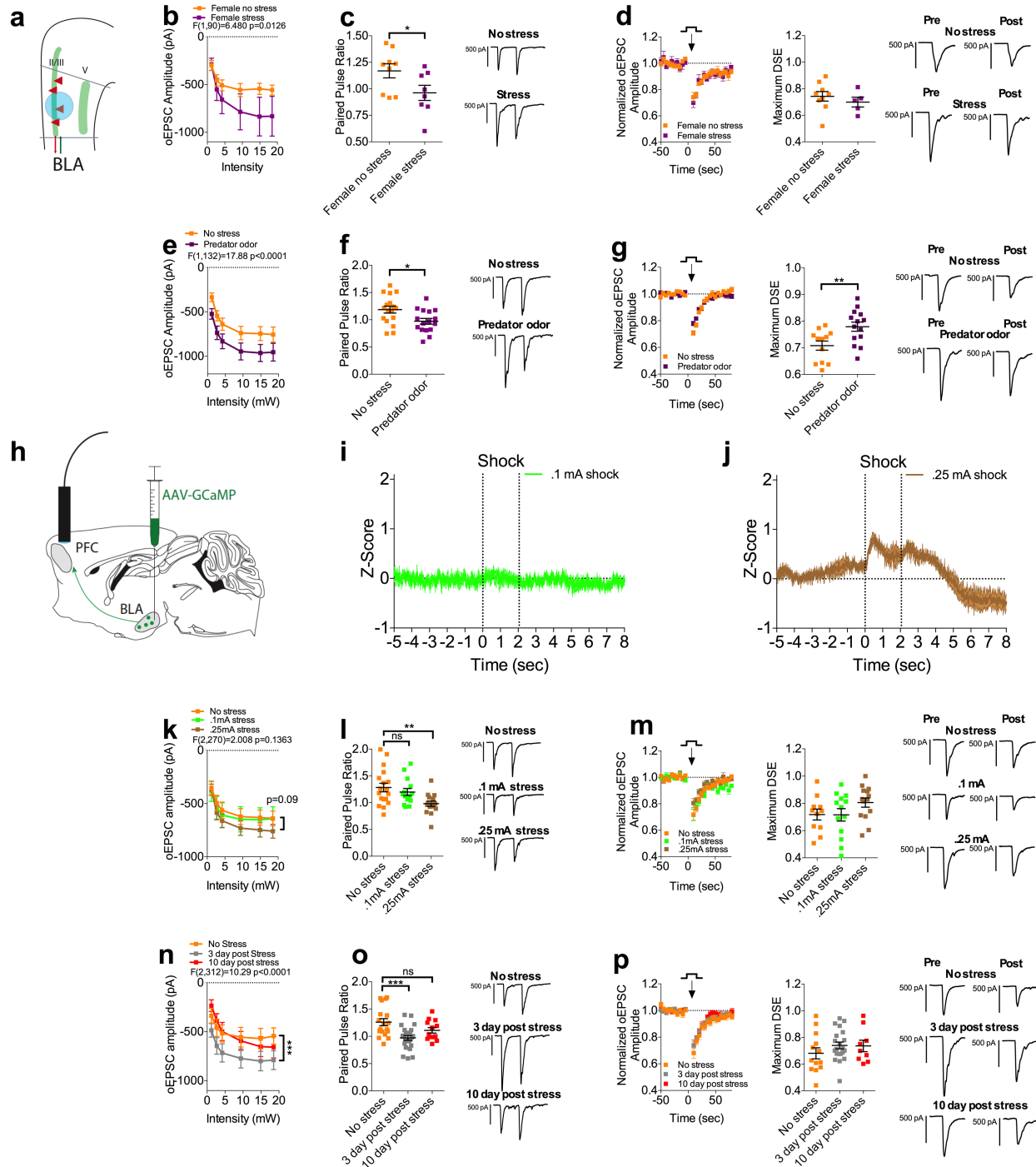


Figure S3, related to figure 2: Dose response, time course, and generalizability of stress induced presynaptic strengthening at BLA-L2 rAAV positive synapses

(a) Schematic for voltage-clamp recordings of oEPSCs from L2/3 rAAV+ neurons.

