Local potentiation of excitatory synapses by serotonin
and its alteration in models of depression

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Supplemental Figures
Supplementary Figure 1: Cai et al.

(a) Schematic diagram of the experimental setup.

(b) Graph showing the effect of Citalopram on fEPSP slope (% control) over time.

(c) Graph showing the effect of Fluoxetine on fEPSP slope (% control) over time.

(d) Graph showing the effect of Imipramine and isamoltane on fEPSP slope (% control) over time.

(e) Fluorescence images of serotonin-depleted samples with scale bar 10 μm.

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Supplementary Figure 1. Serotonin effects in the hippocampus. a, Arrangement of stimulating and recording electrodes in acute hippocampal brain slices. Temporo-ammonic (TA) – CA1 EPSPs (fEPSPs) were recorded with pipettes placed in SLM of area CA1 in response to stimuli delivered in SLM ca. 150 µm away. Schaffer collateral (SC) - CA1 fEPSPs were recorded in SR in response to stimuli delivered in SR at the CA3/CA1 border. Because the saline contained GABA receptor antagonists, area CA3 and DG were cut off, as indicated, to prevent spontaneous epileptiform discharge. SP, stratum pyramidale; SR, stratum radiatum; SLM, stratum lacunosum/molecular. b,c, Promoting accumulation of endogenous serotonin by bath application of the selective serotonin reuptake inhibitors citalopram (10 µM; n=4 slices) or fluoxetine (20 µM; n=5 slices) potentiated TA-CA1 fEPSPs in SLM of area CA1 of acutely prepared hippocampal slices. d, Persistent potentiation of TA-CA1 fEPSPs following acute application of imipramine (2 µM, n=4 slices) is reduced upon application of the 5-HT1B antagonist, isamoltane (10 µM), indicating that it is due to persistent elevation of endogenous serotonin levels and persistent activation of 5-HT1Bs. e, Images of SLM in hippocampal sections from a control rat and two different rats treated with the inhibitor of serotonin synthesis, PCPA, after staining with an antibody against serotonin. PCPA treatment depleted serotonin, as indicated by a lack of the normal dense fiber staining. Anipritoline responses in slices from PCPA treated animals (below) were comparable to that seen in controls (n = 4 slices from 3 PCPA-treated animals).
Supplementary Figure 2: Cai et al.

(a) Imipramine and Wild-type in TA-CA1 fEPSP slope (% control) over time (min).

(b) Fluoxetine showing TA-CA1 fEPSP slope (% control) over time (min).

(c) Imipramine with 5-HT1B R^-/- and + NAN-190 (5-HT1A R antag.)

(d) Before and after aniprtoline comparison of TA and SC.

(e) CP94253 and Y-25130 effects on TA-CA1 fEPSP slope (% control) over time (min).

(f) Acute fluoxetine effect showing TA-CA1 fEPSP slope (% control) for SC and TA.
Supplementary Figure 2. Serotonin-induced potentiation requires 5-HT_{1b}Rs and is selective for TA-CA1 synapses. 

a, Responses to acute application of imipramine (2 μM) were absent in slices from 5-HT_{1b}R–/– mice (red, n = 6 slices), but similar to responses of rats in slices from wild-type 129X1/SvJ mice (black, n = 4 slices). 

b, Similarly, responses to acute application of fluoxetine were absent in slices from 5-HT_{1b}R–/– mice (red, n = 3 slices).

c, Potentiation of TA-CA1 fEPSPs by imipramine in the presence of the 5-HT_{1b}R antagonist NAN-190 (10 μM) (n = 6 slices) is comparable to the potentiation in untreated slices.

d, Anpirtoline (50 μM) selectively enhanced TA-CA1 EPSPs, elicited with stimulation in SLM of area CA1 and recorded in whole-cell current-clamp mode, but not SC-CA1 EPSPs elicited with stimulation in SR (n = 9 cells) (TA-CA1 110±13% vs. SC-CA1 181±12%, t(16) = 4.01, p = 0.01). 

e, The 5-HT_{1b}R agonist, CP94253 (5 μM) potentiated TA-CA1 fEPSPs (n = 4 slices), like anpirtoline, whereas a 5-HT_{3}R antagonist, Y-25130 (5 μM), did not (n = 3 slices). 

f, Acute application of fluoxetine to elevate endogenous serotonin potentiates TA-CA1 fEPSPs, but not SC-CA1 fEPSPs (n = 4,7 slices) (TA-CA1 141±8% vs. SC-CA1 89±22%, t(9) = 2.70, p = 0.024). *p < 0.01, **p < 0.001 compared with before anpirtoline or fluoxetine, paired t-test.
Supplementary Figure 3: Cai et al.

