Archival Report

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Blunted Glucocorticoid Responsiveness to Stress Causes Behavioral and Biological Alterations That Lead to Posttraumatic Stress Disorder Vulnerability

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ABSTRACT

BACKGROUND: Understanding why only a subset of trauma-exposed individuals develop posttraumatic stress disorder is critical for advancing clinical strategies. A few behavioral (deficits in fear extinction) and biological (blunted glucocorticoid levels, small hippocampal size, and rapid-eye-movement sleep [REMS] disturbances) traits have been identified as potential vulnerability factors. However, whether and to what extent these traits are interrelated and whether one of them could causally engender the others are not known.

METHODS: In a genetically selected rat model of reduced corticosterone responsiveness to stress, we explored posttraumatic stress disorder–related biobehavioral traits using ex vivo magnetic resonance imaging, cued fear conditioning, and polysomnographic recordings combined with in vivo photometric measurements.

RESULTS: We showed that genetic selection for blunted glucocorticoid responsiveness led to a correlated multitrait response, including impaired fear extinction (observed in males but not in females), small hippocampal volume, and REMS disturbances, supporting their interrelatedness. Fear extinction deficits and concomitant disruptions in REMS could be normalized through postextinction corticosterone administration, causally implicating glucocorticoid deficiency in two core posttraumatic stress disorder–related risk factors and manifestations. Furthermore, reduced REMS was accompanied by higher norepinephrine levels in the hippocampal dentate gyrus that were also reversed by postextinction corticosterone treatment.

CONCLUSIONS: Our results indicate a predominant role for glucocorticoid deficiency over the contribution of reduced hippocampal volume in engendering both REMS alterations and associated deficits in fear extinction consolidation, and they causally implicate blunted glucocorticoids in sustaining neurophysiological disturbances that lead to fear extinction deficits.

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Posttraumatic stress disorder (PTSD) can develop following exposure to traumatic events, causing notable impairments in patients (1,2) and imposing a substantial economic cost (3). Although therapies have advanced (4,5), many patients exhibit partial or temporary relief, with recurring symptoms over time. A crucial challenge is to understand why only about 25% to 35% of people who are exposed to severe trauma develop PTSD (1,5). Identifying predisposing factors can help with early detection, understanding the mechanisms underlying PTSD onset, and refining treatments (5).

Some preexisting behavioral (impairments in fear extinction) and biological (blunted glucocorticoid levels, small hippocampal volume, sleep disturbances) traits have been identified as potential vulnerability factors for developing PTSD. Behaviorally, difficulty in extinction learning has emerged as both a predisposing factor and a core process underlying PTSD symptoms (6,7–12).

Biologically, low glucocorticoid levels (12–14) and smaller hippocampal volume (15–17) were originally thought to result from trauma (18,19), but they are currently viewed also as potential pretrauma risk factors for PTSD (15,17,20). Establishing their causal role is challenging due to data collection issues and limited animal models (21). Some longitudinal studies have linked reduced cortisol reactivity to PTSD development (22,23), but findings have been inconsistent for baseline cortisol levels (24–26), and the link between prior smaller hippocampal volume and PTSD is still being debated (15,17,27–30). Given that both glucocorticoids (21) and the hippocampus (31–33) play roles in fear extinction, and these traits can be mechanistically interrelated (34–36), their contributions to PTSD vulnerability deserve further attention (37).

Sleep disturbances, especially in rapid-eye-movement sleep (REMS), are also emerging as potential pretrauma PTSD markers (38–41). Sleep, which is critical for memory

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consolidation (42,43) and is affected by glucocorticoid levels (44,45), may be an additional risk factor for the development of PTSD (46).

Here, we explore whether genetic predisposition for blunted glucocorticoid responsiveness 1) influences hippocampal volume, fear extinction, and sleep architecture and 2) predicts and/or causes fear extinction deficits and sleep alterations beyond the contribution of hippocampal volume. We utilized a genetically selected rat model with reduced corticosterone (CORT) responsiveness involving polygenic inheritance (47–50), consistent with the polygenic nature of both human variation in cortisol levels (51,52) and PTSD genetic risk (53).

METHODS AND MATERIALS

A detailed description is provided in the Supplement.

Study Approval

All procedures were conducted in accordance with the Swiss National Institutional Guidelines on Animal Experimentation and approved by the Swiss Cantonal Veterinary Office Committee for Animal Experimentation.

