Supplementary Materials and Methods

Animals and Drugs.

All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee of the State University of New York at Buffalo. SD male rats at the prepubescent or early adolescent period (25-28 days of age) were group-housed in cages with a light/dark cycle of 12 hrs. Stress procedures were performed around 8:30am. For the forced-swim stress,[64,65] rats were placed in a cylindrical glass tank (24.5 cm high x 18.5 cm diameter) filled with water to a depth of 20 cm. Rats were forced to swim in warm water (23-25°C) for 20 min. For the elevated-platform stress,[37,65] rats were placed on an elevated platform (20 x 20 cm, 1.5 m from the floor) for 20 min. The animals displayed signs of stress, including freezing, defecating and shivering. In some experiments, animals were i.p. injected with RU486 at 30 min before stress exposure. After the acute stress procedure, rats were returned to their home cages, if they were not immediately sacrificed for *in vitro* experiments.

Pharmacological agents used include: corticosterone (Sigma), RU486, RU28362, dexamethasone, fluticasone, aldosterone, human/rat corticotrophin releasing factor (all from Tocris), and Akt inhibitor V (Calbiochem). For *in vitro* drug application, concentrated stocks in DMSO or water were made and stored at -20ºC, and stocks were thawed and diluted (1:5000 to $1:10^6$) in the culture medium prior to experiments. The highest concentration of DMSO ($2x10^{-4}$) was used as the vehicle control. Corticosterone was first prepared as a 10 mM stock in DMSO, and then diluted ($1:10^5$) with the culture medium to reach the final working concentration of 100 nM. The same concentration (10^{-5}) of vehicle (DMSO) was added to control groups. For *in vivo* delivery, RU486 was dissolved into 0.1% DMSO-containing saline (0.5 ml, pH adjusted to 7.4), and injected into animals at the concentration of 10 mg/kg. The vehicle control used i.p. injection of 0.1% DMSO-containing saline (0.5 ml, pH = 7.4).

Electrophysiological recording in slices.

Rats were anesthetized by inhaling Halothane (Sigma) for 20-30 sec and decapitated quickly; brains were removed, iced and then blocked for slicing. The tissue was cut in 300 μm coronal slices with a Vibratome (Leica VP1000S) while bathed in a low Ca^{2+}, HEPES-buffered salt solution (in mM: 140 Na isethionate, 2 KCl, 4 $MgCl_2$, 0.1 $CaCl_2$, 23 glucose, 15 HEPES, pH = 7.4, 300-305 mOsm). Slices were then incubated for 1-6 hrs at room temperature (20-22°C) in a $NaHCO_3$-buffered saline bubbled with 95% O_2, 5% CO_2. For evoked EPSC recording, patch electrodes were filled with the following internal solution (in mM): 130 Cs-methanesulfonate, 10 CsCl, 4 NaCl, 10 HEPES, 1 $MgCl_2$, 5 EGTA, 2.2 QX-314, 12 phosphocreatine, 5 MgATP, 0.2 Na_3GTP, 0.1 leupeptin, pH 7.2-7.3, 265-270 mOsm. For evoked IPSC recording, internal solution contained: 100 CsCl, 30 N-methyl-D-glucamine, 4 NaCl, 10 HEPES, 1 $MgCl_2$, 5 EGTA, 2.2 QX314, 12 phosphocreatine, 5 MgATP, 0.2 Na_3GTP, 0.1 leupeptin. PFC slices were placed in a perfusion chamber attached to the fixed stage of an upright microscope (Olympus) and submerged in continuously flowing oxygenated artificial CSF (ACSF, in mM: 130 NaCl, 26 $NaHCO_3$, 3 KCl, 5 $MgCl_2$, 1.25 NaH_2PO_4, 1 $CaCl_2$, 10 Glucose, pH 7.4, 300 mOsm). Bicuculline (10 μM) and CNQX (25 μM) were added in NMDAR-EPSC recordings. Bicuculline (10 μM) and D-APV (25 μM) were added in AMPAR-EPSC recordings. CNQX and D-APV were added in $GABA_A$R-IPSC recordings. Cells were visualized with a 40X water-immersion lens and illuminated with near infrared (IR) light, and the image was detected with an IR sensitive CCD camera. A Multiclamp 700A amplifier was used for these recordings. Tight seals (2-10 GΩ) from visualized pyramidal neurons were obtained by applying negative pressure. The membrane was disrupted with additional suction, and the whole-cell configuration was obtained. Evoked currents were generated with a pulse from a stimulation isolation unit controlled by a S48 pulse generator (Astro Med, West Warwick, RI). A bipolar stimulating electrode (FHC, Bowdoinham, ME) was positioned ~100 μm from the neuron under recording. Membrane potential was held at -70mV during AMPAR-EPSC or $GABA_A$R-IPSC recording. For NMDAR-EPSC measurement, the cell

(clamped at -70 mV) was depolarized to +60mV for 3 s before stimulation to fully relieve the voltage-dependent Mg^{2+} block. To record miniature EPSC in PFC slices, ACSF with 1 mM $MgCl_2$ was used.

