Persistence of learning-induced synapses depends on neurotrophic priming of glucocorticoid receptors

Margarita Arango-Lievano*, Amelie M. Borie*, Yann Dromard*, Maxime Murat*, Michel G. Desarmenien*, Michael J. Garabedianb,1, and Freddy Jeanneteaua,1

*Department of Neuroscience, Institut de Genomique Fonctionnelle, INSERM, CNRS, University of Montpellier, 34090 Montpellier, France; and bDepartment of Microbiology, New York University Langone Medical Center, New York, NY 10016

Edited by Bruce S. McEwen, The Rockefeller University, New York, NY, and approved May 21, 2019 (received for review February 26, 2019)

Stress can either promote or impair learning and memory. Such opposing effects depend on whether synapses persist or decay after learning. Maintenance of new synapses formed at the time of learning upon neuronal network activation depends on the stress hormone-activated glucocorticoid receptor (GR) and neurotrophic factor release. Whether and how concurrent GR and neurotrophin signaling integrate to modulate synaptic plasticity and learning is not fully understood. Here, we show that deletion of the neurotrophin brain-derived neurotrophic factor (BDNF)-dependent GR-phosphorylation (PO4) sites impairs long-term memory retention and maintenance of newly formed postsynaptic dendritic spines in the mouse cortex during motor skills training. Chronic stress and the BDNF polymorphism Val66Met disrupt the BDNF-dependent GR-PO4 pathway necessary for preserving training-induced spines and previously acquired memories. Conversely, enrichment living promotes spine formation but fails to salvage training-related spines in mice lacking BDNF-dependent GR-PO4 sites, suggesting it is essential for spine consolidation and memory retention. Mechanistically, spine maturation and persistence in the motor cortex depend on synaptic mobilization of the glutamate receptor subunit A1 (GluA1) mediated by GR-PO4. Together, these findings indicate that regulation of GR-PO4 via activity-dependent BDNF signaling is important for the formation and maintenance of learning-dependent synapses. They also define a signaling mechanism underlying these effects.

Stress modifies adaptive behaviors such as learning and memory (1). Glucocorticoids are stress hormones that signal via the glucocorticoid receptor (GR) and can either promote or impair learning and memory (2). Whereas prolonged secretion of glucocorticoids during chronic stress disrupts learning and memory, release of glucocorticoids at the time of learning may promote them (3). An acute rise in glucocorticoid levels at the time of learning stimulates the formation and stabilization of new dendritic spines, and eliminates synapses established before learning. New dendritic spines require protein synthesis, which is initiated in the neuronal networks targeted by behavioral experience (4–6). For example, motor learning instructs remodeling of dendritic spines in excitatory neurons of the motor cortex (7–9). Stabilization of new spine synapses forges new learning-induced connectivity that correlates with memory consolidation (10, 11). However, the pathways and molecular mechanisms affecting spine stabilization during learning and memory in vivo are not understood.

Like glucocorticoids via GR, through its receptor TrkB, brain-derived neurotrophic factor (BDNF) stabilizes newly formed synapses and fosters learning and memory (12–14). BDNF is also critical for modulating the impact of stress in the corticollimbic and mesolimbic systems (15, 16). Behavioral actions of glucocorticoids and BDNF are complementary, and play roles in avoidance, fear, coping, and impulse control (17, 18). The influence on GR by the BDNF pathway likely relies on activity-dependent release of BDNF, which is reduced in the BDNF genetic variant Val66Met associated with impaired response to stress (19–21). BDNF signaling through TrkB alters the GR transcriptome through changes in GR-phosphorylation (PO4) and can affect neuronal plasticity (22–24). However, it remains unclear whether BDNF-dependent GR-PO4 mediates the persistence of new spines associated with learning and memory, and if activity-dependent secretion of BDNF is also required.

Here, we used two-photon in vivo microscopy of learning-associated dendritic spine remodeling to examine the effects of the BDNF-dependent GR-PO4 pathway in a newly developed GR-PO4−deficient mouse and in a mouse carrying the Val66Met polymorphism of BDNF. We found that GR-PO4 and BDNF secretion were both important for the formation and maintenance of new spines after learning through the synaptic recruitment of glutamate receptor A1 (GluA1). Our findings uncover an important mechanism for how acute glucocorticoids can direct a cell type-specific response to store and retain new information upon learning. By turning off this mechanism, chronic stress impaired cell type-specific contextual GR response.

Significance

Signal transduction upon activation of receptor tyrosine kinases by neurotrophins and nuclear receptors by glucocorticoids is essential for homeostasis. Phosphorylation (PO4) is one way these receptors communicate with one another to support homeostatic reactions in learning and memory. Using a newly developed glucocorticoid receptor (GR)-PO4−deficient knock-in mouse, we show that consolidation of learning-induced neuroplasticity depends on both GR-PO4 and neurotrophic signaling. Cross-talk between these pathways affects experience-dependent neuroplasticity and behavior, extending previous implications of neurotrophin priming of glucocorticoid response for adaptive plasticity to chronic stress and antidepressant response. Therefore, a disruption of cross-talk between these pathways by, for example, the misalignment of circadian glucocorticoid release and experience-dependent neurotrophic signaling may contribute to the pathophysiology of stress-related disorders.

Author contributions: M.A.-L., A.M.B., M.G.D., and F.J. designed research; M.A.-L., A.M.B., Y.D., M.M., and F.J. performed research; M.A.-L., Y.D., M.I.G., and F.J. contributed new reagents/analytic tools; M.A.-L., A.M.B., Y.D., M.M., and F.J. analyzed data; and M.A.-L., Y.D., M.M., and F.J. performed research; M.A.-L., Y.D., M.M., and F.J. contributed new reagents/analytic tools; M.A.-L., A.M.B., Y.D., M.M., and F.J. analyzed data; and M.A.-L., Y.D., M.M., and F.J. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

1To whom correspondence may be addressed. Email: michael.garabedian@nyulangone.org or freddy.jeanneateau@igh.cnrs.fr.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1903203116/-/DCSupplemental.