RESULTS

Low Glucocorticoid Responsiveness to Stress Leads to a Smaller Hippocampus

We used 3 genetically selected rat lines (47–50) generated from an outbred population of Wistar Han rats and classified them as low-CORT responders (low-CRs), controls with normative CORT responsiveness (Ctrs), and high-CORT responders (high-CRs) at postnatal day 30 (P30) (Figure 1A; Figure S1A) [see (50) and the Supplement]. We studied the progeny of selected breeders (i.e., not stressed themselves) and compared low-CR and Ctr rats. Adult low-CR rats exhibited blunted CORT responsiveness following novelty (open field) (Figure 1B) or restraint (Figure S1B) stress and showed a blunted CORT circadian pattern (Figure S1C).

Ex vivo magnetic resonance imaging whole-brain voxelwise measurements to investigate gray matter volume (Figure 1C) indicated no differences in total brain size (Figure 1D), while hippocampal areas were prominently decreased in low-CR rats (Figure 1C, E, F), including CA1, CA3, and the dentate gyrus (DG) (Figure 1G). We also measured several regions that have been implicated in fear conditioning and extinction (54) and confirmed specific changes circumscribed to the dorsal hippocampus (Figure S1D). Immunohistochemistry measures of hippocampal mineralocorticoid (MR) and glucocorticoid (GR) receptors indicated higher MR levels in the CA1 and a higher MR/GR ratio in the CA1 and DG in low-CR rats (Figure S1E).

Low Glucocorticoid Responsiveness to Stress Leads to Impaired Fear Extinction

Next, we asked whether blunted CORT responsiveness led to core behavioral features of PTSD, such as alterations in fear acquisition and/or extinction (5). We trained animals in an acoustic fear conditioning paradigm and then assessed their fear extinction capacities through exposure to repetitive conditioned stimulus (CS) presentations without an unconditioned stimulus (US) in a different context (Figure 2A).

In males, we found that low-CR rats did not differ from Ctr rats in the memory recall test given 24 hours after training (Figure 2B) or during the subsequent extinction training (Figure 2B). However, low-CR rats exhibited higher freezing levels than Ctr rats in the extinction retrieval session the next day. Their heightened fear response was still observed when testing took place 30 days later (remote extinction retrieval assessment) (Figure 2B). These results indicate that low-CR male rats showed a deficit in the consolidation of fear extinction, a trait that predisposes humans to develop PTSD (6,55–57) and a distinctive hallmark of the disease (7–11,58). These findings are consistent with previously reported deficits in long-term retention and reversal learning in the hippocampus-dependent water maze task in low-CR rats (48). We replicated the extinction memory deficits in another cohort (Figure 2C, D) that was further exposed to a reinstatement protocol (i.e., single US exposure in a novel context). Notably, low-CR rats showed higher susceptibility to fear relapse, as indicated by higher freezing levels in response to the CS in a recall session 24 hours later (Figure 2E).

In females, we found no difference between low-CR and Ctr rats in any of the fear processes that we examined (Figure 2F). Two-way analysis of variance indicated a significant line × sex interaction, supporting the observed differences in males but not in females (Figure S2). Although we cannot exclude the possibility that low-CR female rats may show extinction deficits using other protocols, to advance the mechanistic understanding, the remainder of the study was conducted with male rats.

Postextinction CORT Treatment Facilitates Fear Extinction Memory in Low-CR Rats

To probe the causal implication of blunted CORT responsiveness in the fear extinction deficits observed in male rats, we first confirmed that low-CR rats displayed lower postextinction plasma CORT levels (Figure 3A, B). Then, we injected low-CR rats with either CORT (1 mg/kg, intraperitoneally) or vehicle immediately after extinction training. Extinction retrieval was tested in 2 sessions, days 3 and 5 postextinction acquisition, given that memory effects of exogenous glucocorticoids can take up to 48 hours after administration to emerge (59). Notably, whereas vehicle-injected low-CR rats retained higher freezing levels than Ctr rats during the second extinction retrieval session, low-CR rats injected with CORT showed a significant attenuation of the fear response (Figure 3C, left). This attenuation was long lasting because their freezing levels were still lower than in vehicle-injected low-CR rats at remote testing 30 days after fear acquisition (Figure 3C, right). To assess the efficiency of corticosterone in continuing to support the extinction processes, animals received a second injection of either vehicle or CORT following a remote extinction session (day 30) (Figure 3C). CORT-injected low-CR rats showed attenuated freezing at remote extinction retrieval (day 33) (Figure S3A) and at fear relapse after reinstatement 3 week later (Figure 3D). Therefore, postextinction CORT normalized fear extinction consolidation in low-CR rats. These effects were enduring and specific to extinction memory, with the original fear memory still subject to reinstatement.

