Corticosterone mediates the synaptic and behavioral effects of chronic stress at rat hippocampal temporoammonic synapses

Mark D. Kvarta,1,3,4 Keighly E. Bradbrook,5 Hannah M. Dantrassy,5 Aileen M. Bailey,5 and Scott M. Thompson1,2,3

1Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland; 2Department of Psychiatry, University of Maryland School of Medicine, Baltimore, Maryland; 3Programs in Neuroscience and Membrane Biology, University of Maryland School of Medicine, Baltimore, Maryland; 4Medical Scientist Training Program, University of Maryland School of Medicine, Baltimore, Maryland; and 5Department of Psychology, Saint Mary’s College of Maryland, St. Mary’s City, Maryland

Submitted 10 April 2015; accepted in final form 13 July 2015

Kvarta MD, Bradbrook KE, Dantrassy HM, Bailey AM, Thompson SM. Corticosterone mediates the synaptic and behavioral effects of chronic stress at rat hippocampal temporoammonic synapses. J Neurophysiol 114: 1713–1724, 2015. First published July 15, 2015; doi:10.1152/jn.00359.2015.—Chronic stress is thought to impart risk for depression via alterations in brain structure and function, but contributions of specific mediators in generating these changes remain unclear. We test the hypothesis that stress-induced increases in corticosterone (CORT), the primary rodent glucocorticoid, are the key mediator of stress-induced depressive-like behavioral changes and synaptic dysfunction in the rat hippocampus. In rats, we correlated changes in cognitive and affective behavioral tasks (spatial memory consolidation, anhedonia, and depression) with impaired excitatory strength at temporoammonic-CA1 (TA-CA1) synapses, an archetypical stress-sensitive excitatory synapse. We tested whether elevated CORT was sufficient and necessary to generate a depressive-like behavioral phenotype and decreased excitatory signaling observed at TA-CA1 after chronic unpredictable stress (CUS). Chronic CORT administration induced an anhedonia-like behavioral state and neohyopophagic behavior. Like CUS, chronic, but not acute, CORT generated an impaired synaptic phenotype characterized by reduced α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-preferring glutamate receptor-mediated excitation at TA-CA1 synapses, decreased AMPA-type glutamate receptor subunit 1 protein expression, and altered serotonin-1B receptor-mediated potentiation. Repeatedly blunting stress-induced increases of CORT during CUS with the CORT synthesis inhibitor metyrapone (MET) prevented these stress-induced neurobehavioral changes. MET also prevented the CUS-induced impairment of spatial memory consolidation. We conclude that corticosterone is sufficient and necessary to mediate glutamatergic dysfunction underlying stress-induced synaptic and behavioral phenotypes. Our results indicate that chronic excessive glucocorticoids cause specific synaptic deficits in the hippocampus, a major center for cognitive and emotional processing, that accompany stress-induced behavioral dysfunction. Maintaining excitatory strength at stress-sensitive synapses at key loci throughout corticomisomis disconnects cerebellum appears critical for maintaining normal cognitive and emotional behavior.

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–preferring glutamate receptor; anhedonia; depression; metyrapone; synaptic strength


In animal models, chronic stress triggers depressive-like changes in motivated reward behaviors, such as anhedonia, as well as synaptic and neuronal dysfunction. Chronic, but not acute, antidepressants restore both normal behavior and synaptic function in stressed animals (e.g., FALES ET AL. 2009). In the rodent hippocampus, chronic stress decreases dendritic spine size and number, and decreases AMPA-type glutamate receptor subunit 1 (GluA1) mRNA in pyramidal cells, particularly in the most distal apical dendrites (MAGARITOS AND McEWEN 1995a; SOUSA ET AL. 2000; SCHMIDT ET AL. 2010). At these synapses, formed by inputs from the entorhinal cortex via the temporoammonic (TA) pathway (STEWARD AND SCOVILLE 1976), chronic stress decreases α-amino-3-hydroxy-5-methyl-
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4-isoxazolepropionic acid-preferring glutamate receptor (AMPA)-mediated signaling and alters serotonin-mediated potentiation of excitatory transmission (Cai et al. 2013; Kallarackal et al. 2013; Fischell et al. 2015). This stress-induced synaptic phenotype is accompanied by impaired consolidation of spatial memories (Kallarackal et al. 2013), a function of TA-CA1 synapses (Remondes and Schuman 2004). Taken together with stress-induced decreases in AMPAR-mediated excitation in the PFC (Yuen et al. 2012) and nucleus accumbens (Lim et al. 2012), stress may promote depressive symptoms by weakening excitatory synaptic transmission at multiple specific sites in corticoesolimbic reward circuitry (Thompson et al. 2015).