Published online June 10, 2019.

www.pnas.org/cgi/doi/10.1073/pnas.1903203116
Results
Timing and Specificity of BDNF-Dependent GR-PO4 Expression in Motor Cortex. The corticosterone-GR pathway is required for the acquisition of new motor skills (25), but its dependence on BDNF-dependent GR-PO4 is unknown. To unravel this, we assessed whether learning might affect GR-PO4 using a rotarod learning paradigm. Mice were left untrained or trained for 2 d. Forty-five minutes after the training, both control untrained and trained mice were euthanized, and expression in the cortex of GR-PO4 at the BDNF-dependent sites [S152 and S284 in mice correspond to S155 and S287 in the rat numbering scheme as previously described (23, 26), as well as c-Fos as an index of neuronal response, was determined by immunohistochemistry. Training increased the level of GR-PO4 at S152 and S284 in motor areas of cortex compared with the untrained controls (SI Appendix, Fig. S1A). The Thy1-YFP-marked excitatory and parvalbumin (PV) inhibitory neurons are among the cell types exhibiting high levels of GR-PO4 in M1 primary motor cortex (SI Appendix, Fig. S1B). Both cells types displayed increased c-Fos protein abundance upon training. Training also raised the percentage of cells harboring BDNF-dependent GR-PO4 in all layers of the M1 cortex (SI Appendix, Fig. S1C). In fact, training also raised the proportion of cells double-labeled with GR-PO4 and c-Fos (SI Appendix, Fig. S1D) and increased GR-PO4 in PV and Thy1 neurons (SI Appendix, Fig. S1 E and F). Induction of GR-PO4 was site-specific (S152/S284 versus S234; SI Appendix, Fig. S1A) and did not persist 24 h posttraining, similar to c-Fos in cortical areas relevant for motor skill learning (SI Appendix, Fig. S1G). Therefore, GR-PO4 at BDNF-dependent sites increased with training in the motor cortex and is linked to neuronal c-Fos response.

Deletion of GR-PO4 at BDNF-Responding Sites Impaired Motor Skill Retention. We next determined whether GR-PO4 at the BDNF-dependent sites affected learning and memory, as well as plasticity of dendritic spines. To do this, we generated a conditional allele of GR consisting of exon 2 harboring serine-to-alanine mutations in the BDNF-dependent GR-PO4 sites S152A and S284A [sites S134 and S267 in human GR and sites S155 and S287 in the rat GR numbering scheme (Fig. 1A)]. Recombination into germ cells using the constitutive ROSA26-CRE mouse line resulted in the mutations being stably transmitted to next generations (SI Appendix, Fig. S3). Cortical tissue from the GR-PO4−/−deficient knock-in (KI) mouse showed no staining with GR-PO4−/−specific antibodies compared with littermate wild-type (WT) controls (Fig. 1B). The same protein abundance of total GR was observed between the WT and KI mice (SI Appendix, Fig. S4). Likewise, mineralocorticoid receptor levels were unaffected in KI mice (SI Appendix, Fig. S4). Therefore, the KI mutant mice eliminated GR-PO4 without affecting the abundance of GRs.

Deletion of the BDNF-dependent GR-PO4 sites resulted in adult mice of normal appearance and body weight under standard, stressed, or enriched living conditions. There was no significant difference between the KI and WT mice in their degree of anxiety (as measured by thigmotaxis and an elevated maze test) (SI Appendix, Fig. S5 A and B), despair behaviors (as assessed by tail suspension and a forced swim test) (SI Appendix, Fig. S5 C and D), and locomotor activity when reared in standard living conditions, as well as in stressful or enriched environmental conditions (Fig. 1C), previously shown to activate the corticosterone-GR pathway (27). KI mice exhibited normal learning abilities of new motor skills on the rotarod but impaired retention of the task compared with WT littermates (Fig. 1D). Retention of new rotarod motor skills was also impaired in WT mice if exposed to chronic unpredictable stress immediately after training during the consolidation period, whereas enrichment living had no impact (Fig. 1E). This is consistent with the reduction of BDNF-dependent GR-PO4 in motor cortex of mice reared in chronic stress conditions (Fig. 1F). What is more, training-induced c-Fos expression in the excitatory and inhibitory (PV) neurons was reduced in KI mice compared with WT controls (Fig. 1 H and I). The effect of the KI showed in specific layers of the cortex differently whether in PV or neurogranin (NG) cells, suggesting putative interlayer network compensation in KI mice. Therefore, GR-PO4 disruption impaired activation of M1 cortex, as determined by c-Fos expression, as well as motor skill retention, which both depend on BDNF and corticosterone (25, 28).

GR-PO4 Is Required for the Maintenance of New Dendritic Spines Formed at Training. Previous studies have shown that de novo spine formation and maintenance contribute to the storage of new motor skills by creating new synaptic connections in the M1 region of the motor cortex (29). Therefore, we assessed how learning-associated spine formation in deep layer excitatory neurons (Thy1-YFP) varied with GR-PO4, using transcranial two-photon microscopy (Fig. 2A). As expected, the majority of spines were stable, such that only a small subset of spines was dynamic after training (Fig. 2B). Rates of spine formation (Fig. 2C) and elimination (Fig. 2D) were undistinguishable between WT and KI mice when untrained. However, after training, KI mice exhibited reduced spine formation (Fig. 2C) and excessive spine elimination (Fig. 2D) that corresponded to a net decrease of total spine number compared with WT littermates (Fig. 2E). Spine maintenance was also diminished in KI mice. This included both training-induced new spines and preexisting old spines present before training (Fig. 2F). In fact, the more training-induced new spines that survived the consolidation period, the better was the retention of motor skill performance (Fig. 2G).