(b) Optically-evoked input/output curve from L2/3 rAAV+ neurons from non-stressed (n=9; N=3) and stressed (n=8; N=3) female mice. Stress exposure enhances excitatory input to L2/3 rAAV+ neurons.

- (c) Effect of stress on PPR at BLA-L2/3 rAAV+ synapses (No Stress: n=9, N=3, Stress: n=8, N=3). Stress exposure decreases PPR (p=0.0499).
- (d) Effect of stress on DSE in female mice (No stress: n=9, N=3; Stress: n=5, N=3). Stress does not alter DSE magnitude in female mice (p=0.4560)
- (e) Optically-evoked input/output curve from L2/3 rAAV+ neurons from mice receiving no stress (n=17, N=4) or 10 minutes exposure to the predator odorant, 2MT (n=16, N=4). Predator odor exposure significantly increases excitatory input to L2/3 rAAV+ neurons.
- (f) Effect of predator odor on PPR at BLA-L2/3 rAAV+ synapses (No stress: n=16, N=4; Predator odor: n=17, N=4). Predator odor decreases PPR (p=0.0152).
- (g) Effect of predator odor on DSE at BLA-L2/3 rAAV+ synapses (No stress: n=12, N=4; Stress: n=13, N=4). Stress decreases DSE magnitude (p=0.0078).
- (h) Schematic for *in vivo* fiber photometry recordings of BLA projections to the pIPFC
- (i) Z-score of $\Delta F/F$ signal recorded from GCaMP6s expressing BLA terminals in the pIPFC in response to 2 second 0.1 mA foot shock (N=5 mice, averaged from 20 shocks per mouse).
- (j) Z-score of $\Delta F/F$ signal recorded from GCaMP6s expressing BLA terminals in the pIPFC in response to 2 second 0.25 mA foot shock (N=5, averaged from 20 shocks per mouse).
- (k) Optically-evoked input/output curve from L2/3 rAAV+ neurons from mice receiving no stress (n=19; N=4), 0.1 mA foot-shock stress (n=14; N=4), and 0.25 mA foot-shock stress (n=15, N=4). 0.25 mA stress exposure induces a trend toward increased excitatory input to L2/3 rAAV+ neurons (p=0.0943).
- (l) Effect of 0.1 and 0.25 mA foot-shock stress on PPR at BLA-L2/3 rAAV+ synapses (No Stress: n=19, N=4, 0.1 mA stress: n=14, N=4, 0.25 mA stress: n=15, N=4). 0.25 mA stress exposure decreases PPR (p=0.0058).
- (m) Effect of 0.1 and 0.25 mA foot-shock stress on DSE at BLA-L2/3 rAAV+ synapses (No stress: n=11, N=4; 0.1 mA stress: n=13, N=4; 0.25 mA stress: n=14, N=4). Neither 0.1 mA (p=0.9697) nor 0.25 mA (p=0.2504) foot-shock stress alter DSE magnitude.
- (n) Optically-evoked input/output curve from L2/3 rAAV+ neurons from mice receiving no stress (n=18, N=4), and mice recorded 3 (n=24, N=4) and 10 days (n=13, N=4) following 0.5 mA foot shock stress. Stress exposure increases excitatory input to L2/3 rAAV+ neurons at 3 days post exposure (p=0.0002)
- (o) Effect of 0.5 mA stress on PPR at BLA-L2/3 rAAV+ synapses at 3 and 10 days post exposure (no stress: n=18, N=4; 3 day: n=24, N=4; 10 day: n=13, N=4). PPR is decreased at 3 days post stress exposure (p=0.0003).
- (p) Effect of 0.5 mA stress on DSE at BLA-L2/3 rAAV+ synapses at 3 and 10 days post exposure (no stress: n=13, N=4; 3 day: n=21, N=4; 10 day: n=9, N=4). DSE magnitude is not altered at 3 (p=0.3689) or 10 (p=0.3689) days post stress exposure.