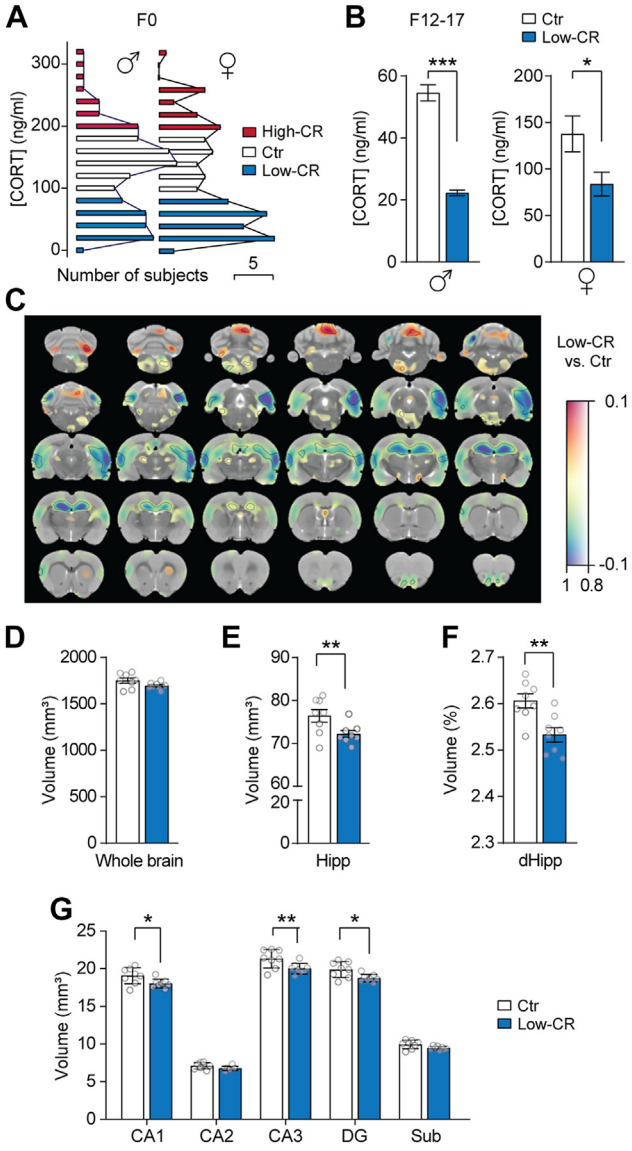

Figure 1. Constitutive differences in glucocorticoid responsiveness to stress are associated with hippocampal structural changes. **(A)** Distribution of plasma CORT levels in response to stress exposure at the peripubertal stage in outbred Wistar male and female rats in the F0 generation reported in (53). Breeders were selected for the generation of lines with constitutive differences in glucocorticoid responsiveness, considering an upper limit of 100 ng/mL for low-CR rats and a lower limit of 200 ng/mL for high-CR rats (color coded). **(B)** Plasma CORT levels measured in the offspring from Ctr and low-CR rats in adulthood after exposure to an open field for generations F12-F14-F17 (males; Ctr, n = 155; low-CR, n = 238) and F13 (females; Ctr, n = 14; low-CR, n = 12). **(C)** A map of voxelwise differences in gray matter volume between low-CR and Ctr rats calculated from ex vivo magnetic resonance images overlaid on the rat brain magnetic resonance imaging template. The colors of the overlay indicate brain volume differences (in mm^3) thresholded at $1 - p > .8$ (cool colors represent reduced volume and warm colors represent increased volume in low-CR compared to that in Ctr rats), and the transparency of the overlay is related to p value; black contours demarcate regions where uncorrected $p < .01$. **(D, E)** Comparison of the volume of the total brain and of the whole hippocampus. **(F)** Comparison of dorsal hippocampus volume normalized to the whole brain. **(G)** Volumetric analysis of hippocampal subregions. All numerical data are means ± SEMs; the number of observations is indicated by datapoints in the bar graphs. Statistical significance was assessed by the Mann–Whitney test **(B)**, unpaired t test **(D–F)**, or two-way analysis of variance **(G)**, with $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$. Nonsignificant comparisons are not indicated. CA, cornu ammonis; CORT, corticosterone; CR, CORT responder; Ctr, control; DG, dentate gyrus; dHipp, dorsal Hipp; Hipp, hippocampus; Sub, subiculum.