To ensure that the BDNF-dependent GR-PO4 effect on experience-dependent spine plasticity was cell-autonomous in excitatory neurons of M1 cortex, we exclusively targeted this set of neurons by in utero electroporation (SI Appendix, Fig. S6 A–C). Substitution of endogenous GR with the PO4−/−deficient GR mutant as previously described (30) decreased spine formation and increased spine elimination in layer 1 of M1 cortex after training, which is consistent with a net decrease of spine density observed in the KI mice. This indicates that the effect of GR-PO4 is cell-autonomous.

Chronic unpredictable stress in WT mice decreased spine formation, increased spine elimination (SI Appendix, Fig. S6 D and E) during training, and reduced spine survival during the consolidation period, thus mimicking the effect of KI on spine dynamics, motor skills learning, and memory. KI mice showed net spine loss exaggerated with training and no further impact of chronic unpredictable stress on spine formation, elimination, and consolidation (Fig. 2F and SI Appendix, Fig. S6F). The lack of any additive effects between chronic stress and deletion of the GR-PO4 sites on spine plasticity indicates a functional redundancy of these pathways. This contrasts with the lack of effects of enrichment living on spine elimination, survival, and motor performance (Fig. 2 F and G). Remarkably, enrichment living reversed spine formation defects in KI mice (SI Appendix, Fig. S6 D and E). However, this did not enhance motor retention, because the new spines were unrelated to the training. This is in agreement with a role of BDNF-dependent GR-PO4 on the maintenance of training-dependent new spines for better retention of motor performance (Fig. 2G).

BDNF-Val66Met Polymorphism Recapitulated Synaptic and Motor Defects of GR-PO4 Deletion. The BDNF-Val66Met polymorphism impairs activity-dependent secretion of BDNF and manifests as defective motor skill training in rodents and humans (28, 31). This phenotype is similar to that observed in the KI mice. Therefore, we tested functional epistasis between KI and BDNF-Val66Met to
determine whether both pathways converge during motor skills learning. We found that BDNF-dependent GR-PO4 was reduced in mouse carriers of the BDNF-Val66Met allele (Fig. 3A). We then crossed heterozygous KI;Thy1-YFP mice with heterozygous BDNF-Val66Met;Thy1-YFP mice to obtain homozygotes. Four groups of mice, WT, KI, M (BDNF-Val66Met), and M;KI (KI:BDNF-Val66Met), were subjected to in vivo imaging of dendritic spines upon motor skill training. M mice exhibited normal acquisition but impaired retention of the motor task. Importantly, the M;KI double-mutant mice showed no additional effect (Fig. 3B). As expected, spine plasticity in the M mice was defective during training but was unaffected in the untrained groups (Fig. 3C). Defects in spine elimination and spine survival in the M mice recapitulated those of the KI mice, with no additive effects in the M;KI double-mutants (Fig. 3C and D). Together, these results indicated that KI mice and M mice (with impaired activity-dependent
BDNF secretion) have a reduction in their ability to stabilize the new training-induced spines and retain new motor skills. This is consistent with the convergence of both pathways toward new spine consolidation.

**Poorer Retention Was Consistent with Weaker Long-Term Potentiation in Motor Cortex upon GR-P04 Deletion.** Potentiation of excitatory synaptic transmission in the M1 motor cortex is required to acquire and retain new motor skills (32). To test whether KI mice showed defects in synaptic plasticity, we performed tetanus-induced long-term potentiation (LTP) 1 d postraining. LTP can be induced in WT and KI mice, regardless of training (Fig. 4 A and B). After three consecutive tetanus stimulations, LTP saturated in the trained cortex of WT but not KI mice (Fig. 4 B and C). These data suggested that training occluded LTP in WT but not KI mice (Fig. 4 D). In fact, LTP occlusion after serial inductions can predict retention of motor skills learning in rodents and humans (33–35). In the KI mice, we find that LTP occlusion was weaker and retention was poorer compared with WT littermates (Fig. 4 E). This indicates that GR-P04 is essential for functional strengthening of M1 synapses postraining.
GR-PO4 Is Required for Synaptic Mobilization of Phosphorylated GluA1. Synaptic delivery of α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA)–type glutamate receptors is thought to contribute to LTP (36). In motor cortex, skill training drives AMPA-type GluA1 to postsynapses, contributing to the dynamic changes of glutamatergic synaptic strength (32). Therefore, we tested whether GR-PO4 and training have an impact on GluA1 synaptic content and PO4 because these regulate AMPA conductance and synaptic strength (37, 38). We first determined if there was a difference in levels of synaptic GluA1 between trained and untrained groups. Synaptosomes were isolated from M1 cortex collected 45 min after training, and synaptic GluA1 levels were determined using Western blot analysis (SI Appendix, Fig. S7). Levels of GluA1 were similar in all groups. However, the level of GluA1 PO4 at S831 increased as a function of training in WT but not KI mice. As GluA1 PO4 at this site has been linked to synaptic potentiation in M1 (39), its lack of increase posttraining could reflect LTP defects observed in KI mice posttraining.

We next investigated cell surface delivery of GluA1 using an established biotinylation assay in cultured cortical neurons (40) (SI Appendix, Fig. S8A). Avidin pulldown of biotin-tagged GluA1 newly inserted at the cell surface was detected by Western blot analysis (Fig. 5A). Upon costimulation of the BDNF and GR pathways, levels of GluA1 and its PO4 isofrom were lower in neurons expressing GR-KI mutant compared with GR-WT (Fig. 5B). This effect was specific for GluA1 (SI Appendix, Fig. S8B). These results suggest that GR-PO4 at the time of training promotes the mobilization of synaptic GluA1. Does this permit the structural maturation and stabilization of training-dependent spines?