How does chronic stress alter behavior and synaptic structure and function? Chronic stress activates the hypothalamic-pituitary-adrenal (HPA) axis and causes elevation of corticosteroids and other stress hormones (Pitman et al. 1988). HPA dysregulation is particularly prevalent in depressed patients with melancholia (up to 90%), as identified using modified criteria relying centrally on anhedonia (Taylor and Fink 2008). Chronic administration of exogenous corticosterone (CORT), the principal corticosteroid in rodents, produces behavioral changes resembling those seen after chronic unpredictable stress (CUS), a validated and widely used intervention to generate a depressive-like behavioral profile of decreased reactivity to rewarding stimuli (Willner et al. 1987; Willner 2005; Gourley and Taylor 2009).

We hypothesized that repeated stress-induced elevations of CORT are necessary and sufficient to cause not only the behavioral changes following CUS, but also the associated synaptic dysfunction. To test this hypothesis, we chronically administered exogenous CORT to unstressed rats. In separate experiments, we administered the CORT synthesis inhibitor metyrapone during CUS to blunt peak stress-induced increases in CORT. In addition to behavioral assays for anhedonia and hyponeophagia [a preference for neophobic behavior over feeding motivation in an ethologically relevant novelty-suppressed feeding (NSF) task], we examined several neurobiological consequences of chronic stress at the synaptic level. We demonstrate that chronic elevations of CORT are necessary and sufficient for decreased GluA1 expression and TA-CA1 excitation, aberrant serotonin-mediated plasticity, anhedonic and hyponeophagic behavior, and impaired spatial memory consolidation induced by chronic stress.

MATERIALS AND METHODS

All protocols were submitted to and approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee.

Subjects. Male Sprague-Dawley rats (3–4 wk old at start; Harlan Laboratories) kept on a regular 12:12-h light-dark cycle were group housed. All rats killed for in vitro electrophysiology and molecular biology experiments were 7–8 wk old and were previously tested for sucrose preference and NSF. In the water maze experiment, rats obtained from Charles River were trained in the water maze at 3–4 wk old. They were tested for memory consolidation at 7–8 wk and were tested weekly for sucrose preference.

Corticosterone administration. CORT was dissolved in tap water (50 μg/ml) and administered in water bottles so as to elevate plasma CORT levels in a manner tied to diurnal activity cycles (Gourley and Taylor 2009). This dose induces several depressive-like behaviors (reduced sucrose intake and impaired forced swim performance) over several weeks, paralleling chronic stress models. After a one-night baseline sucrose preference test, rats were randomized to receive either the CORT solution or regular tap water ad libitum for 3–4 wk. Over the course of the paradigm, rats were exposed to an average dose of 7.1 mg·kg−1·day−1.

CUS and metyrapone administration. Rats were exposed to two stressors per light cycle for 3–4 wk, as described previously (Willner et al. 1992; Cai et al. 2013). Stressors included restraint (30 min), strobe light (30 min), forced swim in cold water (5 min), cage rotation (3 h), cage tilt (3 h), white noise (3 h), and overnight food deprivation. Rats were randomized to receive either metyrapone (MET, 50 mg/kg ip) or vehicle (VEH) (60% sterile saline and 40% polyethylene glycol) before each stressor. Another cohort of rats received injections of either MET or VEH without exposure to stress. These animals were given injections on the same schedule as those exposed to CUS.

Sucrose preference test. While singly housed for a single dark cycle, control and treated rats were presented with two identical bottles placed on the cage top, containing either tap water or a dilute sucrose solution for 16 h. Rats were familiarized with the task once with 2% sucrose, then tested with 1% for subsequent tests including baseline and final readouts. Tests lasted from 2 h before the dark cycle to 2 h after. Single housing during this period is potentially a stressor. Data are quantified as sucrose solution consumed as a percent of total fluid consumption.

NSF test. Rat chow pellets were placed in the center of a brightly lit arena in a dark room, as described previously (Santarelli et al. 2003; Dulawa 2009; Cai et al. 2013). Control and treated rats were placed in the corner of the arena, and latency to feed was measured. After one bite, the rat was returned to the familiar home cage to feed ad libitum for 5 min, to ensure sufficient hunger (all consumed >0.05 g). No group tested fed significantly more or less than any other groups, demonstrating no major effect on appetite overall (P > 0.05, 1-way ANOVA). Rats that did not feed in the arena were assigned the maximum time allowed. These data were therefore treated as ordinal. The chamber was cleaned between rats with 70% ethanol and a 0.01% sodium hypochlorite solution diluted from household bleach. In the chronic CORT experiment (Fig. 1), rats were first food deprived for 24 h to instigate feeding behavior, and the maximum time was 400 s. In the CUS ≥ MET experiment (see Fig. 4), rats were food deprived for 16 h (as part of the CUS paradigm), and the maximum time was 600 s. Food deprivation preceding the task is potentially a stressor.