All error bars represent \pm SEM. “n” represents number of neurons, “N” represents number of mice. P values reported from two-tailed unpaired t-test (c,d,f,g) and one-way ANOVA (l,m,o,p). F and P values from ANOVA shown in relevant panels.

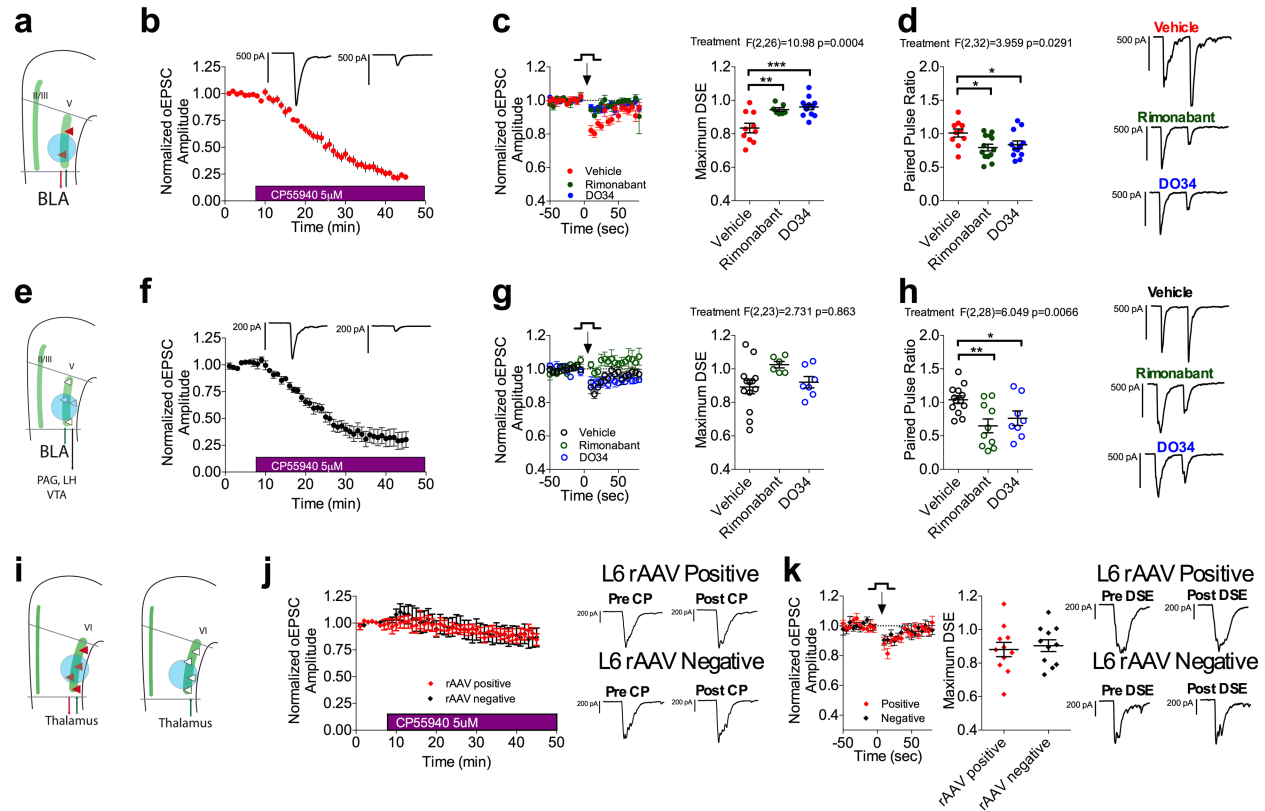


Figure S4, related to figure 3: Phasic and tonic 2-AG signaling broadly regulate BLA-plPFC L5 glutamatergic synapses but not MDT-plPFC synapses

