Chapter 4
Dual Regulation of Glutamatergic Transmission and Cognition by Stress in Prefrontal Cortex

Yan Zhen

Abstract Corticosterone, the major stress hormone, serves as a key controller for neuronal responses that underlie behavioral adaptation, as well as maladaptive changes that lead to cognitive and emotional disturbances in stress-related mental disorders. The molecular and cellular mechanisms underlying the complex actions of corticosteroid stress hormones are largely unknown. Here we demonstrate that acute versus chronic stress exerts opposite effects on glutamatergic transmission in prefrontal cortex (PFC), which leads to opposing effects on PFC-dependent cognitive functions. Acute stress induces synaptic potentiation by increasing surface delivery of N-methyl-D-aspartate (NMDA)-type and α-amino-3-hydroxy-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptor channels via glucocorticoid/serum- and glucocorticoid-inducible kinase (SGK)/Rab4 signaling, resulting in enhanced working memory performance. In contrast, repeated stress induces synaptic depression by increasing the ubiquitin/proteasome-mediated degradation of NMDA and AMPA receptor subunits, resulting in impaired recognition memory.

Abbereviation

AMPA	α-Amino-3-hydroxy-methyl-4-isoxazole propionic acid
NMDA	N-Methyl-D-aspartate
PFC	Prefrontal cortex
GR	Glucocorticoid receptor
MR	Mineralocorticoid receptors
ESPC	Excitatory postsynaptic current
SGK	Serum- and glucocorticoid-inducible kinase
WM	Working memory
TOR	Temporal order recognition
DR	Discrimination ratio

Y. Zhen (✉)
Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY 14214, USA
e-mail: zhenyan@buffalo.edu

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4.1 Introduction

In response to stress, the brain recruits many neuronal circuits to adapt to the demand, leading to the activation of hypothalamic-pituitary-adrenocortical axis, and the production of adrenal corticosterone (cortisol in humans), the major stress hormone (de Kloet et al. 2005). Corticosterone exerts its cellular effects by acting on mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). Importantly, stress hormones have both protective and damaging effects on the body (McEwen 1998). In situations of acute stress, they are essential for adaptation and maintenance of homeostasis, while in response to chronic and repeated stress, they can produce wear and tear on the body (McEwen 2007). Consistently, behavioral studies have found that moderate acute stress facilitates classical conditioning, associative learning, and working memory (WM) (Shors et al. 1992; Henckens et al. 2011), in contrast to the chronic stress-induced deficits in spatial and contextual memory performance and attentional control (McEwen 1999; Liston et al. 2006; Cerqueira et al. 2007). Thus, it has been proposed that the opposing effects that stress has on learning depend on the relative timing of the events (Joëls et al. 2006). Specifically, stress within the context of a learning situation leads to the release of corticosteroids, resulting in focused attention and improvements in memory (Joëls et al. 2006). It has also been suggested that there exists an "inverted U" relationship of stress to cognitive function (Diamond et al. 1992; Joëls 2006), such that a moderate level of glucocorticoids has pro-cognitive effects, while too low or too high glucocorticoid levels are detrimental to cognitive processing.

Given the strong impact of stress hormones on cognition and emotion, it is important to understand the neuronal basis underlying their actions in the brain. One of the primary targets of stress hormones is the prefrontal cortex (PFC), a brain region critical for WM, executive function, and extinction of learning. It has been proposed that glutamate receptor-mediated synaptic transmission that controls recurrent excitation within networks of PFC neurons is crucial for WM (Goldman-Rakic 1995; Lisman et al. 1998). Dysfunction of glutamatergic transmission is considered the core feature and fundamental pathology of stress-related mental disorders with impaired WM (Tsai and Coyle 2002; Moghaddam 2003). Thus, we speculate that NMDA receptors (NMDARs) and AMPA receptors (AMPARs) are potential targets of stress hormones critically involved in the regulation of PFC functions.

Our recent studies have found that acute stress induces a robust and sustained potentiation of glutamate receptor surface expression and excitatory synaptic currents in PFC pyramidal neurons, as well as a significant facilitation of PFC-mediated WM, via a mechanism dependent on serum- and glucocorticoid-inducible kinase (SGK) and the Rab family small guanosine triphosphatases (GTPases) (Yuen et al. 2009, 2011; Liu et al. 2010; Lee et al. 2012). On the other hand, we have found that repeated (subchronic) stress dampens PFC glutamatergic transmission by facilitating glutamate receptor turnover, which causes the detrimental effect on PFC-dependent cognitive processes (Yuen et al. 2012).

4.2 Methods

4.2.1 Stress Paradigm

Prepubertal (25–28 days of age) Sprague Dawley (SD) male rats were exposed to acute stressors of diverse types. For the forced-swim stress, rats were placed in a cylindrical glass tank (24.5 cm high × 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 acute restraint stress, rats were placed in air-assessable cylinders for 2 h. The size of the container was similar to the size of the animal, which made the animal almost immobile in the container. For the elevated-platform stress, rats were placed on an elevated platform (20 × 20 cm) for 20 min. For repeated unpredictable stress (7 days), rats were subjected each day to two stressors that were randomly chosen from six different stressors, forced swim (room temperature (RT), 30 min), elevated platform (30 min), cage movement (30 min), lights on overnight, immobilization (RT, 1 h), and food and water deprivation overnight.

4.2.2 Electrophysiological, Biochemical and Behavioral Experiments

Details can be found in our previous publications (Yuen et al. 2009, 2011, 2012; Liu et al. 2010; Lee et al. 2012; Wei et al. 2013).