To generate the input-output responses, a series of different stimulation intensities (5-9 V) with the same duration of pulses (0.6 ms for NMDAR-EPSC; 0.06 ms for AMPAR-EPSC; 0.1 ms for $GABA_A$R-IPSC) was used to elicit synaptic currents. In other experiments, synaptic currents evoked by the same stimulation intensity were recorded in individual neurons across groups with different manipulations. To minimize experimental variations between cells, the following criteria were used: (1) layer V mPFC pyramidal neurons with comparable membrane capacitances were selected; (2) stimulating electrode was positioned at the same location (layer VI, ~100 μm horizontally) from the neuron under recording, and the electrode tip was cleaned after every recording to allow precise stimulation capacity; (3) recordings from control vs. stressed animals were interleaved throughout the course of all experiments. Data analyses were performed with Clampfit (Axon instruments) and Kaleidagraph (Albeck Software).

Whole-cell recordings in acutely dissociated and cultured neurons.

The internal solution contained (in mM): 180 N-methyl-D-glucamine, 40 HEPES, 4 $MgCl_2$, 0.1 BAPTA, 12 phosphocreatine, 3 Na_2ATP, 0.5 Na_2GTP, and 0.1 leupeptin, pH 7.2-7.3, 265-270 mOsm. The external solution contained (in mM): 127 NaCl, 20 CsCl, 1 $MgCl_2$, 10 HEPES, 5 $BaCl_2$, 12 glucose, 0.001 TTX, pH 7.3-7.4, 300-305 mOsm. For recording NMDAR currents, external solution was modified to contain 0 mM $MgCl_2$, 1 mM $CaCl_2$ and 20 μM glycine. Neurons were held at -60mV for recording NMDAR- or AMPAR-mediated ionic currents, while at -40mV for recording $GABA_A$R-mediated current. NMDA (100 μM), AMPA (100 μM) or GABA (100 μM) was applied for 2 s every 30 s via a gravity-fed ‘sewer pipe’ system. The array of application capillaries (ca. 150 μm i.d.) was positioned a few hundred microns from the cell

under study. Solution changes were effected by the SF-77B fast-step solution stimulus delivery device (Warner Instrument).

Miniature EPSC in cultured PFC neurons were recorded with the same internal solution used for recording evoked AMPAR-EPSC in slices. The external solution contained (mM): 127 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 12 glucose, 10 HEPES, 0.001 TTX, pH 7.3-7.4, 300-305 mosM. Bicuculline (10 μM) and D-APV (25 μM) were added to block $GABA_AR$ and NMDAR activation. The membrane potential was held at -70 mV. Synaptic currents were analyzed with Mini Analysis Program (Synaptosoft, Leonia, NJ).

Western Blot and Co-immunoprecipitation.

Equal amounts of protein from PFC slice were subjected to western blotting analysis. Antibodies used include anti-SGK1, anti-SGK2, anti-SGK3 (all 1:500, abcam), and anti-actin (1:1000, Santa Cruz). For coimmunoprecipitation experiments, each PFC slice was collected and homogenized in 1 ml of lysis buffer (50 mM Tris, 1% deoxycholic acid, 10 mM EDTA, 10 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, and 1 mg/ml leupeptin). Lysates were ultracentrifuged (200,000 x g) at 4°C for 60 min. Supernatant fractions were incubated with anti-Rabaptin-5 (1:100, Santa Cruz) for 4 hr at 4°C, followed by incubation with 50 μl of protein A/G plus agarose (Santa Cruz) for 2 hr at 4°C. Immunoprecipitates were washed three times with lysis buffer containing 0.2 M NaCl, then boiled in 2x SDS loading buffer for 5 min, and separated on 7.5% SDS-polyacrylamide gels. Western blotting experiments were performed with anti-Rab4 and anti-Rab5 (both 1:1000, Santa Cruz).

Immunocytochemical staining.

For the detection of glutamate receptors on the cell surface, PFC cultures were fixed but not permeabilized, blocked and incubated with the primary antibody at 4ºC overnight. For

endogenous GluR1 in PFC cultures (DIV24-30), an antibody against N-terminal extracellular GluR1 (1:500, Upstate) was used. For recombinant GFP-NR2A or GFP-NR2B (GFP tagged at N-terminal) in transfected cultures (DIV12-14), an anti-GFP antibody (1:50, Chemicon) was used. After washing, cultures were incubated with an Alex594-conjugated secondary antibody (1:500, Invitrogen) for 1 hr at room temperature. In some experiments, to co-stain with PSD-95, cells were washed and permeabilized by Triton (0.2%, 20 min), and incubated with anti-PSD-95 antibody (1: 500, Abcam) at 4C° overnight. After 3 washes, they were incubated with the Alex594 (red) or Alex488 (green) conjugated secondary antibody (1:200, Molecular Probe) at RT for 1 hr. After washing in PBS three times, the coverslips were mounted on slides with VECTASHIELD mounting media.