Inhibiting CORT Synthesis During Extinction Memory Consolidation Impairs Extinction Retrieval

As reported above, low-CR rats showed both blunted CORT responsiveness and reduced hippocampal size. To investigate whether reduced glucocorticoid release impacts fear extinction in isolation from any neuroanatomical difference, we assessed the impact of CORT synthesis inhibition in Ctr rats during the consolidation of fear extinction. First, we verified the efficiency of metyrapone (Mety) (100 mg/kg, subcutaneous, 15 minutes prestress), a CORT synthesis blocker (60,61), in decreasing the levels of circulating CORT following exposure to restraint stress. Sixty minutes after stress onset, Mety-treated animals displayed blunted CORT levels compared with vehicle-treated animals (Figure 3E). Accordingly, we administered Mety 15 minutes before extinction training to dampen the levels of circulating CORT during the postextinction period. Freezing levels during extinction training were not affected by Mety (Figure S3B). However, in the extinction retrieval test 24 hours later, Mety-injected rats showed higher freezing levels than vehicle-injected rats (Figure 3F). Therefore, decreasing CORT levels in the postextinction period inhibited the consolidation of extinction memory. When Mety was given to influence CORT levels during extinction training, there were no retrieval deficits (Figure S3C).

High Glucocorticoid Responsiveness Is Not Associated With Fear Extinction Deficits

Given that both blunted and high glucocorticoid levels can lead to reduced hippocampal volume (35,36), we utilized the high-CR line to assess how this trait relates to both hippocampal size and fear extinction. Gray matter volume analyses revealed that high-CR rats displayed smaller hippocampal sizes (Figure S4A–C, E), with reductions restricted to the CA3 region (Figure S4D). Analyses that included the fear conditioning and extinction network of brain regions did not identify any significant difference in other brain areas (Figure S4F).

Importantly, when exposed to a cued fear conditioning and extinction paradigm (Figure 4A), and despite their reduced hippocampal size, high-CR rats showed freezing levels at extinction retrieval, spontaneous recovery (Figure 4B), and fear relapse (Figure 4C) that were not significantly different from those of Ctr rats.

To further investigate the relationship between hippocampal size and glucocorticoid actions on fear extinction, we next assessed the effects of GR blockade with RU486 (10 mg/kg, intraperitoneally) given just before the extinction session in high-CR rats. While this treatment did not affect freezing during extinction training (Figure 4D), RU486-injected high-CR rats exhibited higher freezing responses than vehicle-treated high-CR and Ctr rats in the extinction retrieval test that took place 24 hours later (Figure 4E).

Taken together, these findings strongly support the view that blunted CORT responsiveness during the consolidation of fear extinction acts as a decisive factor in promoting fear extinction deficits, while small hippocampal size per se is not sufficient to determine impairments in fear extinction.

Sleep-Related Signatures Associated With Blunted CORT Responsiveness

Given the emerging hypotheses linking sleep disturbances with PTSD susceptibility (41,62,63), we performed polysomnographic recordings in naïve low-CR and Ctr rats during 48 hours of undisturbed sleep/wake cycles (Figure 5A). Low-