4.3 Results

4.3.1 Differential Effects of Acute Versus Repeated Stress on Glutamate Transmission and Glutamate Receptors in PFC

To study the impact of stress on glutamate transmission, we examined synaptic strength by measuring input-output curves of evoked synaptic responses, such as NMDAR- and AMPAR-mediated excitatory postsynaptic current (EPSC), in PFC pyramidal neurons. Young male rats (4-week-old) were exposed to either a 20-min forced-swim acute stress paradigm, or repeated (7-day) restraint stress or unpredictable stress. As shown in Fig. 4.1a–d, AMPAR- or NMDAR-mediated excitatory synaptic responses were markedly potentiated in neurons from acutely stressed animals at 1–4 or 24 h post stress. No significant difference was found at 5 days post stress. In contrast, AMPAR-EPSC and NMDAR-EPSC amplitudes were markedly reduced in neurons from animals exposed to repeated stress (restraint or

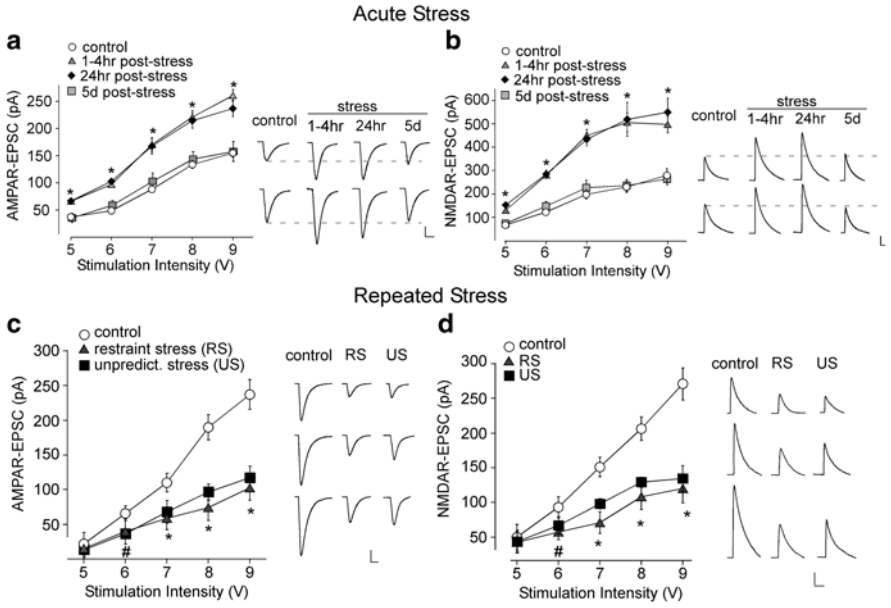

Fig. 4.1 Glutamatergic transmission in PFC pyramidal neurons is enhanced by acute stress, and impaired by repeated stress. **a, b** Summarized input-output curves of α-amino-3-hydroxy-methyl-4-isoxazole propionic acid receptor (*AMPAR*)-excitatory postsynaptic current (*EPSC*) (**a**) or N-methyl-D-aspartate receptor (*NMDAR*)-EPSC (**b**) evoked by a series of stimulus intensities in PFC pyramidal neurons taken from control or animals exposed to acute forced-swim stress (examined at 1–4, 24 h, and 5 days post stress). *Inset:* representative synaptic current traces. *Scale bars:* 100 pA, 100 ms (**a**); 50 pA, 20 ms (**b**). $*p<0.001$. **c, d** Summarized input-output curves of AMPAR-EPSC (**c**) or NMDAR-EPSC (**d**) in response to a series of stimulation intensity in control versus animals exposed to 7 days repeated restraint stress (*RS*) or unpredictable stress (*US*). $*p<0.01$, $**p<0.05$, ANOVA. *Inset*: representative EPSC traces. *Scale bars:* 50 pA, 20 ms (**c**) or 100 ms (**d**). (Adapted from Yuen et al. 2011, 2012)

unpredictable). Injection of the GR antagonist RU486 blocked both the enhancing effect of acute stress and the suppressing effect of repeated stress on glutamatergic responses (data not shown). These results suggest that stress exerts a bi-phasic effect on PFC glutamatergic transmission depending on the duration of stressor.

The alteration of glutamatergic transmission by stress could result from the changed number of glutamate receptors. To test this, we performed Western blotting and surface biotinylation experiments to detect the total and surface level of AMPAR and NMDAR subunits in PFC slices from stressed young male rats. As shown in Fig. 4.2a–d, animals exposed to acute restraint stress (single time, 2 h) showed a significant increase in surface AMPAR and NMDAR subunits, while the total proteins remained unchanged. Animals exposed to 5 or 7-day restraint stress showed a significant decrease in the amount of GluR1 and NR1 subunits. Moreover, repeated stress did not affect the total level of other glutamate receptor subunits, such as GluR2, NR2A, and NR2B, nor the expression of MAP2 (a dendritic

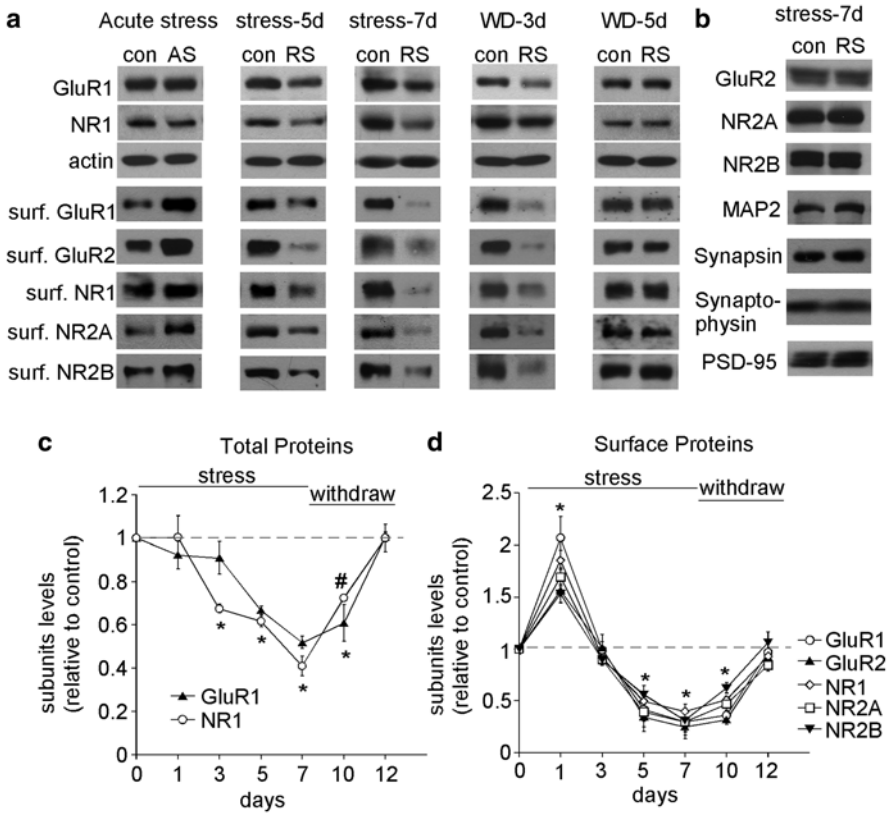

Fig. 4.2 The surface and total levels of AMPAR and NMDAR subunits in PFC are differentially altered by acute versus chronic stress. **a, c, d** Immunoblots (**a**) and quantification analysis (**c, d**) of the total and surface AMPAR and NMDAR subunits in PFC from control (*con*) versus rats exposed to acute restraint stress (*AS*, 1 day, single time of 2 h) or 5–7-day (2 h/day) repeated restraint stress (*RS*). Some animals were withdrawn (*WD*) for different durations (3 or 5 days) after being exposed to 7-day *RS*. $*p<0.01$; $**p<0.05$, *t* test. **b** Immunoblots of the total proteins in PFC from control versus repeatedly stressed (7-day restraint) rats. (Adapted from Yuen et al. 2012)