Acute slice electrophysiology. Standard methods were used to prepare 400-μm-thick transverse slices from middle and ventral hippocampus. Dissection and recording were performed in artificial cerebrospinal fluid (ACSF) containing (in mM) 120 NaCl, 3 KCl, 1.0 NaH2PO4, 1.5 MgSO4·7H20, 2.5 CaCl2, 25 NaHCO3, and 20 glucose and was bubbled with carbogen (95% O2-5% CO2). Slices were then transferred to a submerison-type recording chamber and perfused at 20–22°C (flow rate = 0.5–2 ml/min). Picrotoxin (100 μM) and CGP-52432 (2 μM) were included to block GABA_A and GABA_B receptors, respectively. The DG and CA3 regions were removed via microdissection to prevent retrograde excitation of CA3 and anterograde activation of area CA1.

Synaptic currents at TA-CA1 synapses are most accurately assayed using local extracellular recordings of local field excitatory postsynaptic potentials (fEPSPs) because they are electrotonically remote from CA1 cell somata. Recording pipettes (3–5 MΩ) containing ACSF were placed in stratum lacunosum-moleculare (SLM). fEPSPs were amplified 1,000 times, filtered at 3 kHz, and digitized at 10 kHz. Concentric bipolar tungsten electrodes were placed >500 μm from the stimulating electrodes in SLM to stimulate TA afferents (100-μs stimuli, 0.05 Hz). The stimulus intensity was set to result in 0.1- to 0.3-mV fEPSPs.

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-to-N-methyl-d-aspartate (NMDA) ratios were recorded and quantified as in our previous work (Kallarackal et al. 2013) in Mg2+-free ACSF. Six to 10 consecutive responses were averaged with fiber volley (FV) am-

* J Neurophysiol doi:10.1152/jn.00359.2015 www.jn.org
plitudes nearest to 0.2 mV, which is in the linear range of responses to varying stimulus intensity (Kallarackal et al. 2013). The IEPSP slope in the initial rising phase was calculated over a 2-ms window, 2–5 ms after stimulus, for AMPAR-mediated responses. 6,7-Dinitroquinoxaline-2,3-dione (50 μM; Tocris) was then added to the ACSF perfusion for 15 min, and the slope of the response was calculated over 3–5 ms, at 5–10 ms after its initiation, for quantification of N-methyl-D-aspartate-preferring glutamate receptor (NMDAR)-mediated responses. 2-Amino-5-phosphoelavate (80 μM; Sigma-Aldrich, St. Louis, MO) was used to verify NMDAR-mediated responses. When multiple recordings were made from slices from a single animal, average AMPA/NMDA values in that animal were calculated to avoid the potential confound of nested data.

**Western blotting.** SLM or stratum pyramidale (SP) tissue punches (1 mm diameter) were taken from CA1 in hippocampal slices on a glass slide resting on dry ice and deposited in standard lysis buffer (protease and phosphatase inhibitor cocktail; Sigma-Aldrich). Antibodies used for each sample were performed using a standard Bradford assay (Coomassie reagent; Thermo Scientific, Rockford, IL). Antibodies used were rabbit anti-GluA1 (0.5 μg/ml; Chemicon), antigluocorticoid receptor (GR, 1:1,000; Millipore), and anti-β-actin (1:5,000; Cell Signaling Technology), and a horseradish peroxidase-linked antirabbit IgG secondary antibody (1:1,000; Cell Signaling Technology). Expression is quantified as signal intensity normalized to β-actin. Densitometry may nonlinearly transform data, so we analyzed Western Blots as ordinal, with nonparametric statistics. CORT was extracted with ethanol. After ethanol evaporation, extracts were reconstituted with methanol and diluted 1:20 for ELISA alongside CORT standards (Assaypro, St. Charles, MO). Fecal CORT reflects an integrated measure of several hours of prior serum corticosteroids. Stress-induced CORT in feces is observed within 6–12 h after stress (Bamberg et al. 2001; Harper and Austad 2012). This method is minimally invasive and is less susceptible to ultradian and circadian interference than single time points.

**Morris water maze.** Rats were trained, as described previously (Remondes and Schuman 2004; Kallarackal et al. 2013). Rats received 10 blocks of training (4 trials/block) over 6 days, with the platform in a fixed location. One day after training, rats completed a probe trial, with path length and latency to target measured. They were then subjected to CUS for 3 wk, administered MET or VEH, as above, and then left unstressed for 1 wk. On day 28 after training, long-term consolidation was tested with a probe trial.