- Schematic for voltage-clamp recordings of oEPSCs from L5 rAAV+ neurons at -70mV.
- Effect of 5 μ M CP55,940 on oEPSC amplitude at BLA-L5 rAAV+ synapses (n=8, N=6).
- Depolarization induced suppression of excitation (DSE) at BLA-L5 rAAV+ synapses (n=11, N=5) is blocked by 10 μ M rimonabant (n=7, N=4; One-way ANOVA with Holm-Sidak post-hoc correction, p=0.0023) and 2.5 μ M DO34 (n=12, N=4; p=0.0003).
- PPR at BLA-L5 rAAV+ synapses (n=10, N=5) is reduced by rimonabant (n=13, N=4; p=0.0217) and DO34 (n=10, N=4; p=0.0389).
- Schematic for voltage clamp-recordings of oEPSCs from L5 rAAV- neurons at -70mV.
- Effect of CP55,940 on oEPSC amplitude at BLA-L5 rAAV- synapses (n=7, N=5)
- Minimal depolarization induced suppression of excitation (DSE) is evoked at BLA-L5 rAAV- synapses (n=13, N=5). As such, there is no significant effect of rimonabant (n=6, N=6; One-way p=0.0580) or DO34 (n=7, N=4; p=0.6040)
- PPR at BLA-L5 rAAV- synapses (n=13, N=5) is reduced by rimonabant (n=12, N=5; p=0.0047) and DO34 (n=8 N=4; p=0.0347).
- Schematic for voltage-clamp recordings of oEPSCs from L6 rAAV+ and negative neurons at -70mV.
- Effect of CP55,940 on oEPSC amplitude at MDT-L6 rAAV+ (n=8, N=6) and negative (n=10, N=6) synapses. CP55,940 does not induce significant depression of oEPSC amplitude at MDT-L6 rAAV+ or rAAV- synapses.
- Minimal DSE was evoked at MDT-L6 rAAV+ (n=11, N=3) and rAAV- (n=11, N=3) synapses.

All error bars represent \pm SEM. “n” represents number of neurons, “N” represents number of mice. All post-hoc p values derived from one-way ANOVA with Holm-Sidak multiple comparisons (c,d,g,h) or two-tailed unpaired t-test (k). F and P values for ANOVA shown in relevant panels.

