Supplemental Experimental Procedures

Behavioral testing

The object location task was conducted as previously described (Barker et al., 2007). Each rat was habituated to a rectangular arena for 5 min before the commencement of the behavioral testing. In the sample phase, rats were allowed to explore two identical objects placed in the far corners of the arena for a total of 3 min. After a 5-min delay, the test phase began. In the test phase, one object remained in the same position, while the other object was placed in a new location. To assess the animal's ability to recognize that an object that it has experienced before has changed location, we calculated a discrimination ratio (i.e., the difference in time spent exploring the "moved" and "unmoved" objects divided by the total time spent exploring both objects) during the 3-min test trial.

For open-field tests, animals were placed in an open area (60 cm x 80 cm) for 5 min, and the amount of time that the animal spent in the center (25 cm x 25 cm) was counted. For locomotion tests, animals were placed in a cage for 5 min, and the number of crossing a midline was counted. Behavioral experimenters were blind to the treatments that animals received.

Electrophysiological Recordings

For whole-cell recordings in isolated neurons, PFC neurons were acutely dissociated using procedures similar to those described previously (Yuen et al., 2011). 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. $MgCl_2$-free external solution containing 1 mM $CaCl_2$ and 20 μM glycine was used for recording NMDAR currents. NMDA (100 μM) or AMPA (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 controlled by the SF-77B fast-step solution stimulus delivery device (Warner Instrument). Neurons were constantly held at -60mV for NMDAR- or AMPAR-mediated ionic currents. To measure VDCC, neurons were depolarized with a ramp protocol.

Miniature EPSC in cultured PFC neurons was recorded with the same internal solution used for recording evoked EPSC in slices (Yuen et al., 2011). 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 and D-APV 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).

Biochemical measurement of surface and total proteins

The modified radioimmunoprecipitation assay buffer used in these experiments contained: 1% Triton X-100, 0.1% SDS, 0.5% deoxycholic acid, 50 mM $NaPO_4$, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and 1 mg/ml leupeptin. Western blots were performed on both total and biotinylated (surface) proteins using antibodies against GluR1 (1:500, Millipore, 05-855 or 1:200, Santa Cruz, sc-13152), GluR2 (1:500, Millipore, MAB397), NR1 (1:500, Millipore, 06-311 or 1:500, Millipore, 05-432), NR2A (1:500, Millipore, 07-632), NR2B (1:500, Millipore, 06-600) and actin (1:1000, Santa Cruz, sc-1616). Western blots were also performed to detect other proteins, such as MAP2 (1:1000, Santa Cruz, sc-20172), PSD-95 (1:1000, Abcam, ab-2723), synaptophysin (1:2000, Sigma, S5768), synapsin (1:2000, kind gift from Dr. Paul Greengard), Nedd4-1 (1:1000, Abcam, ab-14592) and Fbx2 (1:500, Abcam, ab-28555).

Immunoprecipitation

The following primary antibodies against glutamate receptor subunits (8 μg) were used: GluR1 (Millipore, 05-855), GluR2 (Millipore, MAB397), NR1 (Millipore, 06-311 or Cell Signaling, 5704), NR2A (Millipore, 07-632), NR2B (Millipore, 06-600). The following primary antibodies against glutamate receptor binding proteins (2 μg) were also used: SAP-97 (Santa Cruz, sc-25661) and PSD-95 (Abcam, ab-2723).

Immunocytochemical Staining

To detect synaptic glutamate receptors, PFC cultures were fixed, permeabilized, blocked and incubated at 4ºC overnight with the primary antibody of GluR1 (1:500, Millipore, 07-660) and PSD-95 (1:500, Abcam, ab2723). After washing, cultures were incubated with an Alex594 (red) or Alex488 (green) conjugated secondary antibody (1:200, Molecular Probe) for 1 hr at RT. After washing three times in PBS, 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 total GluR1, total PSD-95 and their localizations were analyzed using Image J software as previously described (Yuen et al., 2011). All specimens were imaged and analyzed under identical conditions and 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 of four to six neurons (two to three dendritic segments of at least 50 μm length per neuron) was measured. Quantitative analyses were conducted blindly (without knowledge of experimental treatment).