**Statistics.** Data are presented as means ± SE. Statistics were calculated using SPSS (IBM, Armonk, NY) and Graphpad (Graphpad Software, La Jolla, CA). All data tested with parametric tests (t-tests for 2 groups, ANOVA for ≥2 with Bonferroni post hoc tests) were normally distributed and homoscedastic. Nonparametric statistics were used for ordinal data (NSF, blots).

### RESULTS

**Is elevated CORT sufficient to generate the neurobiological correlates of chronic stress?** We first tested whether chronic exogenous administration of CORT for 21 days would mimic the effects of CUS in causing reduced sucrose preference and neohypophagia in unstressed animals. Chronic CORT reduced sucrose preference [group × time interaction F(1,54) = 8.461, n = 57, P = 0.005 2 × 2 mixed ANOVA, P < 0.005 vs. all other groups; Fig. 1A], similar to previous work that reported reduced sucrose intake (Gourley et al. 2008). ELISA of CORT levels from fecal pellets demonstrated that chronic CORT treatment significantly elevates the total CORT in feces, as expected, and shows no difference compared with controls (n = 25, P = 0.36, t-test), SPT, sucrose preference test; ns, not significant.
amount of CORT exposure \((n = 21, P = 0.038)\) Student’s \(t\)-test; Fig. 1B). The total amount of liquid consumed did not differ by group, suggesting no effect of CORT treatment on water balance. Similarly, after 2 wk of treatment, CORT-treated animals exhibited a significantly increased latency to feed in the NSF test \((n = 11, P = 0.007,\) Mann-Whitney \(U\)-test; Fig. 1C). All control rats fed within the allotted time limit, whereas two of six CORT-treated rats never fed (Fig. 1D). The body weight of rats treated chronically with CORT was not different from controls \((n = 25, P = 0.36)\) Student’s \(t\)-test; Fig. 1E). Together with no difference in food consumed in the home cage at the conclusion of the NSF test \((n = 11, P = 0.6)\), we infer that the increased latency to feed is not driven by a change in overall appetite. These results demonstrate that chronic CORT treatment is sufficient to induce anhedonia and neohyphagia.

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CUS causes a decrease in the AMPAR-mediated component of excitatory postsynaptic fEPSPs at TA-CA1 synapses (Kallarackal et al. 2013). As with CUS, chronic CORT reduced AMPA-to-NMDA ratios \((P = 0.010, n = 18)\) Student’s \(t\)-test; Fig. 2, A and B). AMPAR strength was also reduced significantly following CORT administration when normalized to FV, a measure of the number of axons activated \((n = 18, P = 0.042, t\)-test; Fig. 2C). There was no difference in NMDA/FV responses \((n = 17, P = 0.4, t\)-test; Fig. 2C). Western blots revealed an \(-31\%\) reduction in GluA1 protein levels in SLM from CORT-treated animals compared with SLM in untreated animals \((P = 0.009, n = 19)\) Mann-Whitney \(U\)-test). Chronic CORT also downregulated GR protein levels in CA1 SP \((n = 8, P = 0.01,\) Mann-Whitney \(U\)-test), as observed following CUS (Sapolsky et al. 1984; Herman et al. 1995). Thus, chronic administration of CORT in

![Figure 2](http://jn.physiology.org/)

Fig. 2. Chronic CORT elevation decreases \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-preferring glutamate receptor (AMPAR)-mediated signaling at temporoammonic (TA)-CA1 synapses by decreasing GluA1 expression. A: example TA-CA1 field excitatory postsynaptic potentials (fEPSPs) recorded in stratum lacunosum-moleculare (SLM) in Mg\(^{2+}\)-free artificial cerebrospinal fluid (ACSF) before (black) and after (gray) 6,7-dinitroquinoxaline-2,3-dione (DNQX) wash-in, exemplifying representative AMPAR- and N-methyl-D-aspartate-preferring glutamate receptor (NMDAR)-mediated responses, respectively, TA-CA1 fEPSPs from chronic CORT-treated rats exhibited a marked reduction in AMPAR-mediated component relative to control, whereas TA-CA1 fEPSPs in slices from rats treated acutely with CORT were increased. \(\alpha\)-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) -to- N-methyl-D-aspartate (NMDA) ratios were calculated as the slope of the black AMPAR-mediated fEPSP divided by the slope of the gray NMDAR-mediated fEPSP. B: quantification of group data for experiments from A in which comparisons of the slope of each response were measured for responses generating a fiber volley (FV) of \(-0.2 \) mV. Chronic CORT treatment significantly reduced AMPA-to-NMDA ratios compared with controls \((P = 0.010, n = 18)\) Student’s \(t\)-test), whereas acute CORT treatment significantly increased AMPA/NMDA \((P = 0.046, n = 11, Student’s t\)-test). C: chronic CORT also reduced AMPAR-mediated signaling when normalized to FV compared with control \((P = 0.042)\), but not NMDAR-mediated signaling. D: left, chronic CORT decreased GluA1 protein expression in SLM, where TA-CA1 synapses are formed \((P = 0.009, n = 19,\) Mann-Whitney \(U\)-test). Right, in stratum pyramidale (SP), where pyramidal cell bodies reside, glucocorticoid receptor (GR) protein expression is also decreased \((n = 8, P = 0.01,\) Mann-Whitney \(U\)-test). *\(P < 0.05\).
unstressed animals was sufficient to cause deficits in TA-CA1 excitatory synapses that are comparable to those produced by CUS (Kallarackal et al. 2013).