marker), synapsin, synaptophysin (presynaptic markers) or PSD-95 (a postsynaptic marker), suggesting that no general dendritic or synaptic loss has occurred under such conditions. The amount of AMPAR and NMDAR subunits in the surface pool was all significantly decreased by repeated stress, indicating the loss of glutamate receptors at the plasma membrane. To find out how long the effect of repeated stress can last, we exposed animals to 7-day restraint stress, and examined at 3–5 days after stressor cessation. After 3-day withdrawal of stress, the expression of total and surface AMPARs and NMDARs was still at a partially reduced level, but returned to the control level after 5-day withdrawal. These results suggest that stress-induced changes in glutamatergic transmission likely occur through GR-induced modification of postsynaptic NMDA and AMPA receptors in PFC pyramidal neurons.

4.3.2 *Molecular Mechanisms Underlying the Differential Effects of Acute Versus Repeated Stress on Glutamate Receptors*

Next, we examined potential mechanisms underlying the differential effects of acute versus repeated stress on glutamatergic transmission in PFC. The onset kinetics of the acute stress effect (>1 h) suggests that it might require the activation of immediate early genes downstream of GR. One of the most likely candidates is the SGK, which is composed of three isoforms, SGK1, SGK2, and SGK3. To assess the potential involvement of SGK, we first examined whether the expression level of SGK was up-regulated in stressed animals. As shown in Fig. 4.3a, b, the level of SGK1 and SGK3, but not SGK2, was progressively elevated in PFC slices examined at 1–2 h after acute stress. SGK phosphorylates serine and threonine residues in the motif R-X-R-X-X-(S/T) (Lang and Cohen 2001). To further examine the role of SGK in corticosterone regulation of NMDARs and AMPARs, we pretreated PFC neurons with a SGK substrate peptide (RPRAATF), which should competitively block the interaction of all SGK isoforms with their endogenous substrates. This peptide was coupled to the protein transduction domain of the human immunodeficiency virus (HIV) TAT protein (YGRKKRRQRRR), which rendered it cell-permeant. As shown in Fig. 4.3c, intravenous (i.v.) injection of TAT-SGK peptide prevented acute stress from increasing the amplitude of NMDAR-EPSC.

To identify which SGK is involved, we knocked down SGK isoforms in PFC cultures with siRNA transfection. We found that the enhancing effect of short-term corticosterone treatment (100 nM, 20 min) on NMDAR and AMPAR currents was lost in neurons transfected with SGK1 siRNA or SGK3 siRNA, but was unaltered in neurons transfected with SGK2 siRNA. Taken together, these data suggest that the regulation of glutamatergic signaling by acute stress requires the activation of SGK1/3 downstream of GRs.

The acute stress-induced potentiation of NMDA and AMPA responses is accompanied by increased surface NMDAR and AMPAR clusters, suggesting that GR activation might influence the membrane trafficking of glutamate receptors. It is known that the Rab family of small GTPases functions as specific regulators of vesicle transport between organelles, and different Rab members control vesicular fusion at different stages in the exocytic/endocytic cycle (Zerial and McBride 2001). Among them, the most likely candidates are: Rab5, which controls the transport from plasma membrane to early endosomes; Rab4, which controls a rapid direct recycling route from early endosomes to cell surface; and Rab11, which mediates recycling from recycling endosomes to plasma membrane. As demonstrated in Fig. 4.3d, knockdown of Rab4 blocked the increase of NMDAR or AMPAR current density by corticosterone treatment (100 nM, 20 min). In contrast, the enhancing effect of corticosterone was not altered by Rab5 siRNA or Rab11 siRNA. These results suggest that the corticosterone-induced increase in functional glutamate receptors is through a mechanism depending on Rab4-mediated receptor recycling.