Fluorescent images were obtained using a 100X objective with a cooled CCD camera mounted on a Nikon microscope. The surface GluR1, GFP-NR2A and GFP-NR2B clusters were measured using Image J software as what we described before.[15,66] All specimens were imaged under identical conditions and analyzed using identical parameters. To define dendritic clusters, a single threshold was chosen manually, so that clusters corresponded to puncta of at least twofold greater intensity than the diffuse fluorescence on the dendritic shaft. Three to four independent experiments for each of the treatments were performed. On each coverslip, the cluster density, size, and fluorescence intensity of four to six neurons (two to three dendritic segments of at least 50 μm length per neuron) were measured. Quantitative analyses were conducted blindly (without knowledge of experimental treatment).

Behavioral tests.

To test working memory, the T-maze delayed alternation task[67,12] was used. Rats (3-4 week, ~100 g) were subjected to restricted diet and maintained at ~85% of their original weight for 1 week. They were habituated to a T-maze until they voluntarily ate a sucrose pellet placed at the end of each arm. On the first trial, animals were rewarded for entering either arm. Thereafter, for a total

of 11 trials per session, animals were rewarded only if they entered the arm opposite to the one that was previously chosen. Between trials the choice point was wiped with alcohol to remove olfactory clues. In the initial 1-2 training sessions, the delay between trials started at 5 sec, and was subsequently raised in 5 sec intervals. In the later training sessions, the delay was fixed at 30 sec, and animals were examined daily until establishing the baseline performance at ~60% correct for two consecutive days. The first trial was not included in the analysis of correctness. Most animals (~90%) required 2-3 training sessions (with 30 sec delay) to achieve stable (~60% correct) performance. The few animals showing inconsistent and perseverative responses (<50% or >80% correct) after training were not used. On the following day, animals were exposed to 20 min forced-swim stress or 20 min elevated platform stress, and tested with the delayed alternation task (delay: 30 sec) at 4 hrs post-stress and 1 day post-stress. Non-stressed control animals were tested in parallel.

In some experiments, peptides (dissolved in saline) were injected into rat tail veins (200 μl/animal) using 23-25 gauge needles at 30 min before stress exposure. Rats were anesthetized by inhaling Halothane throughout the i.v. injection (~1 min). For local injection of drugs to PFC, animals were implanted with double guide cannulas (Plastics One Inc.) using a stereotaxic apparatus (David Kopf Instruments). The coordinates of PFC were: 2.5 mm anterior to bregma; 0.75 mm lateral; 2.5 mm dorsal to ventral. The injection cannula extended 1.5 mm beyond the guide. After the implantation surgery, animals were allowed to recover for three days prior to the habituation and training sessions. After training, on the day of testing, the TAT-SGK peptide (5 μg/μl or 15 ng/μl, dissolved in saline) was injected via the cannula bilaterally into PFC using a Hamilton syringe (22-gauge needle, 1 μl per side) at 30 min before stress exposure. A scrambled TAT peptide was injected in parallel as controls. Behavioral experimenters were blind to the treatments that animals received.