Quantitative real-time RT-PCR

Total RNA was isolated from rat PFC using Trizol reagent (Invitrogen) and treated with DNase I (Invitrogen) to remove genomic DNA. Then SuperScript III first-strand synthesis system for RT-PCR (Invitrogen) was used to obtain cDNA from the tissue mRNA, followed by the treatment with RNase H (2 U/ μl) for 20 min at 37°C. Quantitative real-time RT-PCR was carried out using the iCycler iQ™ Real-Time PCR Detection System and iQ™ Supermix (Bio-Rad) according to the manufacturer's instructions. In brief, GAPDH was used as the housekeeping gene for quantitation of the expression of target genes (AMPAR and NMDAR subunits) in samples from control vs. stressed rats. Fold changes in the target gene relative to the GAPDH endogenous control gene was determined by: Fold change = $2^{-\Delta(\Delta C_T)}$, where $\Delta C_T = C_{T,\,target} - C_{T,\,GAPDH}$, and $\Delta(\Delta C_T) = \Delta C_{T,\,stressed} - \Delta C_{T,\,control}$. C_T (threshold cycle) is defined as the fractional cycle number at which the fluorescence reaches 10x the standard deviation of the baseline. A total reaction mixture of 25 μl was amplified in a 96-well thin-wall PCR plate (Bio-Rad) using the following PCR cycling parameters: 95°C for 5 min followed by 40 cycles of 95°C for 30 sec, 56°C for 30 sec, and 72°C for 60 sec. PCR products were detected with 2% agarose gels. Quantitative real-time RT-PCR was performed in triple reactions.