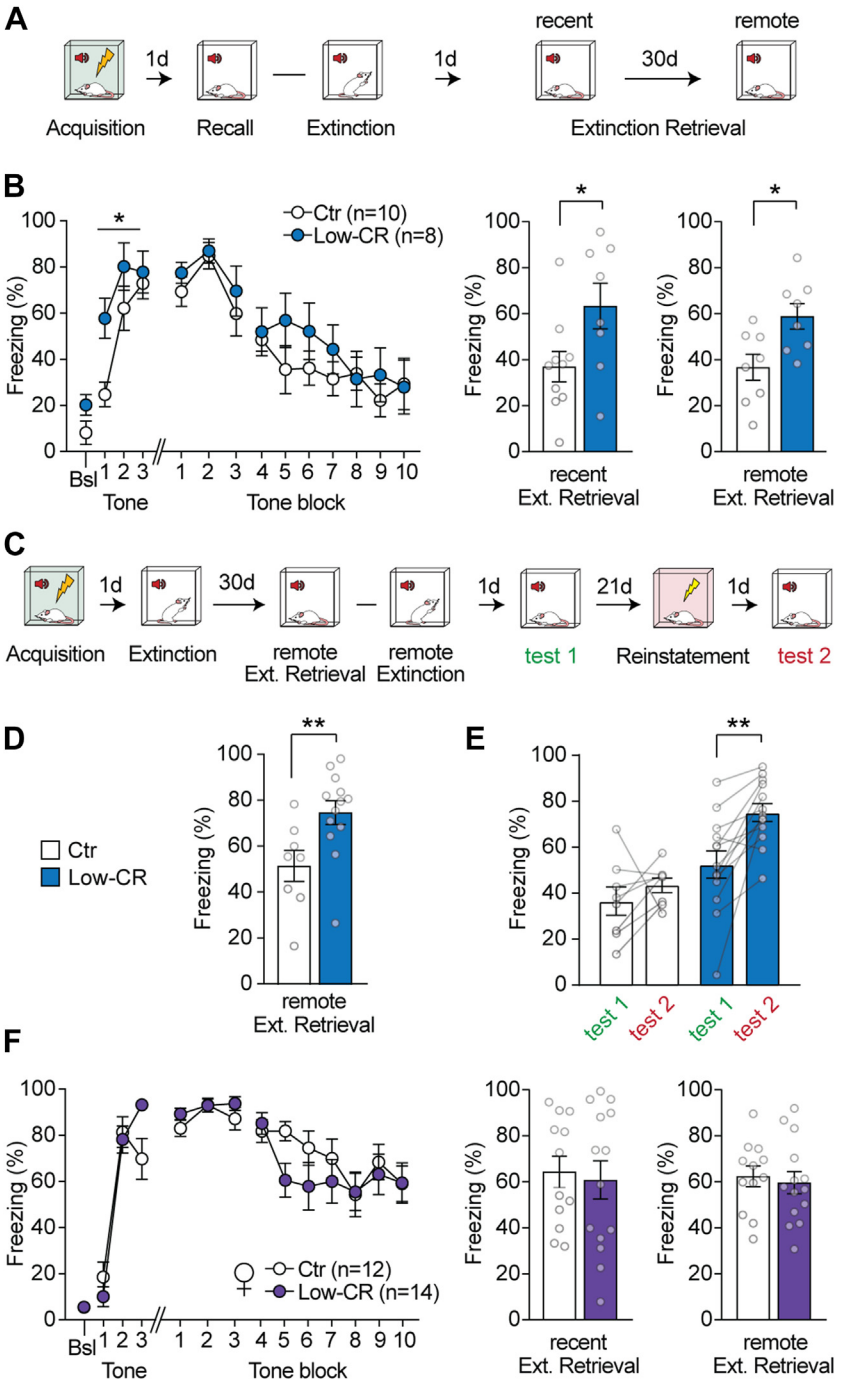

Figure 2. Blunted glucocorticoid responsiveness to stress is associated with impaired fear extinction in male rats. **(A)** Schematic representation of the paradigm for cued fear conditioning and extinction. The cued fear condition was induced by pairing a CS (tone) with an unconditioned stimulus (foot-shock) 3 times. Memory recall and extinction training and retrieval were performed in a different context. Consolidation of extinction was assessed 1 day after extinction training (recent extinction retrieval) and 30 days thereafter (remote extinction retrieval). **(B)** Freezing levels measured in Ctr and low-CR male rats during baseline and during the presentation of the CS in the acquisition phase, average freezing levels over 2 CS presentations (tone block) for recall and extinction training, and average freezing levels at recent and remote extinction retrieval. **(C)** Schematic representation of the paradigm for cued fear conditioning, extinction, and subsequent fear reinstatement. **(D)** Mean freezing levels during remote extinction retrieval of rats subsequently exposed to fear reinstatement. **(E)** Fear relapse was assessed by comparison between the freezing levels during the last exposure to CS after remote extinction (test 1) and the freezing levels at CS presentation (test 2) after a mild shock was delivered in a novel context. **(F)** Same representation as in **(B)** for data from Ctr and low-CR female rats. All numerical data are means ± SEMs; the number of observations is indicated by data points in the bar graphs. Statistical significance was assessed by unpaired *t* tests **(B, D, F)** or repeated-measures two-way analysis of variance **(B, E, F)** following Holm-Sidak's post hoc analysis, with $*p < .05$, $**p < .01$, $***p < .001$. Nonsignificant comparisons are not indicated. Bsl, baseline; CR, corticosterone responder; CS, conditioned stimulus; Ctr, control; Ext., extinction.