References

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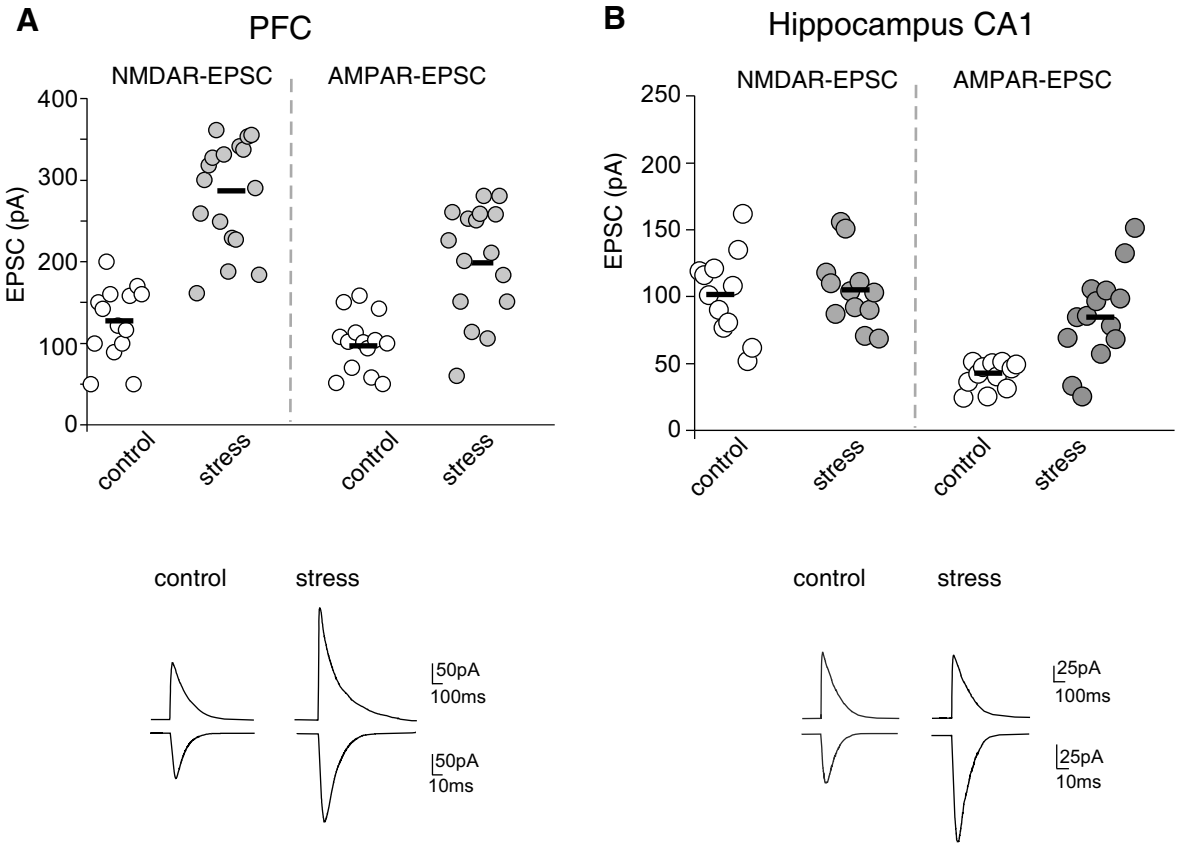
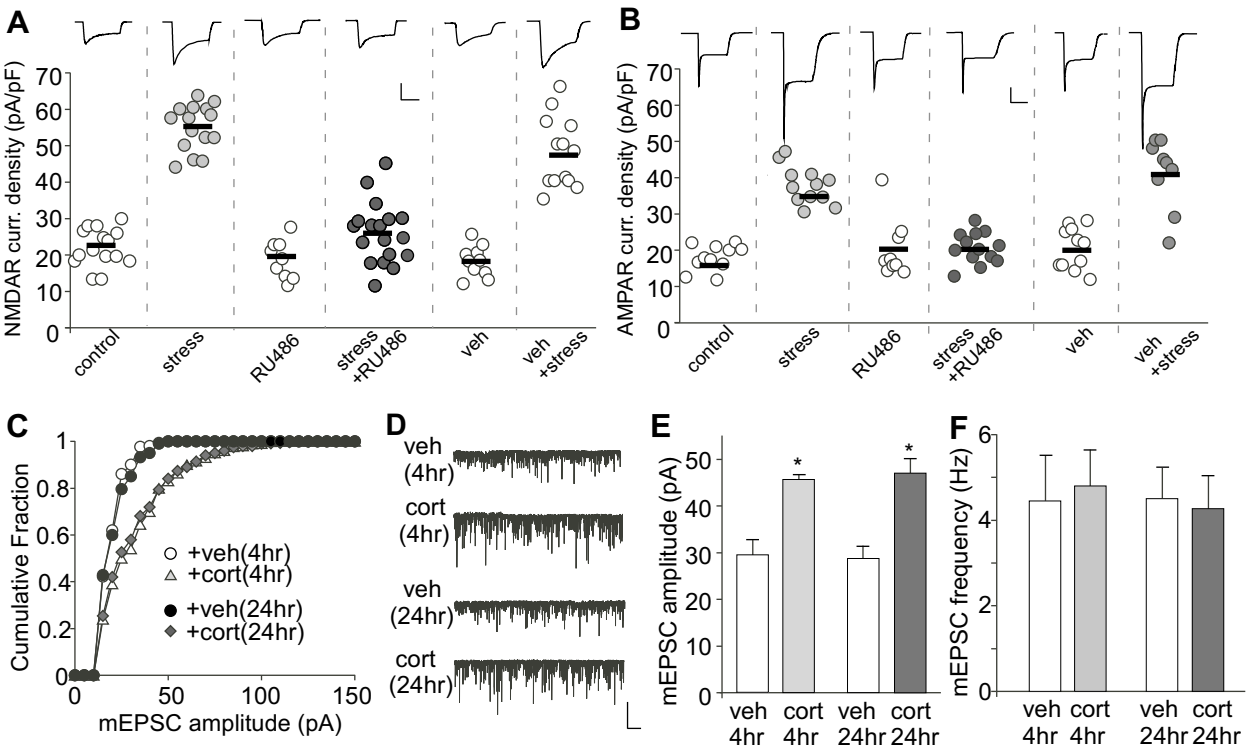
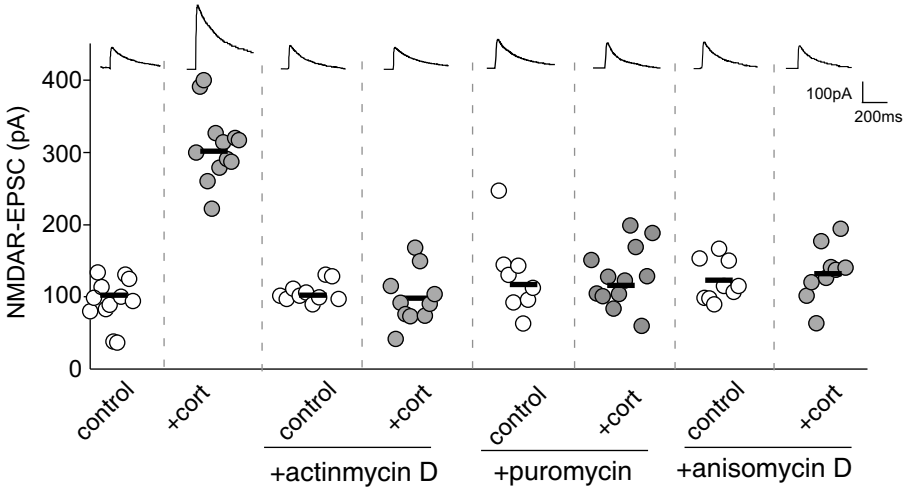