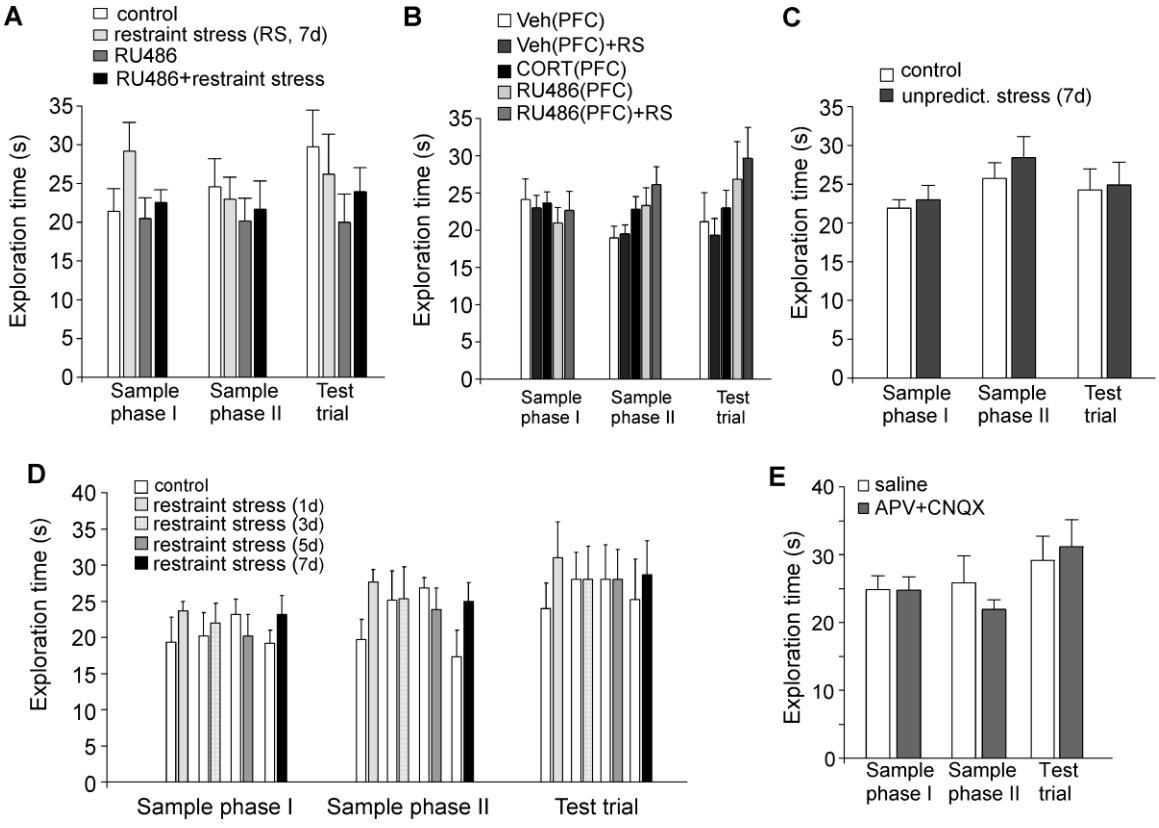

Figure S1, related to Figure 1. The total exploration time in TOR tasks is unchanged by various treatments. A. Bar graphs of the total exploration time from control vs. stressed (7-day restraint) rats without or with systemic injections of RU486. **B.** Bar graphs of the total exploration time from control groups vs. stressed animals (restraint, 7d) with PFC infusions of vehicle or RU486. Another group of animals was given repeated injections of CORT to the PFC. **C.** Bar graphs of the total exploration time from control vs. rats exposed to 7-day unpredictable stress. **D.** Bar graphs of the total exploration time from control vs. stressed (restraint for 1, 3, 5, 7d). **E.** Bar graphs of the total exploration time from animals with PFC infusion of saline vs. APV+CNQX.