GR-PO4 Pathway Is Required for Strengthening Glutamatergic Response Posttraining. Training-induced LTP occlusion has been previously associated with enlargement of dendritic spine heads in upper layers of motor cortex, a process dependent on glutamate (41). Therefore, we tested the role of GR-PO4 on glutamate-dependent structural maturation of dendritic spines in vivo. To this end, we used time-lapse imaging of dendritic spines and two-photon uncaging of 4-methoxy-7-nitroindolyl–caged t-glutamate (MNI-glutamate) directly into M1 cortex through a cranial window (Fig. 5C). Glutamate uncaging on the spine head causes its enlargement without affecting unstimulated neighboring spines (Fig. 5D). Photostimulation of spines in the absence of MNI-glutamate had no effect, confirming the specificity of the glutamate uncaging response. In trained mice, the rate of glutamate-evoked spine enlargement was 79 ± 11% in WT mice and 59 ± 9% in KI mice. Glutamate-evoked spine enlargement is also slower in KI mice than in WT mice (Fig. 5E). Thus, glutamate-induced spine maturation is weaker in KI mice than in WT mice.

Pharmacological blockade of GR with RU486 administered immediately before training impaired the glutamate response of dendritic spines in WT controls, whereas no further inhibition of the response was observed in KI mice (Fig. 5F). The lack of additive effects between the deletion of GR-PO4 and GR inhibition indicated functional redundancy. In contrast, RU486 administered after training had no effect on the glutamate response of dendritic spines (SI Appendix, Fig. S9). This further indicates that BDNF-dependent GR-PO4 at training is required for the strengthening of the glutamatergic response of dendritic spines after training.

Discussion

Using a newly developed GR-PO4 site-deficient KI mouse, we have expanded on our previous report (23) describing the impact of mice on new spines [t(14) = 2.24] and old spines [t(14) = 9.95], and V:WT mice and M:KI mice on new spines [t(14) = 2.15] and old spines [t(14) = 7.91, *P < 0.05].
of BDNF-dependent GR-PO₄ on neuronal plasticity and demonstrated the requirement for BDNF activity-dependent release in remodeling of excitatory synapses in the cortex upon learning, as well as its impact on behavior. Our study reveals that either the lack of GR-PO₄ induced by mutation of the BDNF-dependent PO₄ sites or decreased activity-dependent BDNF secretion modeled in the BDNF-Val66Met mutant reduced the survival of learning-associated new spines and impaired memory retention. Moreover, combining the BDNF-Val66Met with the GR-PO₄ mutant mice does not induce further deficits on spine maintenance and behavior as in the individual mutants, indicating that the effect of GR-PO₄ on spine dynamics occurs via BDNF. In agreement with this finding is an increase in GR-PO₄ in the motor cortex by motor skills training, as well as reduced sensitivity to corticosterone and stress in humans and mice with the BDNF-Val66Met allele (20, 42). The physiological role of GR-PO₄ is revealed when the BDNF and GR signaling pathways are paired (26). Permissive actions of BDNF on GR physiological responses can be explained because GR-PO₄ comes about by the activation of kinases through the BDNF-TrkB pathway and the deactivation of phosphatases by the GR-HSP90 pathway (23). Mice lacking GR-PO₄ sites will be instrumental in unraveling the physiological consequences of BDNF and glucocorticoid secretion alignment (e.g., circadian variations, novelty, learning, stress-related diseases, antidepressant treatment) (18, 43). Timing of BDNF and glucocorticoid secretion is brain region-specific, and could change the neuroplasticity of cells equipped with both GR and TrkB receptors. This is the case in PV and pyramidal cortical neurons, which express high GR-PO₄ levels and in which interconnectivity modulates the dendritic spine elimination response to circadian learning, stress, and antidepressant treatment (25, 44, 45).

Our findings also shed light on the molecular mechanisms underlying how information in the brain is stored (i.e., motor engraving) in response to external stimuli, including stress and enriched environment. We observed that chronic stress increased the turnover of spines in WT mice but not in the GR-PO₄ deletion mice as both stress and GR-PO₄ deletion resulted in a net loss of training-induced new spines in the cortex. This highlights the critical role of BDNF-dependent GR-PO₄ on the maintenance and connectivity of new training-induced spines within a motor engraving. Our findings also predict that physical exercise and/or an enriched environment would promote spine maintenance in cortex, by transforming the training-related new spines into a persistent pool of memory spines, and alleviate the impact of stress on the synaptic engraving (45, 46). Motor learning was also associated with an increase in the active phosphorylated form of the GluA1 into synapses that stabilize and strengthen the neuroplasticity of cells equipped with both GR and TrkB receptors. This is the case in PV and pyramidal cortical neurons, which express high GR-PO₄ levels and in which interconnectivity modulates the dendritic spine elimination response to circadian learning, stress, and antidepressant treatment (25, 44, 45).

Physiological consequences of the BDNF-Val66Met allele, like those of GR-PO₄ deletion, are revealed not in the basal state but in response to experience-driven increase of neural activity
This indicates that GR senses and responds to BDNF by selective GR-PO₄ that alters its activity and promotes learning and memory. Whether this reflects changes in GR genomic and/or nongenomic activity remains to be determined. Evidence obtained from RU486 injections indicates that GR-PO₄ signaling is required hours rather than minutes before dendritic spine maturation via a glutamate-dependent mechanism. Therefore, these effects depend on a slow process, likely genomic, to consolidate new spines. This could have implications for the encoding and persistence of new structural engrams required for adaptive plasticity that either translate to a new behavior or adapt to a changing environment (i.e., stress, learning, enrichment).