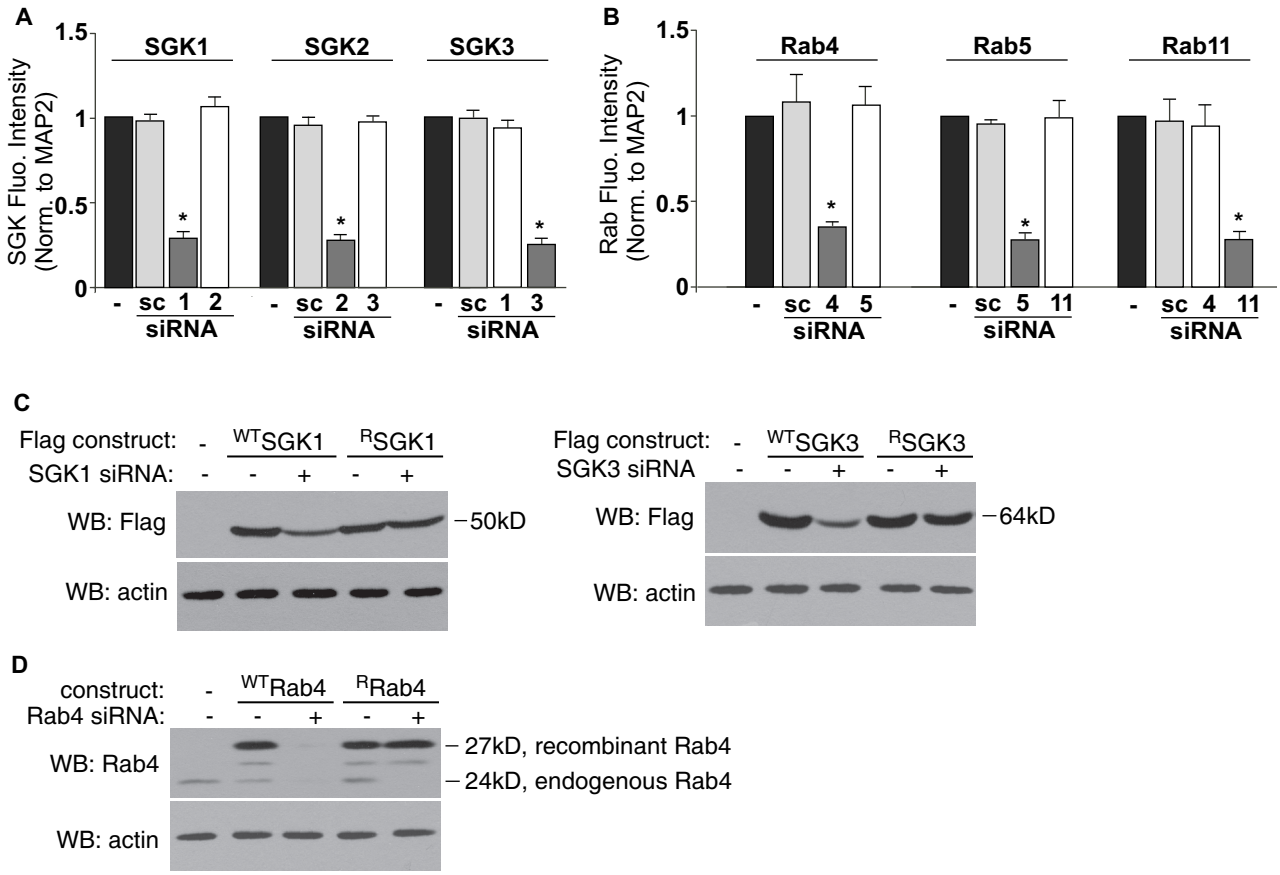
Supplemental Figure 1. Recordings of AMPAR- and NMDAR-mediated EPSCs in the same cells from PFC or hippocampus. Neurons were held at -70 mV to obtain AMPAR-EPSC, followed by +40mV with addition of CNQX (20μM) to record NMDAR-EPSC. Bicuculline (10μM) was present throughout the recording. One neuron per slice was recorded and extensive wash was performed between recordings to prevent contamination of receptor antagonists.
A. In PFC pyramidal neurons, acute stress significantly increased both NMDAR-EPSC (control: 126.2±11.9 pA, n=14; stressed: 282±16 pA, n=17; $p<0.001$, *t* test) and AMPAR-EPSC (control: 100.1±9.2 pA, n=14; stressed: 198.9±16.4 pA, n=17; $p<0.001$, *t* test) to a similar extent. **B.** In CA1 pyramidal neurons, acute stress only increased AMPAR-EPSC significantly (control: 42.0±9.1 pA, n=12; stressed: 85.6±9.2 pA, n=16; $p<0.001$, *t* test), while left NMDAR-EPSC unchanged (control: 102.0±9.1 pA, n=12; stressed: 104±7.8 pA, n=12; $p>0.05$, *t* test).