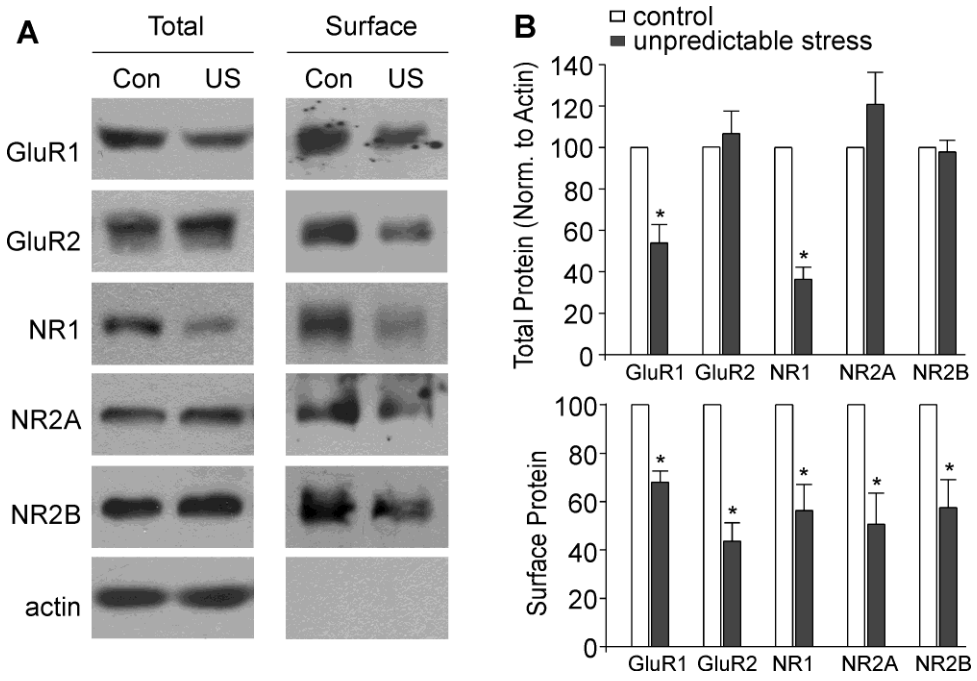

Figure S2, related to Figure 3. Repeated unpredictable stress decreases the total and surface levels of AMPAR and NMDAR subunits in PFC from young male rats. A, B. Immunoblots and quantification analysis of the total and surface AMPAR and NMDAR subunits in PFC from control vs. rats (p28) exposed to 7-day unpredictable stress. *: $p<0.01$. Stressed animals showed a significant decrease in the level of total GluR1 (54.0±9.0% of control, n=4 pairs) and NR1 (36.4±5.8% of control, n=5 pairs), but not GluR2, NR2A or NR2B (n=6 pairs). Surface AMPAR and NMDAR subunits were all significantly decreased by stress (43.7-68.0% of control, n=5 pairs).