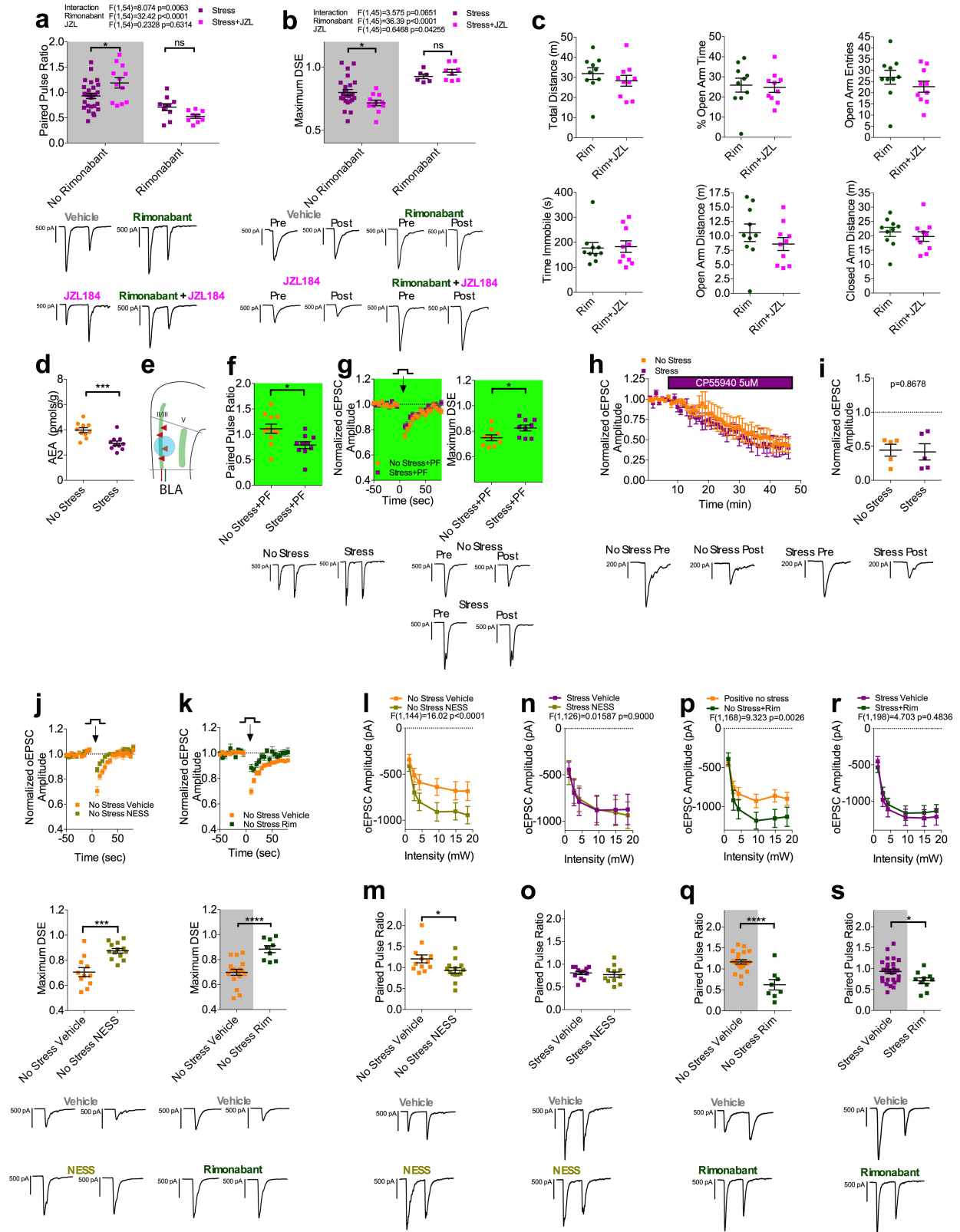


Figure S5, related to figure 4: Rimonabant blocks the effects of JZL184, AEA augmentation does not rescue stress-induced changes in PPR or DSE, stress does not alter CB1 sensitivity, and stress occludes the effects of NESS and rimonabant at BLA-L2/3 rAAV positive synapses

- (a) Effect of JZL and JZL+rimonabant on PPR in stressed mice. 1 μ M JZL reverses the stress-induced decrease in PPR at BLA-L2/3 rAAV+ synapses (Stress: n=27, N=11; Stress+JZL: n=13, N=4, Two-way ANOVA with Holm-Sidak post hoc correction, p=0.0158). JZL does not alter PPR in the presence of 10 μ M rimonabant (Stress+rimonabant: n=10, N=5; Stress+rimonabant+JZL: n=9, N=2; p=0.1452). Stress and Stress+JZL without rimonabant from main text figure shown in grey shading.
- (b) Effect of JZL and JZL+rimonabant on PPR in stressed mice. JZL reverses the stress-induced attenuation of DSE magnitude at BLA-L2/3 rAAV+ synapses (Stress: n=24, N=11; Stress+JZL: n=11, N=4). JZL does not alter DSE magnitude in the presence of rimonabant (p=0.5157). Stress and Stress+JZL without rimonabant from main text figure shown in grey shading.
- (c) Effect of JZL in the presence of rimonabant in the EZM 24 hours after .5 mA foot-shock stress exposure. JZL has no behavioral effect in the presence of rimonabant. (Total Distance: p=0.3809)(Open Arm Time: p=0.7914)(Open Arm Entries: p=0.3042)(Time Immobile: p=0.8655)(Open Arm Distance: p=0.3127)(Closed Arm Distance: p=0.4981).
- (d) Effect of stress on mPFC AEA levels. Stress exposure causes a decrease in mPFC AEA levels (No stress: N=10, Stress: N=10; p=0.0009).
- (e) Schematic for voltage clamp-recordings of oEPSCs from L2 rAAV+ neurons at -70mV.
- (f) Effect of 10 μ M PF3845 on PPR at BLA-L2 rAAV+ synapses. PF3845 does not rescue the stress induced decrease in PPR at BLA-L2/3 rAAV positive synapses (No Stress: n=11, N=8; Stress: n=10, N=6; p=0.0121).
- (g) Effect of PF3845 on DSE at BLA-L2 rAAV+ synapses. PF3845 does not rescue the stress induced decrease in DSE magnitude at BLA-L2/3 rAAV positive synapses (No Stress: n=9, N=6; Stress: n=9, N=6; p=0.0299).
- (h) Effect of 5 μ M CP55,940 on oEPSC amplitude at BLA-L2/3 rAAV+ synapses.
- (i) Quantification of last 5 minutes of CP55,940 application on oEPSC amplitude at BLA-L2 rAAV+ synapses. Stress does not cause a significant change in CP55,940 induced depression of oEPSC amplitude at BLA-L2/3 rAAV+ synapses (No Stress: n=5, N=3; Stress: n=5, N=2; p=0.8678).
- (j) Effect of 1 μ M NESS on DSE at BLA-L2/3 rAAV+ synapses in non-stressed mice (vehicle: n=11, N=4; NESS: n=13, N=4). NESS blocks DSE (p=0.0002).
- (k) Effect of 10 μ M rimonabant on DSE at BLA-L2/3 rAAV+ synapses in non-stressed mice (vehicle: n=18, N=7; NESS: n=9, N=4). Rimonabant blocks DSE (p<0.0001). Grey shading: No Stress Vehicle data taken from main text.
- (l) Effect of NESS on optically-evoked input/output curve from L2/3 rAAV+ neurons from non-stressed mice (vehicle: n=12, N=4; NESS: n=14, N=4). NESS enhances excitatory input.
- (m) Effect of NESS on PPR at BLA-L2/3 rAAV+ synapses from non-stressed mice (vehicle: n=12, N=4; NESS: n=14, N=4). NESS decreases the PPR (p=0.0209).
- (n) Effect of NESS on optically-evoked input/output curve from L2/3 rAAV+ neurons from stressed mice (vehicle: n=12, N=4; NESS: n=11, N=4). NESS does not affect excitatory input following stress.