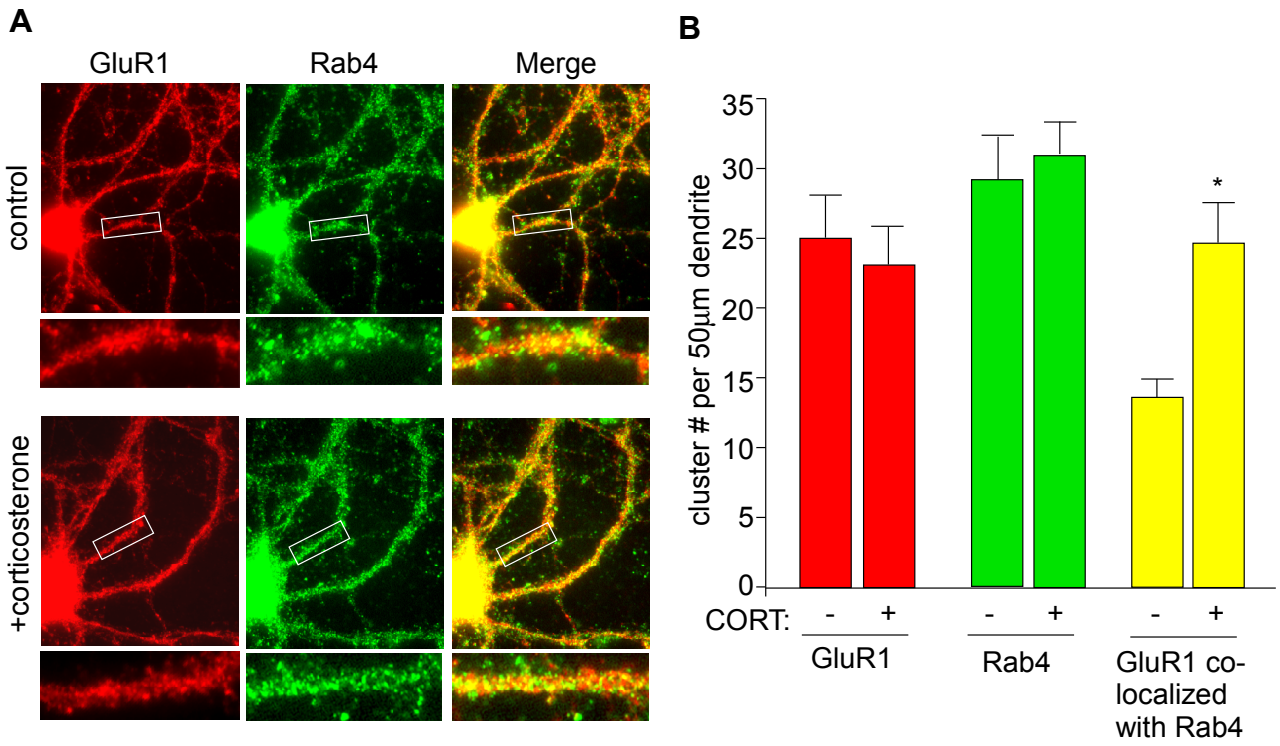
Supplemental Figure 2. AMPAR- and NMDAR-mediated ionic currents and mEPSC after acute stress or corticosterone treatment. A,B. Animals exposed to forced-swim stress had a significantly increased NMDAR and AMPAR current density (pA/pF, NMDA: control: 22.1±1.4, n=15; stressed: 54.9±1.6, n=15; AMPA: control: 16.9±1.1, n=11; stressed: 36.3±1.2, n=12; $p<0.001$, t test), which was abolished by injection (i.p.) of the GR antagonist RU486 (10 mg/kg) (NMDA: RU486: 19.0±1.7, n=9; RU486+stress: 26.2±1.9, n=18; AMPA: RU486: 19.9±2.4, n=10; RU486+stress: 20.5±1.2, n=13; $p>0.05$, t test), but not the vehicle (DMSO) control. **C-F.** The amplitude of mEPSC was significantly increased in PFC cultures at 4 hr or 24 hr after corticosterone treatment (4 hr: vehicle: 29.5±3.2 pA, n=8, cort: 45.6±1.2 pA, n=7; $p<0.001$, *t* test; 24 hr: vehicle: 28.7±2.7 pA, n=8, cort: 47.0±3.2 pA, n=7; $p<0.001$, *t* test). The mEPSC frequency was not altered by corticosterone treatment (4 hr: vehicle: 4.5±1.1 Hz, n=8, cort: 4.8±0.9 Hz, n=7; $p>0.05$, *t* test; 24 hr: vehicle: 4.5±0.7 Hz, n=6, cort: 4.3±0.8 Hz, n=7; $p>0.05$, *t* test).