CR rats spent less time in REMS during the light (inactive) phase (Figure 5B) and more time in the awake state (Figure S5C), without differences in the number of bouts of the different sleep states (Figure S5A, B) or in the amount of time spent in non-REMS (Figure S5D). Notably, REMS bouts were longer in low-CR rats (Figure 5C).

Norepinephrine (NE) is emerging as a key candidate in linking the stress response, memory processing, and sleep physiology (64–68). Excessive locus coeruleus (LC) activity and higher NE levels have been reported in patients with PTSD (69,70), and heightened hippocampal NE signaling during REMS in particular has been proposed to lead to PTSD pathophysiology (71). To investigate whether the altered REMS microarchitecture in low-CR rats is associated with alterations in NE signaling, we combined polysomnographic recordings with fiber photometry using the biosensor $GRAB_{NE}2h$ (65,68,72) expressed in the DG (Figure 5D). At non-REMS–REMS transitions, we observed a pronounced drop in hippocampal NE in Ctr rats, which is consistent with the reported LC silencing during REMS (65,68,73). However, the change in NE levels was attenuated in low-CR rats and was associated with a prolonged decay time compared with that of Ctr rats (Figure 5E), suggesting that a residual level of NE may persist in the DG during REMS. We found no differences in LC area, tyrosine hydroxylase density, or cFos activity in the LC between Ctr rats and low-CR rats (Figure S6), suggesting that the differences in $GRAB_{NE}2h$ signal at non-REM–REM transitions are not due to lower initial levels of NE release during non-REMS in low-CR rats, but rather to a persistence of extracellular NE during REMS.

Taken together, these findings indicate that subjects with low-CORT responsiveness exhibit a constitutive alteration in sleep architecture involving lower amounts of REMS, possibly accompanied by excessive NE signaling in the hippocampus.

CORT Normalizes REMS and Hippocampal NE Dynamics

Finally, we analyzed the sleep profile during the hours following extinction training (performed 5–6 hours into the light phase), i.e., in the period expected to be conducive to the consolidation of fear extinction memory (74,75). Importantly, implanted low-CR rats retained higher freezing levels than Ctr rats in response to the CS during the extinction retrieval test (Figure 6A).

The hippocampus contributes to long-term consolidation during sleep, even for nonclassical hippocampus-dependent memories (76,77). Thus, we examined REMS architecture and hippocampal NE levels in CORT-injected low-CR rats and vehicle-injected low-CR and Ctr rats. The CORT injection (1 mg/kg, intraperitoneally) was administered immediately after extinction training, after which rats were tethered to the electroencephalography/electromyography system and left undisturbed in their home cage. While we found no differences in total sleep time, indicating that rats slept after extinction training and CORT administration in these experiments (Figure S7), Ctr rats showed a surge in REMS in the first 3 hours postextinction, which appeared as an increase in both the number of REMS bouts and the percentage of time spent in REMS, a phenomenon that has been reported to support the consolidation of fear extinction (42,74,75,78) (Figure 6B). However, vehicle-treated low-CR rats failed to show this surge in REMS, and both the bout number and the percentage of