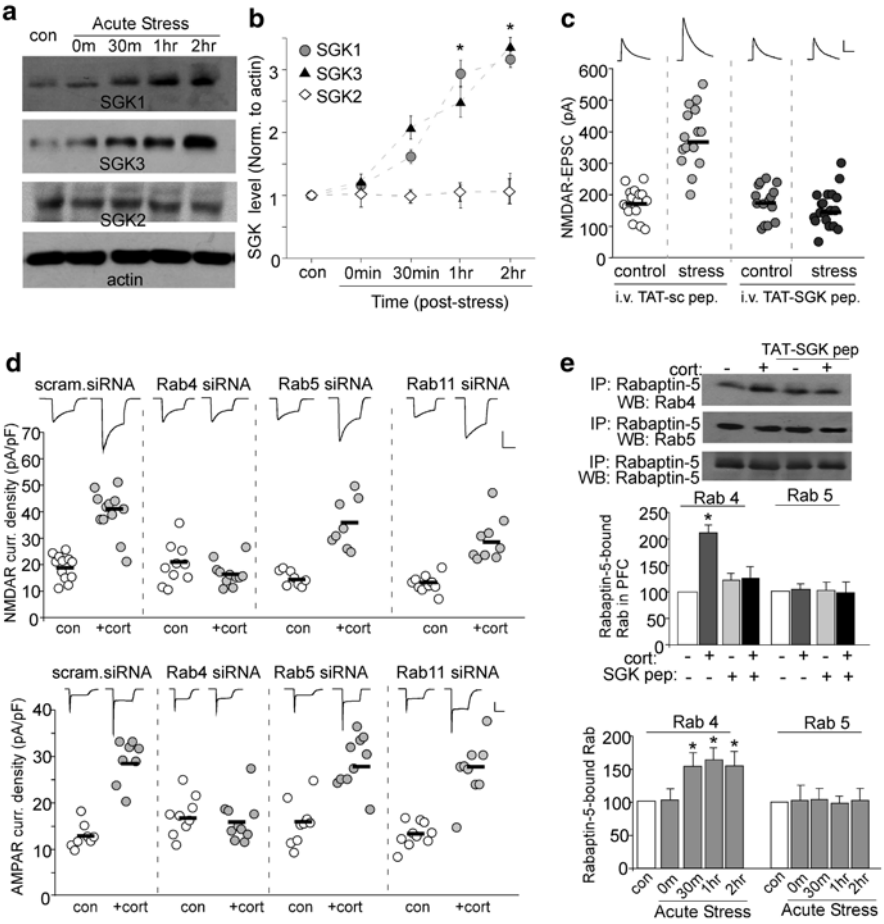

Fig. 4.3 Serum- and glucocorticoid-inducible kinase (*SGK*)/Rab4 signaling is required for acute stress-induced potentiation of glutamatergic transmission. **a, b** Western blots (**a**) and quantification (**b**) of SGKs in lysates of PFC slices taken from control or acutely stressed animals at various post stress time points (0 min, 30 min, 1 and 2 h). *$p < 0.001$. **c** Dot plots of N-methyl-D-aspartate receptor (*NMDAR*)-excitatory postsynaptic current (*EPSC*) recorded in prefrontal cortex (*PFC*) slices from control versus acutely stressed animals i.v. injected with TAT-SGK peptide (0.6 pmol/g) or a scrambled control peptide (TAT-sc, 0.6 pmol/g). Peptides were administered 30 min prior to stress, and recordings were performed at 1–4 h post stress. *Inset*: Representative NMDAR-EPSC traces. *Scale bars:* 100 pA, 100 ms. **d** Dot plots showing the effect of corticosterone (*CORT*) treatment (100 nM, 20 min) on NMDAR or α-amino-3-hydroxy-methyl-4-isoxazole propionic acid receptor (*AMPAR*) current density in PFC cultures transfected with a scrambled siRNA or siRNA against Rab4, Rab5, or Rab11. Recordings were obtained 1–4 h after the treatment. *Inset*: Representative current traces. *Scale bars*: 200 pA, 1 s. **e** Co-immuoprecipitation blots and analysis showing the level of active (Rabaptin-5-bound) Rab4 or Rab5 in PFC slices without or with corticosterone treatment (100 nM, 20 min, collected 1 h after treatment) in the absence or presence of TAT-SGK peptide (50 μM, 30 min prior to corticosterone, *top*), or in PFC slices from control versus swim-stressed animals examined at various post stress time points (*bottom*). *IP* immunoprecipitation, *WB* Western blot. (Adapted from Yuen et al. 2011)

To further test the involvement of Rab4, we examined whether acute stress could increase the activity of this small GTPase. We found that the level of active Rab4 was significantly increased by acute stress or corticosterone treatment (100 nM, 20 min), which was blocked by pretreatment of PFC slices with TAT-SGK peptide (Fig. 4.3e). It suggests that acute stress selectively increases Rab4 activity in PFC via SGK signaling, which may facilitate the recycling of glutamate receptors to plasma membrane.

For repeatedly stressed animals, since the total level of NR1 and GluR1 was reduced, we examined whether it could be due to the decreased synthesis or increased degradation of glutamate receptors. We found that repeated stress did not significantly alter the mRNA level of AMPAR and NMDAR subunits, suggesting that protein synthesis is intact. Thus, the reducing effect of repeated stress on NR1 and GluR1 expression may be due to the increased ubiquitin/proteasome-dependent protein degradation. Consistent with this, the level of ubiquitinated GluR1 and NR1 was significantly increased in animals exposed to repeated restraint stress, which was blocked by injecting the GR antagonist RU486 (Fig. 4.4a, b). The level of ubiquitinated GluR2, NR2A, or NR2B subunits remained unchanged (Fig. 4.4c). Repeated stress also failed to alter the ubiquitination of SAP97 (a GluR1 binding protein) and PSD-95 (an NR1 binding protein, Fig. 4.4c). These results provide direct evidence showing that prolonged GR activation selectively increases ubiquitin conjugation of GluR1 and NR1 subunits in PFC and thus enhances the susceptibility of these proteins to proteasome-mediated degradation.

To further test the role of glutamate receptor degradation in chronic stress-induced reduction of synaptic transmission, we injected the proteasome inhibitor MG132 to PFC via an implanted cannula. As shown in Fig. 4.5a, repeated stress caused a substantial down-regulation of eEPSC amplitude in saline-injected animals, but had little effect in MG132-injected animals. Biochemical measurement of glutamate receptor subunits in PFC slices (Fig. 4.5b) indicated that MG132-injected rats exhibited the normal level of GluR1 and NR1 after being exposed to 7-day restraint stress, which was in sharp contrast to the reduced expression of GluR1 and NR1 in saline-injected rats after repeated stress. Taken together, these results suggest that repeated behavioral stress induces the ubiquitin/proteasome-dependent degradation of GluR1 and NR1, leading to the depression of glutamatergic transmission in PFC.

To find out which E3 ubiquitin ligases are potentially involved in the repeated stress-induced ubiquitination of GluR1 and NR1 subunits in PFC, we focused on two possible candidates, Nedd4–1 (neural-precursor cell-expressed developmentally downregulated gene 4-1), an E3 ligase necessary for GluR1 ubiquitination in response to the agonist AMPA (Schwarz et al. 2010; Lin et al. 2011), and Fbx2, an E3 ligase in the ER that ubiquitinates NR1 subunits (Kato et al. 2005). Nedd4-1 or Fbx2 shRNA lentivirus was delivered to rat frontal cortex via a stereotaxic injection to knockdown these E3 ligases in vivo. As illustrated in Fig. 4.5c, d, repeated stress caused a substantial down-regulation of the eEPSC amplitude in green fluorescent protein (GFP) lentivirus-injected animals, but had little effect on AMPAR-EPSC in Nedd4 shRNA lentivirus-injected animals or on NMDAR-EPSC in Fbx2 shRNA