(a) The graph shows the effect of anpirtoline on [(TA+SC)-TA]/SC (% control) after anpirtoline. The x-axis represents the TA - SC interval (ms), while the y-axis shows the response. The graph indicates a decrease in the [(TA+SC)-TA]/SC (% control) after anpirtoline treatment.

(b) The figure shows the effect of anpirtoline on the TA - SC interval (ms) before and after anpirtoline treatment. The graph illustrates the changes in the TA - SC interval over time.

(c) The graph depicts the effect of anpirtoline on fEPSP slope (mV) before and after treatment with different drugs. The effect of anpirtoline, AP5, DNQX, and AP5 + anpirtoline are shown, with a bar graph illustrating the comparison between control and treated conditions.

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Supplementary Figure 3. Promotion of action potential firing and enhancement of TA-SC interaction by 5-HT1B receptor activation. a, Anpirtoline enhanced TA-SC interaction efficacy and increased the amplitude and duration of the period of superlinear summation. Whole-cell current-clamp recordings were made from CA1 cell somata with the membrane potential set at -60 mV, and two stimulating electrodes were placed in SLM and SR to evoke TA- and SC-EPSPs, respectively. TA- and SC-EPSPs were first elicited separately and then in pairs, with the SC stimulus preceding the TA stimulus by 10 to 320 ms. The protocol was then repeated after bath application of anpirtoline. The amount of summation produced by combined stimulation was calculated by digitally subtracting the response to TA stimulation alone (TA) from the combined response (TA+SC), and then dividing by the response to SC stimulation alone. This ratio was then plotted as a function of the interval between the TA and SC stimuli (ratio = 1 indicates linear summation). We observed a time dependent summation of TA- and SC-EPSPs in control saline. Anpirtoline enhanced supralinear summation at all intervals, indicating that the effect of 5-HT1B receptor activation is to increase the amplitude and duration of the interaction between the two input pathways to CA1 cells. b, Responses of a CA1 cell to combined TA and SC stimulation at various intervals from 0 – 320ms at a holding potential of -55 mV. Anpirtoline greatly increased the probability that the combined stimuli elicited action potential discharge. c, Representative traces showing potentiation of AMPAR-mediated fEPSPs by anpirtoline in the presence of D,L-AP5 (80 μM) (upper traces), but not NMDAR-mediated fEPSPs in Mg2+-free saline containing DNQX (50 μM) (lower traces). Potentiation of AMPAR-mediated fEPSPs by anpirtoline was apparent after washout of DNQX (189±8% before anpirtoline), indicating that anpirtoline potentiated AMPAR- but not NMDAR-mediated responses (F(2,7)=6.992, p=0.021 DNQX vs. control or APV, Bonferroni post-hoc, p<0.05, n=6 control slices, n=3 slices in AP5, n=4 slices in DNQX).
Supplementary Figure 4: Cai et al.

(a) Phosphoprotein levels in the stratum radiatum. (b) Phosphoprotein levels in the 5-HT1BR−/− condition.

(c) Blot analysis showing CaMK and GluA1 levels in the control condition.

(d) Blot analysis showing GluA1 levels in the medial prefrontal cortex.