- (o) Effect of NESS on PPR at BLA-L2/3 rAAV+ synapses from stressed mice (vehicle: n=12, N=4; NESS: n=11, N=4). NESS does not affect PPR following stress (p=0.5873).
- (p) Effect of rimonabant on optically-evoked input/output curve from L2/3 rAAV+ neurons from non-stressed mice (vehicle: n=21, N=8, rimonabant: n=8, N=4). Rimonabant enhances excitatory input.
- (q) Effect of rimonabant on PPR at BLA-L2/3 rAAV+ synapses from non-stressed mice (vehicle: n=21, N=8, rimonabant: n=8, N=4). Rimonabant decreases the PPR (p<0.0001).
- (r) Effect of rimonabant on optically-evoked input/output curve from L2/3 rAAV+ neurons from stressed mice (vehicle: n=27, N=10; rimonabant: n=10, N=4). Rimonabant does not affect excitatory input following stress.
- (s) Effect of rimonabant on PPR at BLA-L2/3 rAAV+ synapses from stressed mice (vehicle: n=27, N=10; rimonabant: n=10, N=4). Rimonabant decreases the PPR following stress (p=0.0297).

All error bars represent \pm SEM. “n” represents number of neurons, “N” represents number of mice. P values reported from two-tailed unpaired t-test (c,d,f,g,i,j,k,m,o,q,s) and post-hoc p values reported from two-way ANOVA with Holm-Sidak multiple comparisons (a,b). F and P values for ANOVA shown in relevant panels.