Our findings also have translational implications. Imbalances in the integration of BDNF-GR signaling axes would be predicted to trigger maladaptive processes that contribute to the pathophysiology observed in many neurological and other diseases (18, 24, 43, 55). For example, GR insufficiency is associated with a state of low BDNF levels in diseases presenting an activated inflammatory system that is relevant for patients with major depression, schizophrenia, glucocorticoid resistance syndromes, and Alzheimer’s disease (56–59). Antidepressant therapies that successfully increase BDNF levels also correct GR-PO₄ and GR sensitivity (23, 60). GR-PO₄ has been suggested to be a mechanism contributing to glucocorticoid resistance in multiple disease models (61). Although many GR-PO₄ sites are glucocorticoid-dependent (18), our data indicate that GR-PO₄ can also be glucocorticoid-independent. This implies that GR activity is influenced by contextual signals in addition to glucocorticoids (22, 26, 62). This applies, for example, to the maintenance of sensory motor engrams as long as activity-dependent BDNF secretion and glucocorticoid oscillatory pulses are synchronized and stimulate the GR-PO₄ pathway. For that reason, aligning glucocorticoid treatment to neurotrophin release should be considered to promote neuroplasticity for sensory motor rehabilitation.

Methods
All experiments were carried out in accordance with the Directive by the Council of the European Communities (86/609/EEC). All protocols complied with the French Ministry of Research institutional guidelines (approved protocol 00651.01) and ethics committee at the University of Montpellier for the care and use of laboratory animals. All tools are listed in SI Appendix, Table S1.

Animals. The PO₄-deficient GR mouse (Flex GR-A152/A284 KI; Ozgene, Pty. Ltd.) was generated in a C57Bl6 background and crossed with the constitutive ROSA26-FLP line [Gt(Rosa)26Sor(tm2(FLP)Sor)] to remove the neomycin cassette and the constitutive ROSA26-CRE line [Gt(Rosa)26Sor(tm1Sor)] to produce a general deletion of GR-PO₄ sites (details are provided in SI Appendix, Fig. S1). Thy1-YFP transgenic mice [B6.Cg-Tg(Thy1-YFP)Hrjrs/J], BDNF-Val66Met
mice [BrdU+ /+ , MGI:3664862], and WT mice in C57BL6 background (The Jackson Laboratory) were housed under a 12-h light-dark cycle (on 6:00 AM, off 6:00 PM) with unrestricted access to food and water. Chronic unpredictable mild stress includes one of the following daily stressors (wet bedding, no bedding, food deprivation, crowded cage, 2-h or 6-h restraint, tilted cage, shaking, 24-h light cycle, forced swim, or tail suspension) for 10 consecutive days. Enrichment consisted of larger cages with toys of various colors and textures (tubes, tunnels, ladders, Lego pieces, beads, or nest) for 10 consecutive days. Homozygous males produced by heterozygous breeding schemes were used in all protocols. All efforts were made to minimize animal suffering and to reduce the number of mice utilized in each experiment.

Open Field. One-month-old mice freely explored an arena (50 cm × 50 cm) for 10-min video-recorded sessions, and total distance traveled was determined with Ethovision XT software (Noldus). Thigmotaxis represents the time spent in the center of the arena (29 cm × 29 cm).

Elevated Plus Maze. Mice at 3 mo of age freely explored the arms (elevation of 50 cm × 50 cm × 20 cm) for 10-min video-recorded sessions to score the number of entries and time spent in each arm.

Forced Swim. Mice at 3 mo of age were subjected to a forced swim test for 9 min in a beaker (15 cm × 25 cm) filled with tap water at room temperature during the stress protocol [once a week between postnatal day (PND) 23 and 36], and the trial was video-recorded. Trial data represent an ac- quired behavioral response in the stress groups but a novel response in the control groups reared in standard conditions.

Tail Suspension. Mice at 3 mo of age were subjected to a video-recorded tail suspension test for 6 min during the stress protocol (once a week between PNDs 23 and 36). Trial data represent an acquired behavioral response in the stress groups but a novel response in the control groups reared in standard conditions (63).

Motor Learning. Mice were habituated for 30 min (15 trials) on the non-accelerating rotarod (2 rpm for 1 min, followed by 30-s rest intertrials) for two consecutive days at glucocorticoid circadian oscillation peak (7:00 PM) in mice at 3 mo of age freely explored the arms (elevation of 50 cm × 50 cm × 20 cm) for 10-min video-recorded sessions to score the number of entries and time spent in each arm.

9 min in a beaker (15 cm × 25 cm) filled with tap water at room temperature before (image 1 at PND23) and after (image 2 at PND26) training, as well as dendritic spines reliably in the dendritic shaft, irrespective of apparent shape, were counted. Spines were considered de- leted if they disappeared into the haze of the dendrites (less than five pixels in length); spines were considered formed if they clearly protruded from the dendritic shaft or more than five pixels in length). To calculate spine brightness, the pixel values containing the spine head were summed. Background fluores- cence, calculated over the same-sized box adjacent to the spine, was sub- tracted. Since dendritic shaft diameters were constant and relatively uniform, we used them to correct fluorescence levels. Average shaft pixel intensity was calculated over the same-sized box adjacent to the spine. The background-subtracted pixel value for each spine was divided by the average shaft pixel value as previously described (67). The resulting relative brightness is expected to be proportional to the spine volume. The fraction of spines gained sub- tracted of the spines lost between imaging sessions, was calculated as the net ratio = (Ntime + Nbase)/2N (49), where N is the population size of spines.

Live Slice Preparation. Mice were decapitated at PDN26, 12–15 h after the last training session as described (39), and brains were immersed in ice-cold oxygenated ACSF containing 127.25 mM NaCl, 1.75 mM KCl, 1.25 mM KH2PO4, 1 mM MgCl2, 2 mM CaCl2, 26 mM NaHCO3, and 10 mM glucose. Coronal slices (400 μm), including the M1 area (1.5–3.5 mm anterior to bregma, 2–4 mm lateral), were transferred to a temperature-controlled chamber and perfused with oxygenated ACSF at a rate of 1 mL/min . Slices were allowed to recover for at least 1 h before recordings.