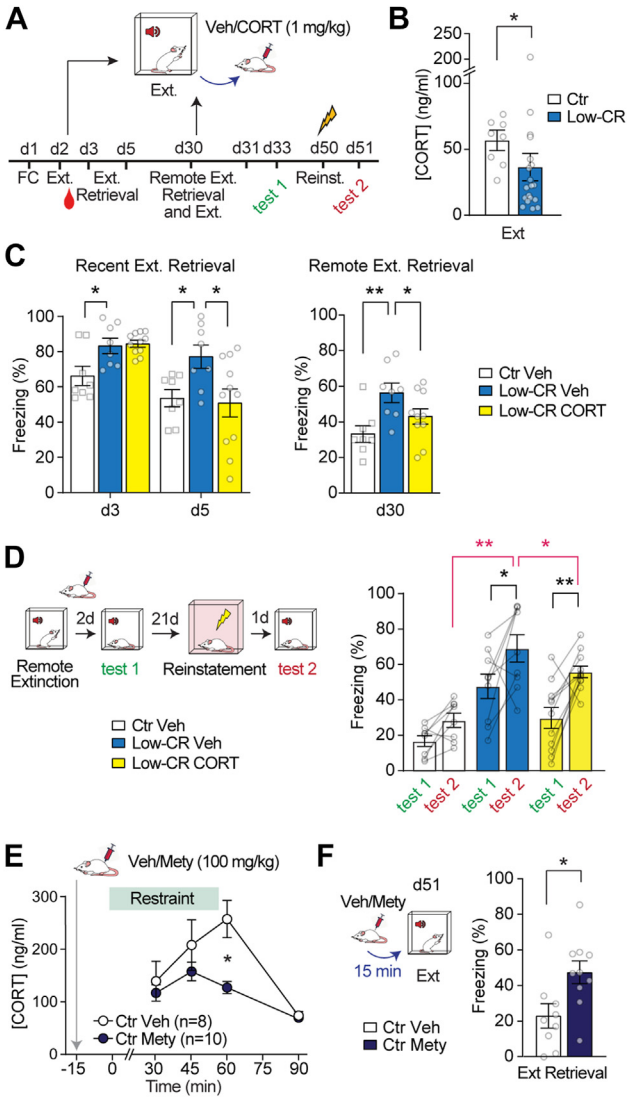

Figure 3. Modulation of glucocorticoids impacts fear extinction consolidation. **(A)** Schematic representation of the paradigm for cued FC and extinction with postextinction administration of CORT in low-CR rats. **(B)** Plasma CORT levels measured in Ctr and low-CR rats after the first extinction training. **(C)** Mean freezing levels during recent extinction retrieval (d3 and d5) and remote extinction retrieval (d30). **(D)** Mean freezing levels prior to (test 1) and after (test 2) fear reinstatement. **(E)** Time course of plasma levels of CORT in Ctr rats receiving an injection of vehicle/metyrapone (100 mg/kg) 15 minutes prior to exposure to restraint stress. **(F)** Mean freezing levels during extinction retrieval in Ctr rats injected with vehicle or metyrapone before extinction training. All numerical data are means ± SEMs; the number of observations is indicated by data points in the bar graphs. Statistical significance was assessed by unpaired *t* test **(F)**, Mann-Whitney test **(B)**, one-way **(C)** or repeated-measures two-way **(D, E)** analysis of variance followed by Holm-Sidak's post hoc analysis, with $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$. Nonsignificant comparisons are not indicated. CORT, corticosterone; CR, CORT responder; Ctr, control; d, day; Ext., extinction; FC, fear conditioning; Mety, metyrapone; Reinst., reinstatement; Veh, vehicle.

time spent in REMS reached a plateau once sleep was stabilized after the first hour postextinction training. Notably, CORT treatment in low-CR rats significantly affected these parameters, which were normalized toward levels found in Ctr rats.

Photometric recordings in the hippocampus revealed that the NE levels during REMS in low-CR rats remained more elevated than those in Ctr rats during the postextinction training period (Figure 6C, D), expanding our baseline observations (Figure 5E). Importantly, CORT treatment reduced NE levels during REMS, leading to a normalization of the excess in hippocampal NE observed in low-CR rats.

Taken together, these data underscore the relevance of REMS during the postextinction training phase for the consolidation of extinction memory and support the implication of blunted CORT responsiveness in the alterations of REMS architecture and hippocampal NE signaling that are detrimental for memory consolidation.

DISCUSSION

We showed here that genetic selection for blunted glucocorticoid responsiveness (47–50) engenders a distinct phenotype that encompasses several pivotal PTSD vulnerability traits, including impaired fear extinction, small hippocampal volume, and REMS disturbances, thus revealing that these traits are biologically interconnected. This is quite remarkable because most studies have focused on 1 (maximum 2) risk factor(s) at a time, considering them to be independent risk factors. Moreover, we demonstrated a causal role for a deficiency in mounting a glucocorticoid response in both fear extinction deficits and concomitant REMS disruptions, which were normalized by CORT administration during the postextinction period.