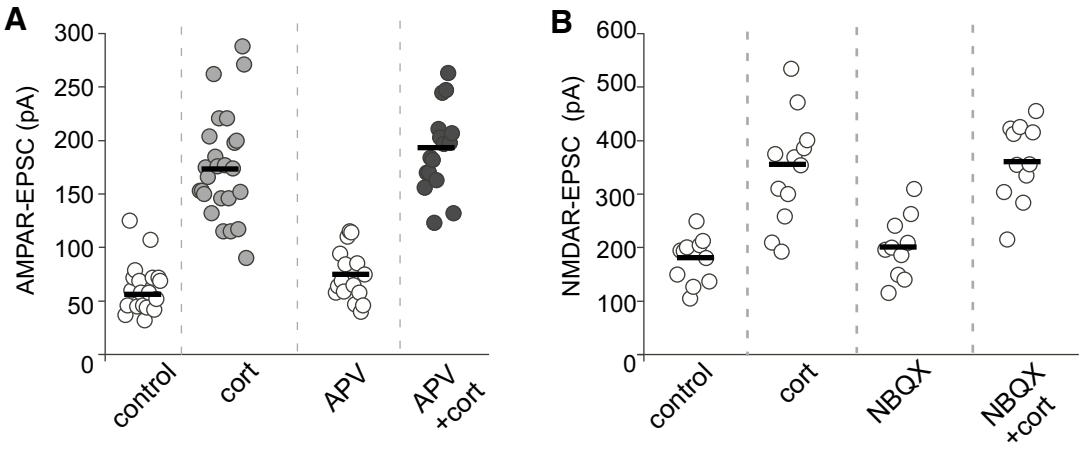
Supplemental Figure 3. The effect of corticosterone on NMDAR-EPSC in the presence of transcription or translation inhibitors. The enhancing effect of corticosterone (100 nM, 20 min) on NMDAR-EPSC in PFC slices (control: 103.5± 11.1 pA, n=15; cort: 309 ± 14.4 pA, n=12; $p<0.001$, t test) was abolished by pre-treatment with the transcription inhibitor actinmycin D (50 μM, 30 min; 96.3 ±11.9 pA, n=10) or puromycin (100 μM, 30 min, 127.5±12.1 pA, n=12). Pre-treatment of PFC slices with translation inhibitor anisomycin D (2 μM, 30 min) also blocked the effect of corticosterone on NMDAR-EPSC (132.2±12.9 pA, n=9).