Electrophysiological Recordings. Stimulation electrodes were positioned in L2/3, 1.5–1.8 mm lateral to the midline, and recording electrodes were placed 500 μm laterally. Field potentials were evoked by stimulation of 0.2 ms at 0.03 Hz. For induction of LTP, stimulus intensity eliciting 50% of the maxi- mum amplitude was used for all measurements before and after LTP in- duction paired with a touch application of bicuculline methiodide (3.5 mM, gamma-aminobutyric acid type A antagonist) as described (33). Baseline amplitudes were recorded using single stimuli applied every 30 s. Following LTP induction, the stimuli were applied at 1 Hz until the LTP amplitude reached 150% of baseline. The LTP amplitude was calculated as the net difference between the baseline and the peak LTP amplitude. The LTP amplitude was calculated as the net difference between the baseline and the peak LTP amplitude.
Immunohistochemistry. Mice were anesthetized at PND26 or PND36 with pentobarbital (50 mg/kg intraperitoneal (i.p.); Ceva Santé Animale) and perfused at a rate of 3 ml/min through the ascending aorta with 30 ml of ice-cold 0.9% NaCl before decapitation. Brain hemissections were fixed with 4% ice-cold paraformaldehyde for 2 h and sectioned with a vibratome. Free-floating coronal sections rinsed in phosphate-buffered saline (PBS) were blocked in 3% normal donkey serum, PBS, and 0.1% Triton X-100 for 2 h at 25 °C. Antibodies are listed in S1 Appendix, Table S1. Images were acquired with a confocal microscope (LSM780; Carl Zeiss) and 10x and 20x dry objectives. An 40x oil-immersion objective to capture dendrites spine for counting densities. Excitation and acquisition parameters were unchanged during the acquisition of all images. More than 26,000 NG neurons, 7,000 PV neurons, 24,000 GR cells, and 2,200 Thy1-YFP neurons were counted in all 5 groups to determine the proportions of cells coabeled with c-Fos and GR-Po.

Synaptosome Preparation. Mice were anesthetized at PND26 with pentobarbital (50 mg/kg i.p.; Ceva Santé Animale) and perfused at a rate of 3 ml/min through the ascending aorta with 30 ml of 0.9% NaCl before decapitation. Motor cortex from brain hemissections was harvested from 200-μm-thick sections dissected with a tissue punch (Stoelting Co.). Potter was used to make homogenates in ice-cold 0.32 M sucrose, 1 mM ethylendiaminetetraacetic acid (EDTA), 10 mM Hepes (pH 8), and 1 mg/mL bovine serum albumin (BSA) complemented with protease and phosphatase inhibitors, and centrifuged twice (1,000 x g for 1 min) to clear debris. Particles were centrifuged (14,000 x g for 12 min), and pellets were suspended in ice-cold 45% Percoll diluted in 140 mM NaCl, 5 mM KCl, 25 mM Hepes (pH 8.0), 1 mM EDTA, and 10 mM glucose plus inhibitors. Synaptosome fractions were collected at the surface after centrifugation (14,000 x g for 2 min) and rinsed in ice-cold 0.32 M sucrose, 1 mM EDTA, and 10 mM Hepes (pH 8). After centrifugation (14,000 x g for 12 min), synaptosomes were lysed in 2% sodium dodecyl sulfate (SDS).

DNA Transfection. In utero electroporations [30 V; pO/N, 50 s; pOFF, 950 s; five pulses with NEPA21 (NepaGene); 1 μg of DNA] were performed at embryonic day 15 on mouse embryos and newborns developed for 1 mo of age (23). Mice were anaesthetized with 4% isoflurane/oxygen and maintained at 1.5% (23). Mice were anesthetized with 4% isoflurane/oxygen and maintained at 2% isoflurane (Abbott Laboratories) throughout surgery using TEC3N Touch (Bio-Rad Laboratories).

Statistics. Parameters used to quantify imaging data include the following: (i) formation/deletion/survival of dendritic spines, (ii) density of dendritic spines, (iii) spine head diameter, (iv) double-labeled cells, and (v) cortical layers visualized with 4,6-diamidino-2-phenylindole stain and Thy1-YFP in L5. Parameters used to quantify motor performance data include the following: (i) latency to fall from the rotarod platform and (ii) the escape index of learning and intertrial improvement as an index of retention. Representation of N for each dataset is indicated in the figure legends. All data collected in animals were from littermate controls and were averaged per experimental group. We used the Student’s t test to compare two groups or time points and Pearson correlation for linear associations between datasets with Prism 8.0 software (GraphPad). We used factorial ANOVA to compare multiple groups (training, genotype, stress, and enrichment), followed by post hoc pairwise comparison with the Tukey test for corrections. All data are shown as mean ± SEM. Significance level is set at P ≤ 0.05. No data were removed from analyses, including statistical outliers. Estimates of sample size were calculated by power analysis based on preliminary data. Sample size was chosen to ensure 80% power to detect the prespecified effect size. Preestablished criteria for stopping data collection included the following: (i) mice reaching ethical endpoint limits, (ii) unexpected mortality (e.g., anesthesia), (iii) crack of the thin skull preparation that would cause unwanted inflammation, and (iv) brains badly perfused and unusable for histology.

ACKNOWLEDGMENTS. We thank F. S. Lee (Weill Cornell Medical School) for providing the Val66Met mice and W. B. Gan (New York University School of Medicine, Weill Cornell Medical School, Liston (Weill Cornell Medical School) for recruiting technicians, W. M. Lambert (Opelousas, Louisiana) for advice, and M. F. G. for animal care and data collection for this study. We also thank the National Institute of Mental Health (Grant 1R01MH75817) for funding the field work and for the use of the Perkin Elmer UltraSpec 210 apparatus.