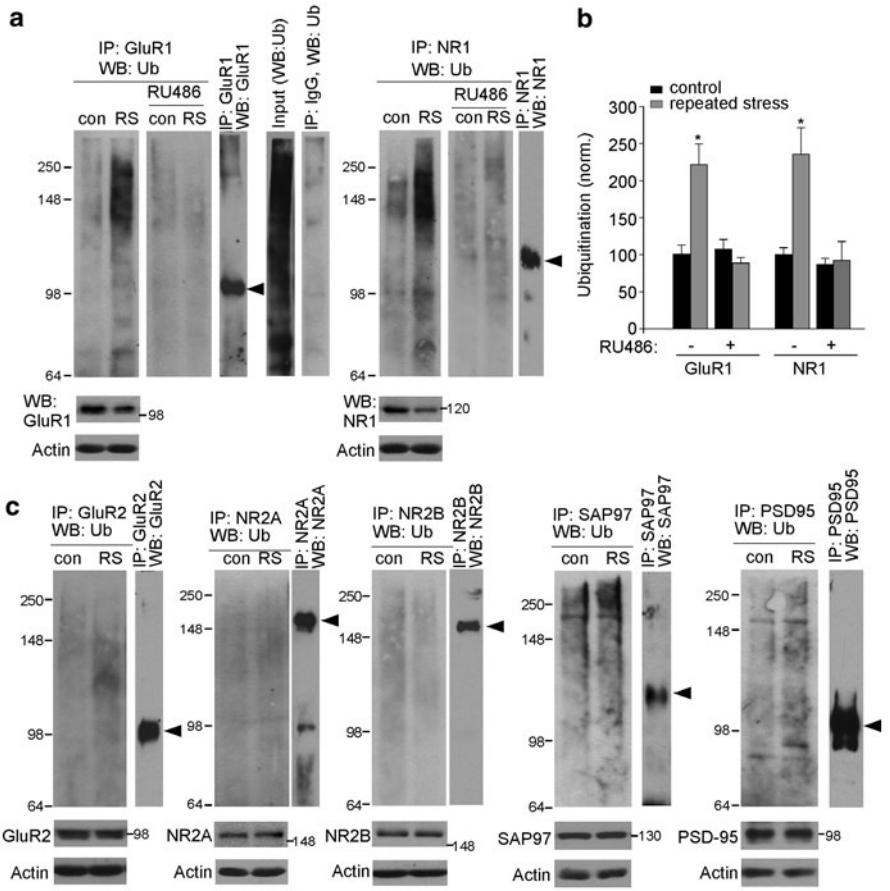

Fig. 4.4 Repeated stress increases the ubiquitination level of GluR1 and NR1 subunits. **a, b** Representative blots (**a**) and quantification (**b**) showing the ubiquitination of GluR1 and NR1 subunits in control versus stressed (7-day restraint) animals without or with RU486 injection (10 mg/kg). $*p<0.01$, *t* test. Lysates of PFC slices were immunoprecipitated with an antibody against GluR1 or NR1, and then blotted with a ubiquitin (*Ub*) antibody. Also shown are the input control, the immunoprecipitation control, and the immunoblots of total proteins in control versus stressed animals. Note, in stressed rats, the immunoprecipitated GluR1 or NR1 showed ubiquitin staining at a molecular mass heavier than the unmodified protein itself. The ladder of ubiquitinated GluR1 or NR1 is typical of proteins that are polyubiquitinated to signal their degradation. (**c**) Ubiquitination of GluR2, NR2A, NR2B, SAP97, and PSD-95 in control versus stressed (7-day restraint) animals. *IP* immunoprecipitation, *WB* Western blot, *RS* restraint stress, *IgG immunoglobulin G*. (Adapted from Yuen et al. 2012)

lentivirus-injected animals. Nedd4-1 shRNA or Fbx2 shRNA lentivirus-injected rats also failed to exhibit the increased level of ubiquitinated GluR1 or NR1 after being exposed to 7-day restraint stress (data not shown). These results suggest that Nedd4-1 and Fbx2 mediate the repeated stress-induced downregulation of AMPAR and NMDAR responses in PFC, respectively.