We compared these results with the effects of acute CORT administration (Joëls 2006). In contrast to CUS and chronic CORT, acute (~16 h) CORT increased AMPA-to-NMDA ratios at TA-CA1 synapses (n = 11, P = 0.046, Student’s t-test; Fig. 2, A and B, right). Acute and chronic CORT elevations thus exert opposing actions on excitatory strength at TA-CA1 synapses.

Endogenous serotonin and the serotonin-1B receptor (5-HT_{1B})-selective agonist anpirtoline potentiate TA-CA1 synapses as a result of calcium/calmodulin-dependent kinase II-mediated phosphorylation of GluA1 (Cai et al. 2013). In brain slices from animals subjected to chronic stress, 5-HT_{1B}-mediated potentiation becomes greater in magnitude and irreversible (Cai et al. 2013). In slices from control rats, anpirtoline (50 μM) induced an average potentiation of fEPSP slope to 125 ± 8% of the baseline, which reversed to 95 ± 11% of baseline after 1 h of washout. In slices from chronic CORT rats, TA-CA1 fEPSPs increased to a maximum of 210 ± 32%, and remained at 196 ± 35% after washout [n = 18, interaction effect F(2,32) = 6.87, P < 0.05 2 × 3 mixed ANOVA, P < 0.05 Bonferroni post hoc CORT peak and washout vs. CORT baseline and all control; Fig. 3]. Therefore, chronic CORT was sufficient to alter TA-CA1 response to serotonergic modulation from reversible to persistent, as occurs after CUS.

Taken together, these results show that chronic CORT elevation is sufficient to mimic both the behavioral effects of CUS and the corresponding neurophysiological and molecular changes in excitatory synaptic transmission at TA-CA1 synapses. Elevated CORT alone is thus sufficient to mimic the behavioral and synaptic consequences of chronic stress in this model.

Is elevated CORT necessary to generate the neurobiological correlates of chronic stress? CUS causes a chronic elevation of average CORT levels and repeated spikes in CORT levels in response to each individual stressor (Sapolsky et al. 1984; Magariños and McEwen 1995a), as part of a complex constellation of concerted neuromodulator interactions (reviewed in Joëls and Baram 2009). HPA axis responses to unpredictable or varied stress do not habituate even after 3 wk (e.g., Fig. 1 in Magariños and McEwen 1995a; reviewed in Herman 2013). We predicted that if chronic stress-induced elevations in CORT are necessary for the changes triggered by CUS, then partially inhibiting CORT synthesis before each stressor with MET (50 mg/kg ip), an inhibitor of the adrenocortical enzyme 11β-hydroxylase (Holsboer 2000), would prevent the CUS-induced synaptic and behavioral deficits. During CUS, we tested whether MET attenuated the increase in basal CORT caused by stress-induced dysregulation of the HPA axis (Johnson and Yamamoto 2009). CORT was extracted from fecal pellets collected overnight, reflecting CORT levels integrated over a day of stress. The CUS-induced increase in CORT, observed in VEH-treated rats, was prevented by MET as measured by ELISA of fecal pellet extractions [2 × 2 ANOVA interaction F(1,37) = 4.45, P = 0.04, P < 0.05 Tukey’s honest significant difference (HSD) post hoc for CUS + VEH vs. all other groups; Fig. 4A]. In unprotected t-tests, MET treatment alone reduced fecal CORT compared with all other groups (P < 0.02). Furthermore, MET blunted peak plasma CORT during an individual restraint stressor (n = 11, P = 0.0008, Student’s t-test; Fig. 4A), as measured by radioimmunoassay.