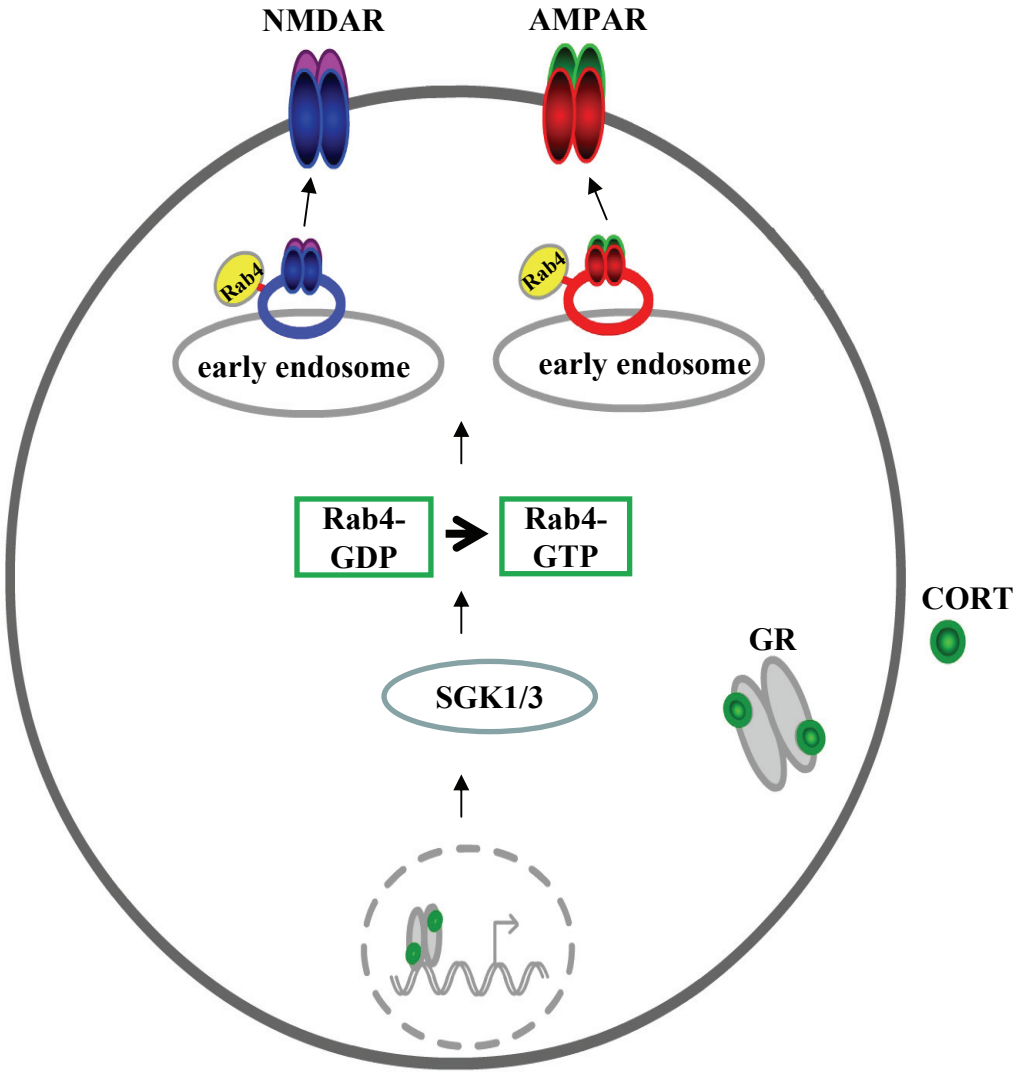
Supplemental Figure 4. Quantification data showing the target-specific gene knockdown by various siRNAs. PFC cultures were transfected with a scrambled control siRNA (sc) or the siRNA against SGK1-3 (A), or Rab4/5/11 (B). GFP was co-transfected. Neurons were co-stained with anti-MAP2 and an antibody against SGK1-3 or Rab4/5/11. The fluorescent intensity (normalized to MAP2) in GFP+ neurons was quantified and compared. **A.** SGK1 was reduced to 0.29±0.04 of control in SGK1 siRNA-transfected cells (n=13), but not altered in cells transfected with scrambled (n=11) or SGK2 siRNA (n=10). SGK2 was reduced to 0.28±0.03 of control in SGK2 siRNA-transfected cells (n=12), but not altered in cells transfected with scrambled (n=10) or SGK3 siRNA (n=14). SGK3 was reduced to 0.27±0.04 of control in SGK3 siRNA-transfected cells (n=13), but was not altered in cells transfected with scrambled (n=10) or SGK1 siRNA (n=13). *: $p<0.001$. **B.** Rab4 was reduced to 0.35±0.03 of control in Rab4 siRNA-transfected cells (n=15), but was not altered in cells transfected with scrambled (n=9) or Rab5 siRNA (n=14). Rab5 was reduced to 0.19±0.02 of control in Rab5 siRNA-transfected cells (n=13), but was not altered in cells transfected with scrambled (n=11) or Rab11 siRNA (n=9). Rab11 was reduced to 0.21±0.04 of control in Rab11 siRNA-transfected cells (n=12), but was not altered in cells transfected with scrambled (n=12) or Rab4 siRNA (n=13). *: $p<0.001$. **C, D.** Representative Western blots in HEK293 cells transfected with wild-type (WT) or siRNA-resistant (R) SGK1, SGK3 (subcloned to pcDNA3.1 vector) or Rab4 (subcloned to plenti6/V5 vector) in the absence or presence of the corresponding siRNA.



Supplemental Figure 5. Measurement of co-localization of GluR1 and Rab4 in PFC cultures treated with or without corticosterone. **A.** As indicated by yellow puncta along dendrites, AMPA receptors can be seen in Rab4-positive internal vesicles. **B.** Cort-treated neurons show more GluR1 clusters co-localized with Rab4 (control: 13.7±1.4, n=10; CORT: 24.8±2.9, n=10; $p<0.01$, *t* test). No significant changes were found with total GluR1 (control: 25.1±3.4; GluR1-CORT: 23.2±2.7, n=10; $p>0.05$, *t* test) or Rab4 (control: 29.2±3.1; CORT: 31.0±2.3, n=10; $p>0.05$, *t* test).



Supplemental Figure 6. The effect of corticosterone on AMPAR-EPSC when NMDAR activation is blocked, and the effect of corticosterone on NMDAR-EPSC when AMPAR activation is blocked. The NMDAR antagonist APV (100 μM) or the AMPAR antagonist NBQX (10 μM) was added 15 min before and during corticosterone treatment (20 min). Recordings were performed at 1-4 hrs after washing off these compounds. **A.** The corticosterone-induced increase of AMPAR-EPSC was not blocked by APV (APV: 73.9±6.1 pA, n=16; APV+cort: 190±9.8 pA, n=16, $p<0.001$, *t* test). **B.** The corticosterone-induced increase of NMDAR-EPSC was not blocked by NBQX (NBQX: 203.3±18.6 pA, n=10; NBQX+cort: 358.1±22.2 pA, n=11, $p<0.001$, *t* test).



Supplemental Figure 7. A diagram showing the potential molecular and cellular mechanism underlying corticosterone regulation of NMDAR and AMPAR trafficking and function. Upon GR activation, SGK1/3 is upregulated, leading to the activation of Rab4. Consequently, the recycling of NMDARs and AMPARs from early endosome to plasma membrane is enhanced. The extra-synaptic glutamate receptors are moved to synaptic surface presumably via lateral diffusion. At synapses, glutamate receptors are endocytosed for recycling or degradation.