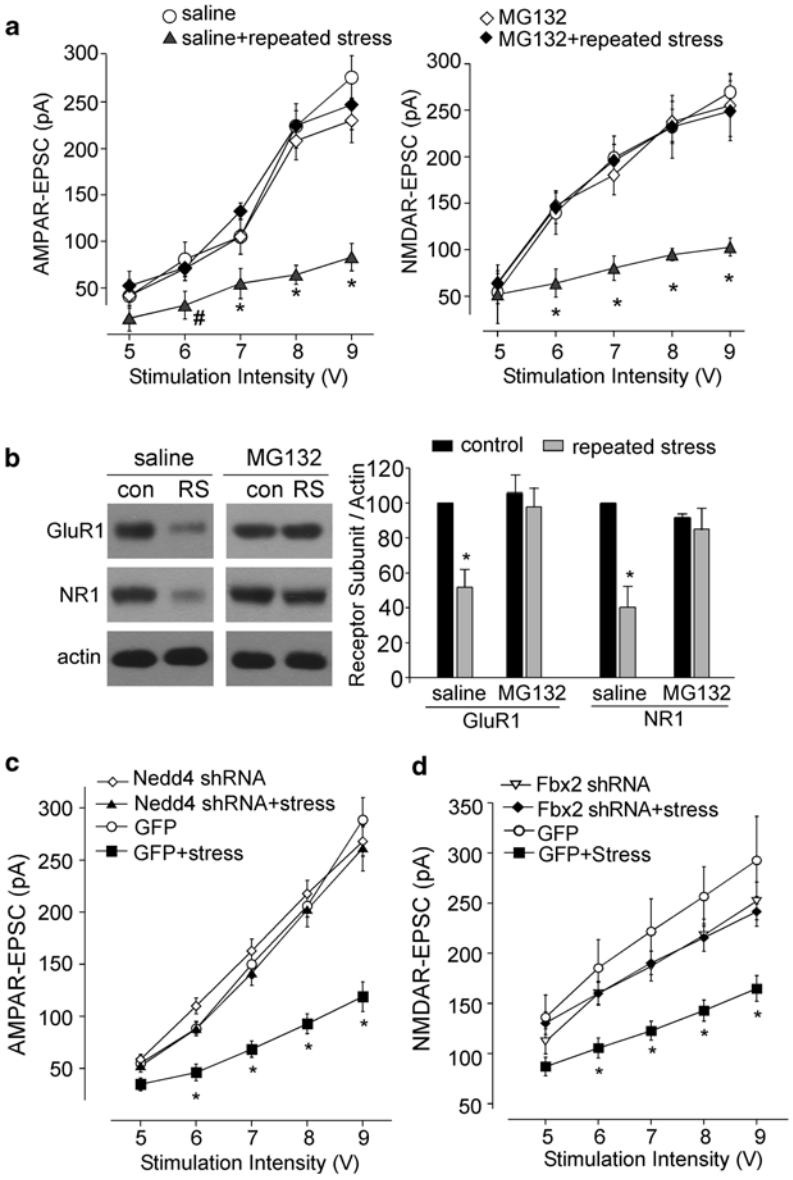

Fig. 4.5 PFC infusion of a proteasome inhibitor or knockdown of the E3 ubiquitin ligases Nedd4-1 and Fbx2 prevents the loss of glutamate receptors by repeated stress. **a** Summarized input-output curves of α-amino-3-hydroxy-methyl-4-isoxazole propionic acid receptor (*AMPAR*)-EPSC or N-methyl-D-aspartate receptor (*NMDAR*)- excitatory postsynaptic current (*EPSC*) in control versus repeatedly stressed (7-day restraint) animals with local injection of the proteasome inhibitor MG132 or saline control. $*p<0.01$, $^{\#}p<0.05$, ANOVA. **b** Immunoblots and quantification analysis of GluR1 and NR1 expression in control versus repeatedly stressed animals with PFC infusion of MG132 or saline. $*p<0.01$, *t* test. **c, d** Summarized input-output curves of AMPAR-EPSC (**c**) or NMDAR-EPSC (**d**) in control versus repeatedly stressed (7-day restraint) rats with the PFC injection of Nedd4-1 shRNA lentivirus (**c**), Fbx2 shRNA lentivirus (**d**), or GFP lentivirus control. $*p<0.01$, ANOVA. *RS* restraint stress, *GFP* green fluorescent protein. (Adapted from Yuen et al. 2012)

4.3.3 Behavioral Consequences of the Dual Effects of Stress on Glutamate Transmission

Since AMPAR- and NMDAR-mediated synaptic transmission at recurrent synapses in PFC networks is crucial for WM, the acute stress-induced enhancement of glutamatergic responses could be linked to improved WM in animals exposed to acute stress. Thus, we performed behavioral tests using the delayed alteration task in the T-maze, a well-established protocol for PFC-mediated WM. As shown in Fig. 4.6a, animals exposed to the acute forced-swim stress performed significantly better when examined at 4 h post stress or 1 day post stress. This difference disappeared at 2 day post stress. Except for the correctness, other parameters, such as the completion time and locomotor activity, were not significantly different between control versus stressed groups. These results indicate that acute stress facilitates WM within the time frame of a few hours to 1 day.

To test whether acute stress enhances WM via GR signaling, we injected (i.p.) animals with RU486 at 30 min prior to the stress procedure, and compared behavioral performance at 4 h or 1 day post stress. As shown in Fig. 4.6b, acutely stressed animals injected with saline showed better performance in the delayed alternation task. Injection of RU486 abolished the enhancing effect of acute stress on WM. These data suggest that the acute stress-induced enhancement of WM is mediated by GR activation.

To provide a "causal link" between stress-induced changes in glutamatergic transmission and WM, we tested whether TAT-SGK peptide, which blocked the effect of acute stress on glutamatergic transmission in vitro, could influence the effect of acute stress on WM. TAT-SGK peptide was stereotaxically injected into PFC prelimbic regions bilaterally via an implanted guide cannula. As shown in Fig. 4.6c, the enhancing effect of acute stress on WM was blocked by TAT-SGK peptide completely. These data suggest that GR/SGK-mediated enhancement of glutamatergic transmission within PFC may underlie the positive effect of acute stress on WM.

To test the impact of repeated stress on cognitive functions, we measured the recognition memory task, a fundamental explicit memory process requiring judgments of the prior occurrence of stimuli based on the relative familiarity of individual objects, the association of objects and places, or the recency information (Ennaceur and Delacour 1988; Dix and Aggleton 1999). Lesion studies have shown that medial PFC plays an obligatory role in the temporal order recognition (TOR) memory (Barker et al. 2007), so this behavioral task was used. Young (4-week-old) male rats, which had been exposed to 7-day repeated behavioral stressors, were examined at 24 h after stressor cessation.

The control groups spent much more time exploring the novel (less recent) object in the test trial, while the repeatedly stressed rats (restraint, 2 h/day, 7 days) lost the preference to the novel object. The discrimination ratio (DR), an index of the object recognition memory, indicated a profound impairment of TOR memory by repeated stress, which was blocked by injection of the GR antagonist RU486 (Fig. 4.7a). In contrast to the impaired TOR memory, rats exposed to repeated restraint stress showed no changes in anxiety-related behavior or locomotive activity (data not shown).