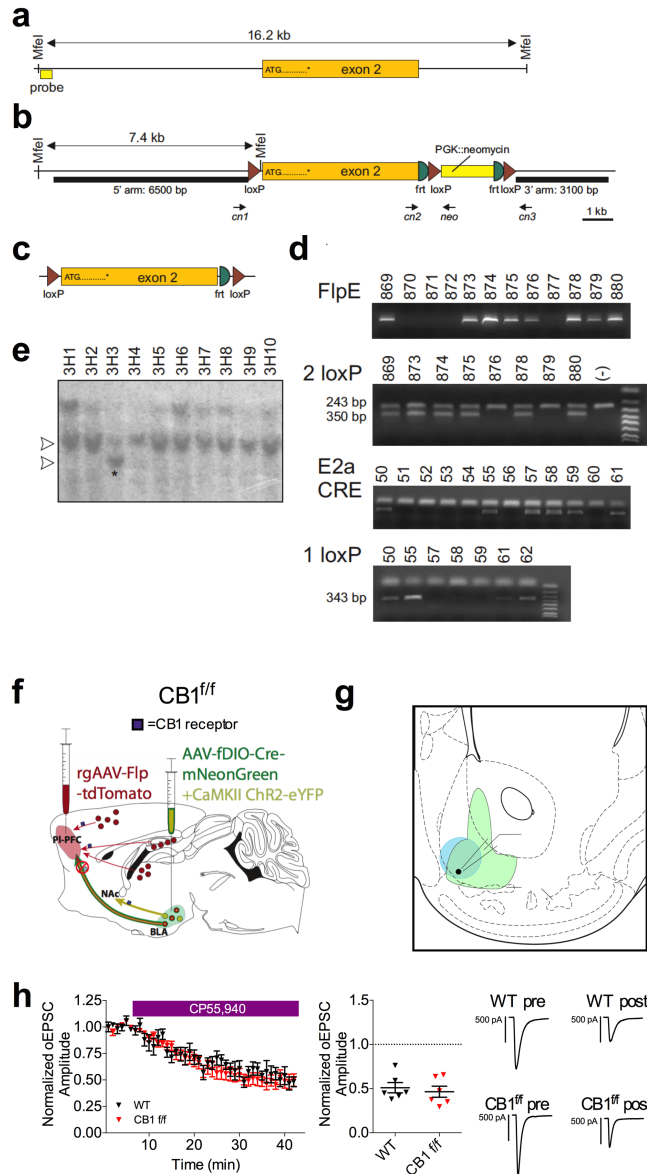


Figure S6, related to figure 6: INTRSECT approach leads to selective deletion of the CB1 receptor from BLA neurons that project to the pIPFC and not from BLA neurons that project to other brain regions

- (a) Schematic representation of the *Cnr1* gene with exons 1 and 2 separated by large intron.
- (b) Schematic representation of the *Cnr1* gene after homologous recombination. Three loxP and 2 FRT sites are highlighted around the coding exon. A PGK-driven neomycin resistance gene cassette is also included. The 5' and 3' arms of recombination are also highlighted. Note the location of PCR primers used to genotype the mice: Primers cn2 – neo were used to amplify a 389 bp fragment from the mutant allele only; primers cn2 and cn3 amplify a 342 and 350 bp fragments from wild-type allele and 2 loxP allele, respectively; primers cn1 and cn3 amplify a 343 bp fragment once exon 2 has been deleted.

- (c) Structure of the mutant allele after FlpE-mediated recombination. This recombination eliminates the neomycin cassette and leaves exon 2 flanked by 2 loxP sites.
- (d) Southern blot analysis of 10 ES cell clones showing the presence of a 16 kb band in all samples and a smaller 7.4 kb fragment in clone 3H3.
- (e) PCR analysis of progeny of 3 loxP mice crossed with FlpE mice giving rise to mice demonstrating a 350 bp fragment originating from the 2 loxP allele. Also shown is a PCR analysis of progeny of 2 loxP mice crossed with E2a-CRE mice giving rise to mice demonstrating a 343 bp fragment originating from the 1 loxP allele.
- (f) Schematic for deletion of CB1 from pIPFC projecting BLA neurons and expression of CaMKII-ChR2-eYFP in BLA projection neurons.
- (g) Schematic for recording of BLA evoked oEPSCs in the Nucleus Accumbens (NAc) Shell
- (h) Effect of CP55,940 on oEPSC amplitude at BLA-NAc shell synapses. WT and CB1^{fl/fl} INTRSECT mice have equal sensitivity to CP55,940 induced depression of BLA evoked oEPSCs in the NAc shell (WT: n=6, N=3; CB1^{fl/fl} n=6, N=2; p=0.6048).

All error bars represent \pm SEM. “n” represents number of neurons, “N” represents number of mice. P values reported from two-tailed unpaired t-test (h).