1. C. C. Chen, J. Lu, Y. Zuo, Spatiotemporal dynamics of dendritic spines in the living brain. Front. Neuroanat. 8, 28 (2014).
2. C. Finsterwald, C. M. Alberini, Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: From adaptive responses to psychopathologies. Neurobiol. Learn. Mem. 112, 17–29 (2014).
3. L. Schwabe, M. Joëls, B. Rozenbaum, D. T. Wolf, M. S. Oitzl, Stress effects on memory: An update and integration. Biol. Psychiatry 74, 1743–1749 (2013).
4. M. Fu, Y. Zuo, Experience-dependent spine plasticity in the cortex. Trends Neurosci. 34, 177–187 (2011).
5. C. R. Bramham, Local protein synthesis, actin dynamics, and LTP consolidation. Annu. Rev. Neurosci. 26, 445–462 (2003).
6. J. Tanaka et al., Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. Science 319, 1683–1687 (2008).
7. T. Xu et al., Rapid formation and selective stabilization of synapses for enduring motor memories. Nature 462, 915–919 (2009).
8. G. Yang, F. Fan, W. B. Gan, Stably maintained dendritic spines are associated with learning and are resistant to the anesthetic 2,2,2-aminopyridine. Neuron 99, 620–634 (2018).
9. L. Ma et al., Experience-dependent plasticity of dendritic spines of layer 3/5 pyramidal neurons in the mouse cortex. Dev. Neurobiol. 76, 277–286 (2016).
10. C. C. Chen, J. Lu, Y. Zuo, Spatiotemporal dynamics of dendritic spines in the living brain. Front. Neuroanat. 8, 28 (2014).
11. K. P. Berry, E. NDLI, Spine dynamics: Are they all the same? Neuro 96, 43–55 (2017).
12. A. E. Autry, L. M. Monteggia, Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol. Rev. 64, 238–258 (2012).
13. S. C. Harvard et al., Autocrine BDNF-TrkB signalling within a single dendritic spine. Nature 538, 99–103 (2016).
14. F. J. Janneteau, M. V. Choi, “Neurotrophins and synaptogenesis” in Cellular Migration and Formation of Neuronal Connections: Comprehensive Developmental Neuroscienc e, J. Rubenstein, P. Rakic, Eds. (Elsevier, ed. 2, 2013), pp. 639–659.
15. J. Wook Koo et al., Essential role of mesolimbic brain-derived neurotrophic factor in chronic social stress-induced depressive behaviors. Biol. Psychiatry 80, 469–478 (2016).
16. E. G. Pitts, D. C. Li, S. L. Gourley, Bidirectional coordination of actions and habits by TrkB in mice. Sci. Rep. 8, 4495 (2018).
17. E. T. Barbier, S. L. Gourley, Prefrontal cortical trkB, glucocorticoids, and their interactions in stress and developmental contexts. Neurosci. Biobehav. Rev. 95, 535–558 (2018).
18. F. J. Janneteau, A. Borte, M. M. Moa, M. Garabedian, Bridging the gap between brain-derived neurotrophic factor and glucocorticoid effects on brain networks. Neuroen- docrinology 2011:59:4009639 (2012).
19. M. F. Egan et al., The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human hippocampal function. Cell 112, 257–269 (2003).
20. H. Yu et al., Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. J. Neurosci. 32, 4092–4101 (2012).
21. I. Shalev et al., BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. Psycho- neuroendocrinology 34, 382–388 (2009).
22. W. M. Lambert et al., Brain-derived neurotrophic factor signaling rewrites the glucocorticoid transcriptome via glucocorticoid receptor phosphorylation. Mol. Cell. Biol. 33, 3700–3714 (2013).
J. Neurosci. 28, 1045–1059 (2018).

M. Arango-Lievano et al., Topographic reorganization of cerebrovascular mural cells under seizure conditions. Cell Rep. 23, 1045–1059 (2018).

M. Arango-Lievano, P. Giannoni, S. Claeysen, N. Marchi, F. Jeanneteau, Longitudinal in vivo imaging of the cerebrovasculature: Relevance to CNS diseases. J. Vis. Exp. 10, e57914 (2015).

J. Arango-Lievano, P. Giannoni, S. Claeysen, N. Marchi, F. Jeanneteau, Longitudinal in vivo imaging of the cerebrovasculature: Relevance to CNS diseases. J. Vis. Exp. 10, e57914 (2015).

J. Arango-Lievano et al., Topographic reorganization of cerebrovascular mural cells under seizure conditions. Cell Rep. 23, 1045–1059 (2018).

M. Arango-Lievano et al., Treadmill exercise suppressed stress-induced dendritic spine elimination in mouse barrel cortex and improved working memory via BDNF/TrkB pathway. Transl. Psychiatry 7, e1069 (2017).

T. C. Hill, K. Zito, LTP-induced long-term stabilization of individual nascent dendritic spines. J. Neurosci. 33, 678–686 (2013).

H. Makino, R. Malinov, AMPA receptor incorporation into synapses during LTP: The role of lateral movement and exocytosis. Neuron 64, 381–390 (2009).

E. Y. Yuen et al., Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. Neuron 73, 962–977 (2012).

P. F. Durieux, S. N. Schiffmann, A. de Kerchove d’Exaerde, Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct doral striatum subregions. EMBO J. 31, 640–653 (2012).

L. Guo et al., Dynamic reweiring of neural circuits in the motor cortex in mouse models of Parkinson’s disease. Nat. Neurosci. 18, 1299–1309 (2015).

L. Froux et al., D5 dopamine receptors control glutamategic AMPA transmission between the motor cortex and subthalamic nucleus. Sci. Rep. 8, 8858 (2018).

K. Sun, Y. Zhao, M. E. Wolf, Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. J. Neurosci. 25, 7342–7351 (2005).

J. A. Klein et al., BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. Nat. Neurosci. 8, 735–737 (2005).

N. P. Daskalakis, E. R. De Kloet, R. Yehuda, D. Malaspina, T. M. Kranz, Early life stress effects on glucocorticoid-BDNF interplay in the hippocampus. Front. Mol. Neurosci. 8, 68 (2015).