Unlike VEH-treated rats subjected to CUS, MET-treated rats subjected to CUS (CUS + MET) maintained a high succes preference that was not different from the sucrose preference of unstressed MET- or VEH-treated (CUS + VEH) rats [n = 36, F(1,20) = 18.6, 4 × 2 mixed ANOVA P < 0.001, P < 0.05
CUS+VEH vs. baseline and all other groups; Fig. 4B). In the NSF test, CUS + MET rats exhibited a shorter latency to feed than CUS + VEH rats and were not different from unstressed VEH or MET rats (Kruskal-Wallis $H = 9.52$, $P = 0.0231$, $n = 33$, CUS + VEH U-test post hoc $P < 0.05$ vs. all others; Fig. 4C). Fewer CUS + VEH rats fed within the allotted time limit than any other group ($P < 0.05$, Fisher’s exact test; Fig. 4D). We found a main effect of CUS on body weight [$F(1,33) = 43.21$, $P < 0.0001$], but no effect of MET [$F(1,33) = 0.02$, $P = 0.89$; Fig. 4E], consistent with previous reports of lack of role of CORT in the weight loss associated with chronic stress (Magarinos and McEwen 1995b). Together with no difference in food consumed in the home cage at the conclusion of the NSF test ($n = 19$, $P = 0.4$, data not shown), we infer that the increased latency to feed is not driven by a change in overall appetite. In summary, limiting stress-induced increases in CORT prevented the stress induction of anhedonia and neohyphagia.

We predicted that MET would also prevent the changes observed at TA-CA1 synapses after CUS. AMPA-to-NMDA ratios were significantly lower in slices from CUS + VEH rats compared with CUS + MET and unstressed MET and VEH rats [2 × 2 ANOVA main effect of CUS [$F(1,54) = 14.71$, $P = 0.0003$] and MET [$F(1,54) = 11.93$, $P = 0.0011$, MET × CUS interaction $F(1,54) = 11.84$, $P = 0.0011$] $P < 0.01$ Tukey’s HSD post hoc, $n = 57$; Fig. 5, A and B). AMPA/FV responses were also lower in slices from CUS + VEH than CUS + MET rats and both non-CUS groups [main effect of MET $F(1,56) = 7.43$, $P = 0.0085$], whereas NMDAR/FV responses were not significantly different ($P > 0.05$ for both CUS and MET main effects). TA-CA1 AMPA/NMDA strength correlated significantly with hedonic state, measured by sucrose preference [$R = 0.52$, $r(29) = 3.293$, $P = 0.002$; Fig. 5B, right].

GluA1 expression in SLM decreased by 25% in tissue from CUS + VEH rats compared with CUS + MET rats ($n = 14$, $P = 0.049$, Mann-Whitney $U$-test; Fig. 5D). GR expression was also lower in SP of CUS + VEH rats compared with CUS + MET ($n = 8$, $P = 0.02$, Mann-Whitney $U$-test).

Finally, anipirtoline potentiated TA-CA1 eEPSP slope to 155 ± 5% of baseline after 60 min in slices from vehicle-injected CUS rats, peaked at 199.5 ± 14%, and remained elevated (166 ± 11%) after washout ($n = 32$, mixed ANOVA interaction effect $F(2,54) = 7.95$, $P = 0.009$. $P < 0.05$...
MET, CUS/H11001

CUSH11001

potentiation peaked at 149 ± 12% and reversed to 106 ± 27% after washout. MET thus prevented the exaggerated, persistent response to anpirtoline at TA-CA1 synapses observed normally after washout.

Taken together, these results show that blunting the elevation of CORT produced by the CUS stressors is sufficient to prevent the behavioral effects of CUS and the corresponding neurophysiological and molecular changes in excitatory synaptic transmission at TA-CA1 synapses.

Does MET prevent a CUS-induced TA-CA1 synapse-specific cognitive deficit? TA-CA1 synapses are required for long-term consolidation of spatial memory (Remondes and Schuman 2004), and CUS impairs this function (Kallarackal et al. 2013). Impaired spatial memory has been described in depressed humans (Gould et al. 2007). We asked whether blunting CUS-induced elevation of CORT with MET treatment during consolidation despite CUS exposure.

Because MET treatment prevented the effects of CUS on three separate behaviors (anhedonia, neohypophagia, and impaired spatial memory consolidation) and the neurophysiological and molecular deficits of excitatory synaptic transmission at a key stress-sensitive synapse, we conclude that chronically elevated CORT is a necessary mediator of the adverse effects of chronic stress at this pathway.