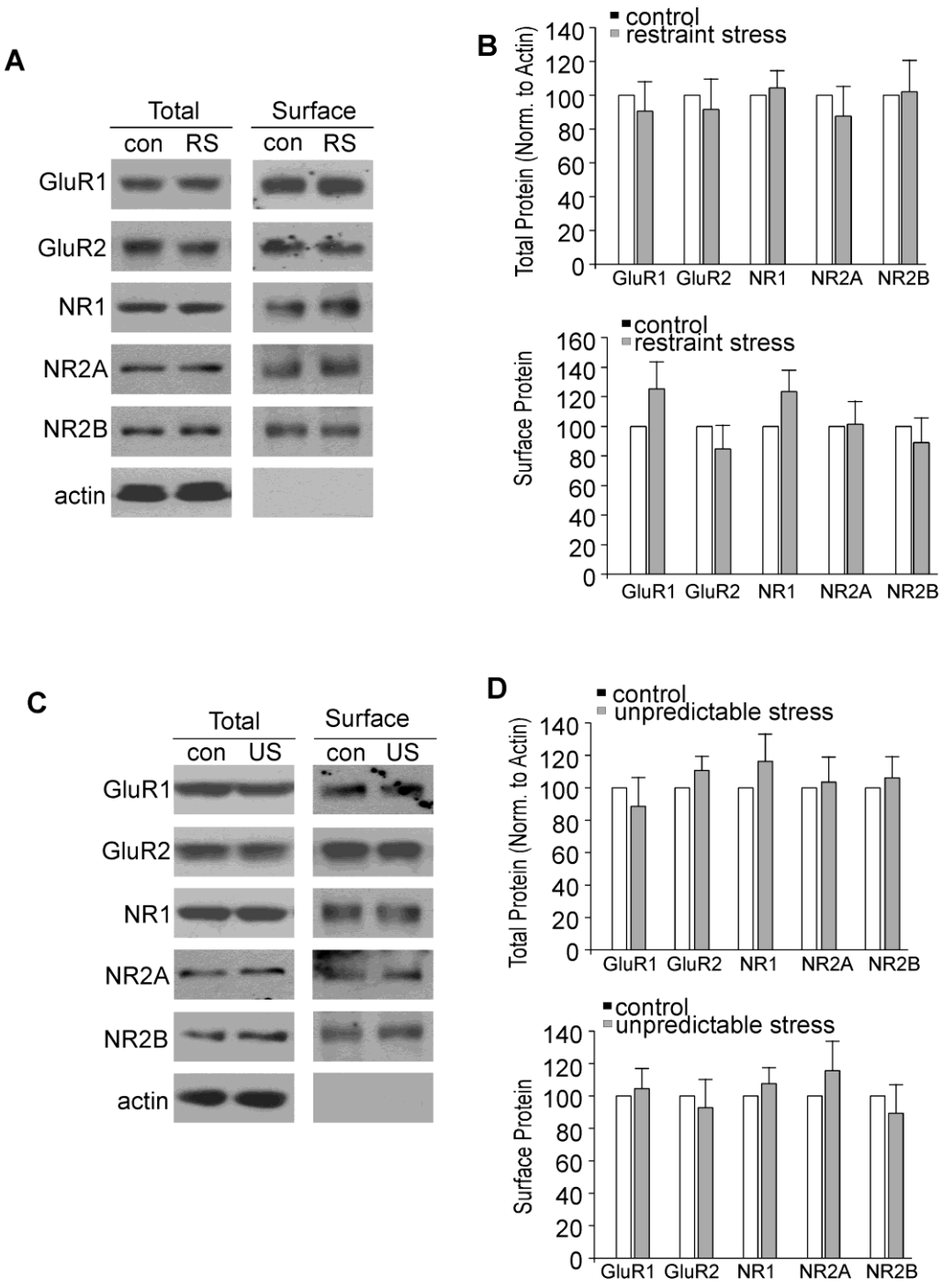

Figure S3, related to Figure 3. Adult male rats exposed to 1-wk repeated stress have normal levels of total and surface AMPAR and NMDAR subunits in PFC. **A-D.** Immunoblots and quantification analysis of the total and surface GluR1, GluR2, NR1, NR2A, and NR2B subunits in PFC from rats (p49) exposed to 7-day of repeated restraint stress (A, B, n=7 pairs, starting at p42) or unpredicatable stress (C, D, n=4 pairs, starting at p42) vs. age-matched controls. No significant changes were found with these stressors.

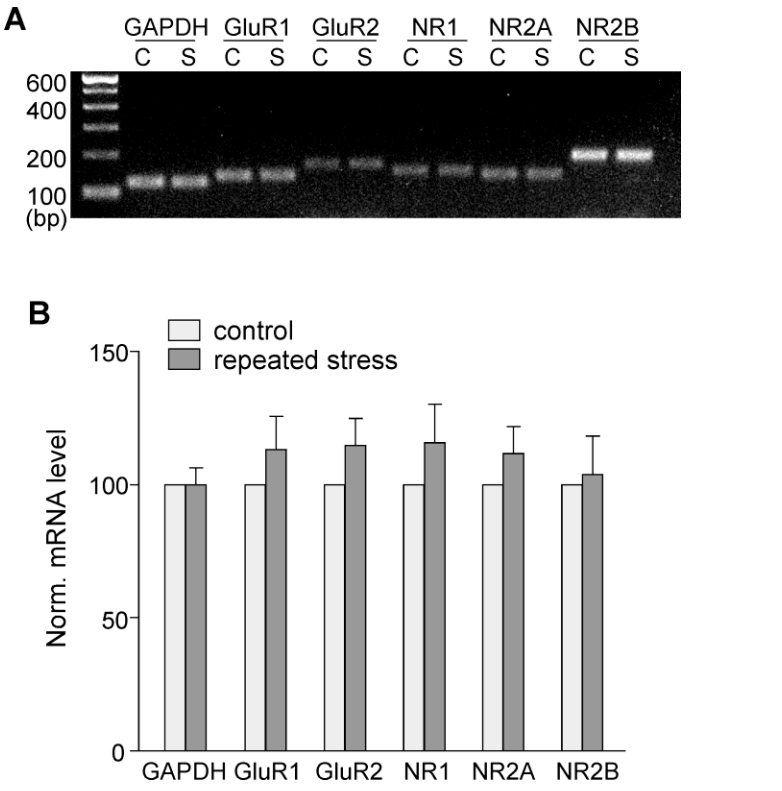

Figure S4, related to Figure 5. Repeated stress does not significantly change the mRNA level of glutamate receptor subunits. A, B. Representative gels and statistic summary of the quantitative real-time RT-PCR products of GluR1, GluR2, NR1, NR2A, NR2B and GAPDH in PFC areas from control (C) or stressed (7-day restraint) young male rats (S).