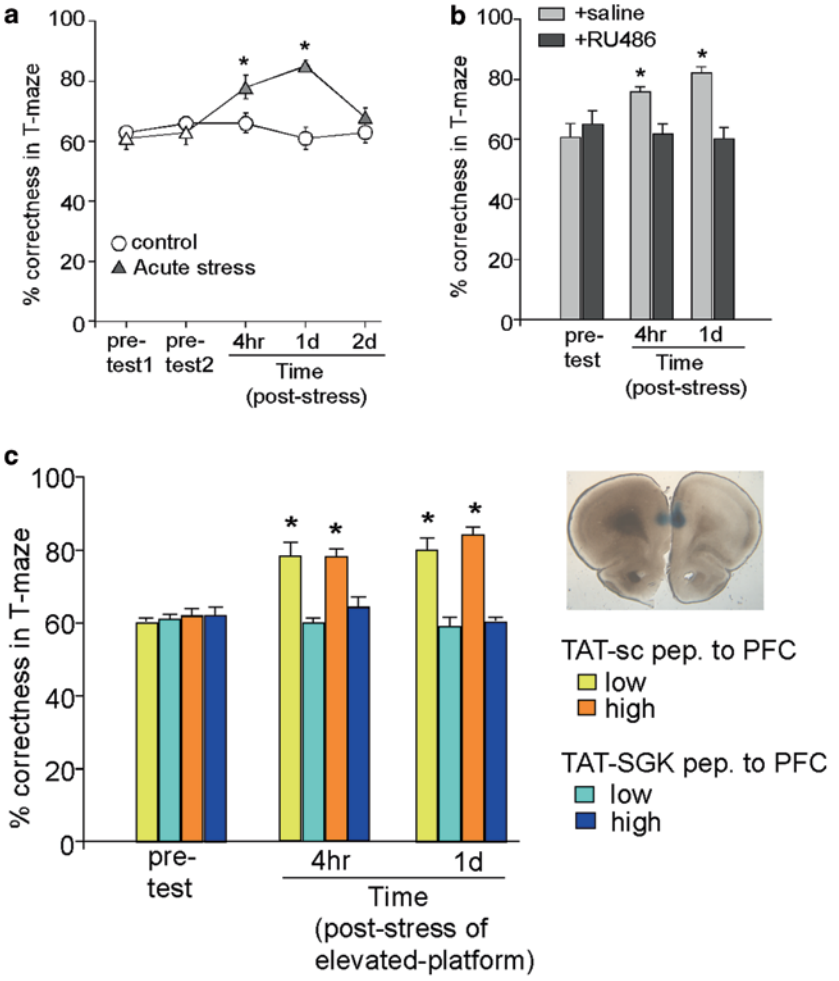

Fig. 4.6. Acute stress enhances working memory via a GR/serum- and glucocorticoid-inducible kinase (*SGK*)-dependent mechanism. **a** Cumulative data (mean ± SEM) showing percentage correct of responses in T-maze tests in control versus stressed (forced-swim) rats examined at various pre and post stress time points. *$p<0.01$, ANOVA. **b** Cumulative data (mean ± SEM) showing percentage correct in T-maze tests before and after forced-swim stress in rats injected with saline versus RU486. *$p<0.01$, ANOVA. **c** Cumulative data (mean ± SEM) showing the percentage correctness in T-maze tests before and after elevated platform stress in rats locally injected to prefrontal cortex (*PFC*) with TAT-SGK peptide versus scrambled TAT-sc peptide (high dose: 40 pmol/g; low dose: 0.12 pmol/g). *Inset:* A photograph showing the slice with a local injection of ink to PFC prelimbic regions to confirm the appropriate location of the cannula. *$p<0.01$, ANOVA. (Adapted from Yuen et al. 2009, 2011)

To test whether glutamatergic transmission in PFC is critical for the object recognition memory, we gave animals a stereotaxic injection of the NMDAR antagonist 2-amino-5-phosphonopentanoic acid (APV) and AMPAR antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to PFC prelimbic regions bilaterally. As

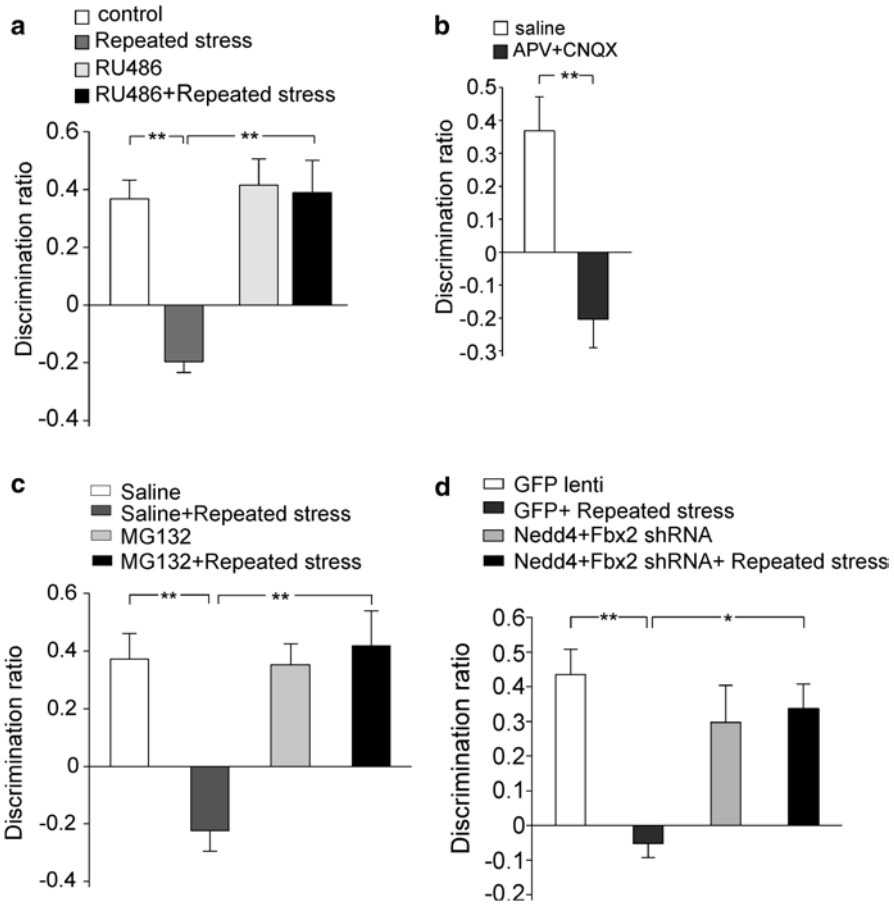

Fig. 4.7 Repeated stress impairs TOR memory, which involves the ubiquitin/proteasome-mediated degradation of glutamate receptors. **a** Bar graphs showing the DR of TOR tasks in control groups versus animals exposed to 7-day restraint stress without or with RU486 injection (10 mg/kg, i.p. daily at 30 min before stress). $^{**}p<0.001$, ANOVA. **b** Bar graphs showing the DR of TOR tasks in animals with PFC infusion of saline versus glutamate receptor antagonists (2-amino-5-phosphonopentanoic acid; *APV*: 1 mM, 6-cyano-7-nitroquinoxaline-2,3-dione, *CNQX*: 0.2 mM, 1 µl each side). The infusion was performed via an implanted cannula at 20 min before behavioral experiments. $^{**}p<0.001$, *t* test. **c** Bar graphs showing the discrimination ratio of TOR tasks in control groups versus repeatedly stressed animals (7-day restraint) with stereotaxic injections of saline or MG132 (0.5 µg each side; 21 pmol/g b.w., daily at 1 h before stress) into PFC via an implanted cannula. $^{**}p<0.001$, ANOVA. **d** Bar graphs showing the discrimination ratio of TOR tasks in control groups versus repeatedly stressed animals (7-day restraint) with PFC injection of GFP lentivirus or Nedd4-1 shRNA+Fbx2 shRNA lentiviruses. $^{**}p<0.001$, $^{*}p<0.01$, ANOVA. *GFP* green fluorescent protein. (Adapted from Yuen et al. 2012)

shown in Fig. 4.7b, APV+CNQX-injected animals lost the normal preference to the novel (less recent) object, similar to the animals exposed to repeated stress. Taken together, it suggests that repeated stress has a detrimental effect on recognition memory, which may be due to the loss of glutamatergic transmission in PFC.