DISCUSSION

Physical and psychosocial stressors are significant risk factors for depression, in addition to anxiety and other mood disorders. Stress affects the hippocampus, a critical region for cognitive processing, mood, and other complex behaviors that are altered in depression, as well as synapses in the PFC and nucleus accumbens, two other regions involved in processing reward. Potential mediators of stress include endocannabinoids (Gray et al. 2013), corticotropin-releasing hormone (Chen et al. 2012), and other neuropeptides, opioids such as dynorphin (Mague et al. 2003; McLaughlin et al. 2003), monoamines such as serotonin and norepinephrine, glutamate (Palucha and...
7.95, potentiation was elicited in slices from CUS rats treated with MET as in unstressed controls. Repeated-measures mixed ANOVA interaction effect generated a significantly greater peak TA-CA1 response in slices from vehicle-treated rats, and it persisted during washout, whereas a reversible modest

10 min before the beginning of anpirtoline application. Chronic CORT administration induces effects of chronic stress. Chronic CORT was also sufficient to decrease AMPAR-mediated excitation and GluA1 subunit expression at TA-CA1 synapses in unstressed animals. The effect was specific for AMPARs, since there was no accompanying change in NMDAR-mediated fEPSPs. Conversely, MET administration during CUS prevented this dysfunction, illustrating that chronic stress-induced elevations of CORT are necessary for these effects. A decreased AMPA-to-NMDA ratio at these synapses is consistent with results from synapses in the PFC and nucleus accumbens (Lim et al. 2012; Yuen et al. 2012), and we suggest that CORT elevation during chronic stress is a potential mediator at those synapses, as well. We suggest further that weakening of synaptic excitation at multiple synapses in corticomesolimbic reward circuits contributes to depressive-like signs after chronic stress. Corresponding restoration of synaptic strength at these stress-sensitive synapses is a critical mechanism of antidepressant action in these models (Popoli et al. 2012; Tizabi et al. 2012; Kallarackal et al. 2013; Musazzi et al. 2013).

Chronic stress also alters serotonin-induced potentiation at TA-CA1 synapses, both qualitatively and quantitatively (Cai et al. 2013). Mediated by 5-HT\(_{1B}\)Rs and phosphorylation of P5c 2005), and glucocorticoids (GCs) (Joëls and Baram 2009). The stress-sensitive TA-CA1 synapse provides a tractable locus at which to examine the neurobiological effects of the stress response and better understand how chronic stress causes altered behavioral phenotypes by changing synapses and circuits. Generalizing the current results from TA-CA1 synapses, we conclude that chronic CORT elevation is both sufficient and necessary to induce the chronic stress phenotype at molecular, synaptic, and behavioral levels.

CORT is necessary and sufficient to mediate the synaptic effects of chronic stress. Chronic CORT administration induces depressive-like behaviors, including anhedonia and increased helplessness, that are reversed by chronic antidepressants (Gourley and Taylor 2009). We replicated these previous behavioral results and further observed that chronic CORT induced hyponeophagia in the NSF task, an ethologically relevant choice between feeding motivation and neophobic behavior. These tests are sensitive to chronic, but not acute, selective serotonin reuptake inhibitor (SSRI) administration (Dulawa et al. 2004). MET treatment significantly reduced the peak CORT response to a stressor and the average CORT levels during CUS. Because CORT administration mimicked, and MET treatment prevented, anhedonia-like and neophobic behaviors in our experiments, we conclude that stress-induced CORT elevation is sufficient and necessary to generate these behavioral changes in the CUS model.

Adolescence and early adulthood present vulnerable time windows to stress that may increase the likelihood of cognitive impairment or psychiatric disease (Eiland and Romeo 2013; Holder and Blaustein 2014). The rats we tested were no older than postnatal day 60 when they were killed for in vitro studies. It is possible that chronic stress or CORT may be affecting pubertal trajectory or testosterone levels (Almeida et al. 2000; Pervanidou and Chrousos 2012). Further studies would be necessary to determine whether this has any effect on mood-related behavior and TA-CA1 physiology.

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Effects on structural organization may contribute to anhedonia. Depression is common in patients with primary abnormalities of GC production and patients receiving exogenous GluA1, serotoninergic modulation covaries with depressive-like behavior and changes in AMPAR function. Conversely, chronic antidepressant treatment restores normal serotonin responses at TA-CA1 synapses in conjunction with restoration of normal behavior and synaptic strength. In the present study, the aberrant stress-induced anpirtoline response was mimicked by chronic CORT treatment alone, and prevented by MET treatment in rats subjected to CUS.

TA-CA1 synapses are required for spatial memory consolidation, and impaired spatial memory has been described in humans (Gould et al. 2007). The ability of MET treatment to preserve TA-CA1 synaptic strength and memory consolidation in rats subjected to CUS demonstrates that CORT-induced changes in synaptic strength likely underlie this stress-induced cognitive deficit.

Structurally, chronic restraint stress reduces the number of spines in CA1 cell distal apical dendrites (Pawlak et al. 2005; Maras et al. 2014). Functionally, chronic stress and CORT administration impair long-term potentiation at proximal CA1 synapses in vitro in some studies (Alfarez et al. 2003), but not all (for review, see Joëls et al. 2012). Chronic stress or chronic CORT cause atrophy of terminal segments of CA1 dendrites (Sousa et al. 2000), which are more vulnerable to stress than more proximal synapses, but are often overlooked in stress-related studies. A history of chronic CORT persistently simplifies CA1 basal dendritic arbors, illustrating that lasting effects on structural organization may contribute to anhedonia via impaired processing of reward-related contextual stimuli (Gourley et al. 2013). Loss of distal dendrites and TA-CA1 synapses may contribute significantly to the atrophy observed after chronic stress in rodents and contribute to the hippocampal volume loss in severe human depression.