D. W. Cain, J. A. Cidlowski, Specificity and sensitivity of glucocorticoid signaling in health and disease. Best Pract. Res. Clin. Endocrinol. Metab. 29, 545–555 (2015).

R. A. Quax et al., Glucocorticoid sensitivity in health and disease. Nat. Rev. Endocrinol. 9, 670–686 (2013).

C. M. Pariante, Why are depressed patients inflamed? A reflection on 20 years of research on depression, glucocorticoid resistance and inflammation. Eur. Neuropsychopharmacol. 27, 554–559 (2017).

J. M. Garabedian, C. A. Harris, F. Jeanneteau, Glucocorticoid receptor action in metabolic and neuronal function. F1000 Res. 6, 1208 (2017).

M. S. Lee et al., Temporal variability of glucocorticoid receptor activity is functionally important for the therapeutic action of fluoxetine in the hippocampus. Mol. Psychiatry 21, 252–260 (2016).

A. J. Gallilhe-Beckley, J. A. Cidlowski, Emerging roles of glucocorticoid receptor phosphorylation in modulating glucocorticoid hormone action in health and disease. IUBMB Life 61, 979–986 (2009).

A. J. Gallilhe-Beckley, J. G. Williams, J. A. Cidlowski, Ligand-independent phosphorylation of the glucocorticoid receptor integrates cellular stress pathways with nuclear receptor signaling. Mol. Cell. Biol. 31, 4663–4675 (2011).

F. Jeanneteau et al., The stress-induced transcription factor NRR1A1 adjusts mitochondrial function and synapse number in prefrontal cortex. J. Neurosci. 38, 1335–1355 (2018).

M. Arango-Lievano et al., Neurotrophic-priming of glucocorticoid receptor signaling is essential for neuronal plasticity to stress and antidepressant treatment. Proc. Natl. Acad. Sci. U.S.A. 112, 15737–15742 (2015).

B. S. McEwen, Preserving neuroplasticity: Role of glucocorticoids and neurotrophins via phosphorylation. Proc. Natl. Acad. Sci. U.S.A. 112, 15544–15545 (2015).

C. Liston et al., Glucocorticoid oscillations promote learning-dependent synapse formation and maintenance. Nat. Neurosci. 16, 698–705 (2013).

M. Arango-Lievano, F. Jeanneteau, Timing and crosstalk of glucocorticoid signaling with cytokines, neurotransmitters and growth factors. Pharmacol. Res. 113, 1–17 (2016).

K. M. Zanca et al., Environmental enrichment increases glucocorticoid receptors and decreases GluA2 and protein kinase M zeta (PKMζ) trafficking during chronic stress: A protective mechanism? Front. Behav. Neurosci. 9, 303 (2015).

B. Fritsch et al., Direct current stimulation promotes BDNF-dependent synaptic plasticity: Potential implications for motor learning. Neuron 66, 198–204 (2010).

A. Hayashi-Takagi et al., Labelling and optical erase of synaptic memory traces in the motor cortex. Nature 525, 333–338 (2015).

M. Arango-Lievano et al., Deletion of neurophin signaling through the glucocorticoid receptor pathway causes tau neuropathology. Sci. Rep. 6, 37231 (2016).

S. A. McHughen et al., BDNF val66met polymorphism influences motor system function in the human brain. Cereb. Cortex 20, 1254–1262 (2010).

H. Kida et al., Motor training promotes both synaptic and intrinsic plasticity of layer II/III pyramidal neurons in the primary motor cortex. Cereb. Cortex 26, 3494–3507 (2016).

M. S. Rioul-Pedotti, D. Friedman, J. P. Donoghue, Learning-induced LTD in neocortex. Science 290, 533–536 (2000).

G. Cantareno, A. Lloyd, P. Celnik, Reversal of long-term potentiation-like plasticity processes after motor learning disrupts skill retention. J. Neurosci. 33, 12862–12869 (2013).

S. J. Martin, R. G. Morris, Cortical plasticity: It’s all the range! Curr. Biol. 11, R57–R59 (2001).

R. Malinov, R. C. Malenko, AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. 25, 103–126 (2002).

H. K. Lee, M. Barbarosie, K. Kameyama, M. F. Bear, R. L. Huganir, Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405, 955–959 (2000).

A. S. Kristensen et al., Mechanism of Ca2+/calmodulin-dependent kinase II regulation of AMPA receptor gating. Nat. Neurosci. 14, 727–731 (2011).

R. Padmashri, B. C. Reiner, A. Suresh, E. Spartz, A. Dunaevsky, Altered structural and functional synaptic plasticity with motor skill learning in a mouse model of fragile X syndrome. J. Neurosci. 33, 19715–19723 (2013).

F. Jeanneteau et al., A functional variant of the dopamine D3 receptor is associated with risk and age-at-onset of essential tremor. Proc. Natl. Acad. Sci. U.S.A. 103, 10753–10758 (2006).

K. J. Harms, M. S. Rioul-Pedotti, D. R. Carter, A. Dunaevsky, Transient spine expansion and learning-induced plasticity in layer 1 primary motor cortex. J. Neurosci. 28, 5686–5690 (2008).

M. Zhao et al., BDNF Val66Met polymorphism, life stress and depression: A meta-analysis of gene-environment interaction. J. Affect. Disord. 227, 226–235 (2018).

F. Jeanneteau, M. V. Chao, Are BDNF and glucocorticoid activities calibrated? Neuroscience 233, 173–195 (2013).

L. H. L. Ng et al., Ketamine and selective activation of parvalbumin interneurons inhibit stress-induced dendritic spine elimination. Transl. Psychiatry 8, 272 (2018).

C. C. Chen, J. Lu, R. Yang, J. B. Ding, Y. Zuo, Selective activation of parvalbumin interneurons prevents stress-induced synapse loss and perceptual defects. Mol. Psychiatry 23, 1614–1625 (2018).