To find out whether the proteasome-dependent degradation of glutamate receptors induced by repeated stress may underlie its detrimental effect on cognitive processes, we examined the TOR memory in animals with stereotaxic injections of MG132 into PFC bilaterally. As shown in Fig. 4.7c, MG132-injected animals exposed to repeated stress had normal TOR memory.

To find out the role of Nedd4-1 and Fbx2 in the repeated stress-induced detrimental effect on cognitive processes, we examined the TOR memory in animals with in vivo knockdown of both E3 ligases in PFC. As shown in Fig. 4.7d, the stress-induced TOR deficit was blocked in animals injected with both Nedd4-1 and Fbx2 shRNA lentiviruses to PFC. These behavioral data suggest that the cognitive impairment by repeated stress may be due to the Nedd4-1 and Fbx2-dependent loss of glutamate receptors in PFC.

4.4 Discussion

It is known that stress exerts complex influence on learning and memory processes, which is usually dependent on the action of stress hormones in combination with neuronal activities within the key target areas (Shors 2006). Mounting evidence has suggested that corticosteroid stress hormones induce divergent changes in different brain regions (de Kloet et al. 2005; McEwen 2007). In addition to the region specificity, the outcome is also determined by the duration and severity of the stressor (de Kloet et al. 2005; Joëls 2006). We have found that acute stress induces a long-lasting potentiation of glutamatergic transmission in PFC and facilitate WM (Yuen et al. 2009, 2011), which is in contrast to the strong suppression of PFC glutamatergic transmission and impairment of object recognition memory by repeated stress (Yuen et al. 2012). Thus, glutamate receptors seem to be a neural substrate that underlies the biphasic effects of stress and glucocorticoids on synaptic plasticity and memory (Diamond et al. 1992; Groc et al. 2008; Krugers et al. 2010; Popoli et al. 2012).

We show that acute stress facilitates WM in young rodents, which is correlated with the increased PFC glutamatergic transmission and glutamate receptor surface expression by acute stress (Yuen et al. 2009). Inhibiting SGK, which blocks stress-induced enhancement of glutamatergic transmission, also blocks stress-induced facilitation of WM, suggesting that the GR/SGK/Rab4-induced glutamate receptor trafficking in PFC may underlie the WM improvement by acute stress (Yuen et al. 2011). These results (Fig. 4.8a) have identified a form of long-term potentiation of synaptic transmission induced by natural stimuli in vivo, providing a potential molecular and cellular mechanism for the beneficial effects of acute stress on cognitive processes subserved by PFC.

On the other hand, the loss of glutamate receptors after repeated stress may involve the increased ubiquitin/proteasome-mediated degradation of GluR1 and NR1

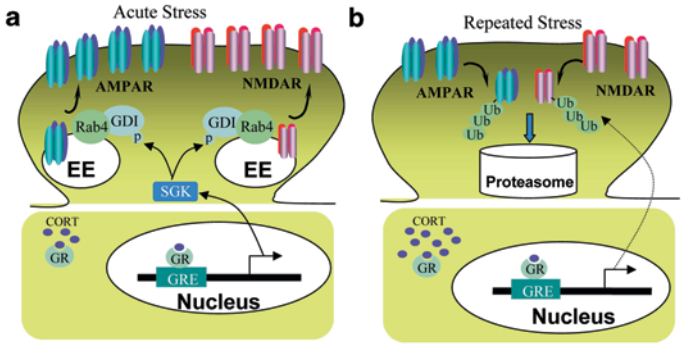

Fig. 4.8 A diagram illustrating the stress-induced changes in glutamate receptor trafficking and function in PFC. **a** In response to acute stress, glucocorticoid receptor (*GR*) activation triggers the upregulation of SGK1/3 (Yuen et al. 2011), leading to the phosphorylation of GTP dissociation inhibitor (GDP dissociation inhibitor *(GDI)*) and increased formation of GDI-Rab4 complex (Liu et al. 2010). Consequently, the functional cycle of Rab4 is facilitated and the Rab4-mediated recycling of N-methyl-D-aspartate receptors (*NMDARs*) and α-amino-3-hydroxy-methyl-4-isoxazole propionic acid receptors (*AMPARs*) from early endosome to plasma membrane is enhanced, resulting in the increased glutamate receptors at the synaptic membrane and potentiated glutamatergic transmission (Yuen et al. 2009, 2011). **b** In response to chronic stress, GR activation leads to the increased ubiquitination of NR1 and GluR1 subunits, probably via activating the E3 ubiquitin ligase Fbx2 and Nedd4, respectively. Consequently, the proteasome-mediated degradation of NMDARs and AMPARs is enhanced, leading to the loss of glutamate receptors from the synaptic membrane and depressed glutamatergic transmission (Yuen et al. 2012). *CORT* corticosterone, *EE* early endosome, *GRE* glucocorticoid response element. (Adapted from Popoli et al. 2012)

subunits. Posttranslational modification through the ubiquitin pathway at the postsynaptic membrane has emerged as a key mechanism for remodeling synaptic networks and altering synaptic transmission (Mabb and Ehlers 2010). Abnormalities in the brain ubiquitin/proteasome system have been implied in a variety of neurodegenerative and mental disorders (Ciechanover and Brundin 2003; Middleton et al. 2002), however little is known about the circumstances under which AMPAR and NMDAR ubiquitination occurs under normal and disease conditions. We demonstrate that the ubiquitination of GluR1 and NR1 subunits, but not their anchoring proteins, is specifically increased in PFC slices upon GR activation following repeated stress. The effect of repeated stress on glutamatergic responses and GluR1/NR1 expression is blocked by the specific inhibitors of proteasomes. This suggests that GR-induced ubiquitination of GluR1 and NR1 subunits tags them for degradation by proteasomes in the cytoplasm, therefore fewer heteromeric AMPARs and NMDARs channels are assembled and delivered to the synaptic membrane (Fig. 4.8b). Interestingly, infusion of a proteasome inhibitor into PFC prevents the loss of recognition memory in stressed animals, providing a potential approach to block the detrimental effects of repeated stress. The identification of E3 ligases involved in the effects of repeated stress provides drug targets for preventing chronic stress-induced impairment of cognitive processes.

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