(e) Graph showing the effect of fluoxetine on TA-CA1 fEPSP slope.
Supplementary Figure 4. 5-HT1B activation phosphorylates serine 831 at TA-CA1 synapses but not SC-CA1 synapses and is required for potentiation by fluoxetine. a, Western blot analyses revealed no activation of CaMK (Friedman’s ANOVA, \( \chi^2=6.67, df=4, p=0.16 \)) and no phosphorylation of S831 of GluA1 (\( \chi^2=0.675, df=4, p=0.95 \)) in response to anpirtoline application in tissue wedges prepared from stratum radiatum. b, Anpirtoline induces phosphorylation of S831 of GluA1 in SLM tissue from control C57BL6j mice, but not in SLM tissue from 5-HT1B\\(-/\\)- mice. c, Acute administration of fluoxetine (50 \( \muM \), 60 min) results in an activation of CaMK (left graph, \( n=6 \) samples each, Kruskal-Wallis H, \( \chi^2=10.986, df=4, p=0.027 \)) and phosphorylation of S831 of GluA1 (right graph, \( n=4 \) samples each, \( \chi^2=10.602, df=4, p=0.031 \)) in tissue from SLM that persists after 60 min of washout, whereas both recover after washout of anpirtoline (50 \( \muM \), 60 min). *, significantly different from control (post-hoc Mann-Whitney U tests, \( p<0.05 \)). d, As in SLM of the hippocampus, phosphorylation of S831 of GluA1 is increased by acute anpirtoline in tissue from the rat medial prefrontal cortex. e, Acute application of fluoxetine potentiated TA-CA1 fEPSPs in hippocampal slices from wild type C57BL6j mice, but not in slices from GluA1 S831A knock-in mice (\( n = 6 \) control, 5 knock-in slices). Full-length blots are presented in Supplementary Figure 7.
Supplementary Figure 5: Cai et al.

(a) Sucrose preference (%), (b) Latency to feeding (s), (c) Food consumption (g/kg), (d) TA-CA1 fEPSP slope (% control), (e) TA-CA1 fEPSP slope (% control).

CUS animals

Cus animals + fluoxetine

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Supplementary Figure 5. Altered behavior in animals subjected to CUS is accompanied by a qualitative and quantitative change in responses to anpirtoline. a,b, Animals subjected to CUS lose their normal preference for sucrose-containing solutions and take longer before eating food in the center of a brightly lit arena in the novelty suppressed feeding test. Subsequent treatment with fluoxetine for 3 weeks restores normal sucrose preference (n=8-9 (F2,22)=6.101, p=0.008) and novelty suppressed feeding behaviors (n=7-8 animals, (F2,19)=9.376, p=0.001)(*, p< 0.05 vs. control, Bonferroni post-hoc). Food consumption was not altered under any condition (F(2,19)=0.571, p=0.58.

c, Potentiation of fEPSP slope at TA-CA1 synapses is large and irreversible in hippocampal slices from animals subjected to CUS (n=4 slices). d, Potentiation of fEPSP slope by anpirtoline remains selective for TA-CA1 synapses in hippocampal slices from animals subjected to CUS in a two pathway design experiment (n=5 slices). e, Persistent potentiation of TA-CA1 fEPSPs in slices from CUS animals (n=3 slices) is not eliminated upon application of the 5-HT_{1B} antagonist, isamoltane (10 \mu m), indicating that it is not due to persistent binding of anpirtoline to 5-HT_{1B}Rs.
Supplementary Figure 6: Cai et al.

(a) Before 36 h Fluoxetine

Sucrose preference (%)

Control  CUS  Control  CUS

(b) 36 h Fluoxetine

TA-CA1 fEPSP slope (% control)

Anipirtoline

Time (min)

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Supplementary Figure 6. Effects of brief administration of fluoxetine. a, Sucrose preference was lower in rats subjected to CUS for three weeks than in controls (n=6). Administration of fluoxetine via the drinking water (80 mg/l) for only 36 hr failed to restore sucrose preference to CUS animals (no main effect of treatment F(1,4)=0.004, p=0.95). b, Anpirtoline (50 μM) irreversibly enhanced TA-CA1 fEPSPs, elicited with stimulation in SLM of area CA1, in hippocampal slices (n=3) taken from the CUS rats receiving fluoxetine for 36 hrs, unlike in rats treated with fluoxetine for 3 weeks (see Fig. 6d). We conclude that both the behavioral effects of CUS and this synaptic correlate of CUS are reversed only with chronic AD administration.
Supplementary Figure 7. Uncropped western blots from panels in indicated figures.
Figure 3e

Phospho-S831 GluA1

ANP  Control

Total GluA1

ANP  Control
Figure 3e

Supp. Fig 4c

phospho-Thr286 CaMKII

Control ANP

Fluoxetine Wash Control ANP Wash

Total CaMKII

NAc SLM

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