**Acute vs. chronic effects of CORT.** In contrast to the impairment of AMPAR-mediated transmission produced by chronic CORT (approximately weeks), acute CORT (approximately hours) increased transmission at TA-CA1 synapses (Fig. 2, A and B), consistent with previous observations at Schaffer collateral synapses (Karst et al. 2005). This short-term effect of CORT to increase CA1 excitability is consistent with the well-established dichotomy between acute and chronic stress and with the transient mood-boosting effects of steroid treatment (Joëls 2006). Acute stress increases plasticity in short time windows via nongenomic mineralocorticoid receptor (Karst and Joëls 2005; Wiegert et al. 2006; Olijslagers et al. 2008), promoting stress-salient memories. In contrast, chronic stress dampens plasticity via GR-mediated genetic regulation (Joëls 2008). We suggest that the synaptic consequences of chronic CORT elevation described here are likely to be mediated by chronic excess GR activation.

**GCs in human disease.** There is compelling evidence of a causal link between altered HPA function and a large subset of severely depressed patients, particularly with regard to anhedonia. Depression is common in patients with primary abnormalities of GC production and patients receiving exogenous...
GCs (Quarton et al. 1955; Jeffcoat et al. 1979). HPA dysregulation is correlated with severity of symptoms and is common (~80% of patients) in treatment-resistant depression (Anacker et al. 2011). The correlation is even greater in melancholia patients (up to 90%), identified using modified criteria relying primarily on anhedonia (Taylor and Fink 2008). Abnormal HPA function is also correlated with a ninefold increase in suicide risk (Coryell and Schlesser 2001). HPA dysregulation may be both a causative factor and a consequence of depression.

The dose of MET used here produced effects that are similar to those produced by clinically relevant doses in humans, effectively halving circulating corticosteroids (O’Dwyer et al. 1995). In stressed rats, MET treatment preserved the normal response of TA-CA1 synapses to serotonergic modulation, suggesting that MET treatment protected the serotonin-innervated excitatory synapses upon which SSRIs act (Sigalas et al. 2012; Cai et al. 2013). MET also prevented normal stress-induced downregulation of GRs, suggesting protection from HPA dysregulation. There is clinical evidence that MET enhances antidepressant response (Sigalas et al. 2012; McAllister-Williams et al. 2013) and is effective as a stand-alone therapy in hypercortisolemic patients with depressive symptoms (Jeffcoat et al. 1979). Our results suggest several mechanisms by which treatment with MET should oppose pro depressive changes caused by stress, suggesting potential efficacy as a clinical add-on, particularly for depressed patients with HPA or GC abnormalities.

In conclusion, we have demonstrated that GCs play a critical role as mediators of the synaptic effects of chronic stress, as well as behavioral changes in rats, which are similar to the mood and cognitive changes characterizing human depression, strengthening the hypothesis that excitatory synaptic dysfunction contributes to the symptoms of depression (Kruger et al. 2010; Joels et al. 2012; Popoli et al. 2012; Duman 2014; Fischell et al. 2015; Thompson et al. 2015). That is, pathological chronic elevation of GCs causes depressive-like behavioral changes because it weakens specific subsets of synapses in corticomesolimbic circuits, such as the hippocampus, PFC (Duman and Aghajanian 2012; Yuen et al. 2012), and ventral striatum (Lim et al. 2012), thereby resulting in the anhedonia that is a prominent feature of human depression. Conventional antidepressants like SSRIs, fast-acting antidepressants like ketamine, and nonpharmacological treatment like electroconvulsive therapy all act to oppose the deleterious actions of chronically elevated GCs on excitatory synapses (Berman et al. 2000; Tizabi et al. 2012; Kallarackal et al. 2013; Zarate et al. 2013). Protecting and restoring excitatory strength at stress-sensitive synapses at key loci throughout the reward systems appears critical for maintaining normal cognitive and emotional behavior.

ACKNOWLEDGMENTS

We thank Leelah Jaberi, Kaitlin Gaylor, Jennifer Marsh, and Pamela Gorgei for technical assistance, Drs. Bruce Krueger and Matthew Trudeau for access to imaging equipment and software, and Dr. Tara LeGates, Adam Van Dyke, and Dr. Thomas Blanpied and his laboratory for helpful comments and criticisms.

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