Patients with PTSD frequently show smaller hippocampal volume. Several human studies have proposed that reduced hippocampal volume is a PTSD vulnerability factor (15,17,79), while others have not supported this view (28), suggesting that the reduction is mainly due to environmental effects, such as the stress of combat (27). Our study supports a preponderant role for glucocorticoid deficiency in engendering both REMS alterations and associated deficits in fear extinction consolidation while minimizing the contribution of reduced hippocampal volume per se. The reduction in hippocampal volume in low-CR rats is consistent with a bidirectional relationship between glucocorticoid levels and hippocampal structure (34–36). Given that the observed reductions were in animals that were not exposed to stress or trauma, they underscore the importance of difference in hippocampal size as a further constitutive trait to consider in interpreting our data.

Low-CR rats showed a flattened circadian pattern but, importantly, it was sufficient to provide a corticosterone treatment during the fear extinction consolidation period to correct their impaired fear conditioning. These data help explain clinical data on pretrauma cortisol indicating that whereas blunted responsiveness to life stressors predicts PTSD development well (22,23), basal cortisol levels do not (24–26). Fear extinction deficits in patients with PTSD are typically not manifested during extinction training but are particularly observed in subsequent extinction recall sessions (9,57,80), pointing to a specific deficit in the consolidation of

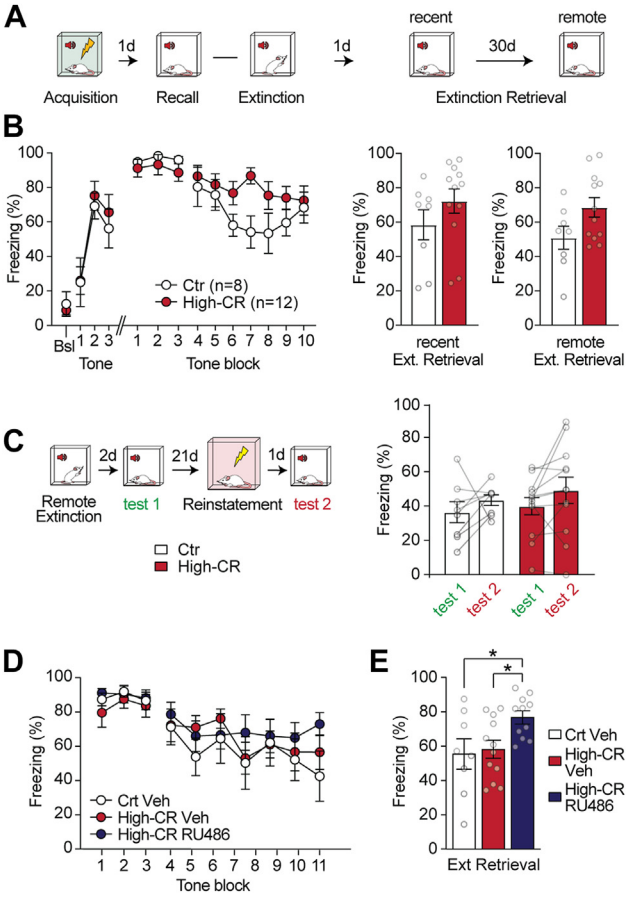

Figure 4. Elevated glucocorticoid responsiveness to stress is not associated with fear extinction impairments. **(A)** Schematic representation of the paradigm for cued fear conditioning and extinction. **(B)** Freezing levels measured in Ctr and high-CR male rats during baseline and during the presentation of the conditioned stimulus for the acquisition phase and average freezing levels over 2 conditioned stimulus presentations (tone block) for the postacquisition phases. **(C)** Schematic representation of the paradigm for fear reinstatement and mean freezing levels at subsequent recall for Ctr and high-CR rats. **(D)** Schematic representation of the paradigm for glucocorticoid receptor blockade using pre-extinction administration of RU486 (10 mg/kg) and freezing levels measured in Ctr and high-CR rats subsequently exposed to fear recall and extinction training. **(E)** Mean freezing levels during extinction retrieval in Ctr and high-CR rats injected with vehicle and high-CR rats injected with RU486 before extinction training. All numerical data are means ± SEMs; the number of observations is indicated by data points in the bar graphs. Statistical significance was assessed by unpaired *t* test **(B)**, one-way **(E)** or repeated-measures two-way **(C)** analysis of variance followed by Holm-Sidak's multiple comparison test, with $^*p < .05$. Nonsignificant comparisons are not indicated. Bsl, baseline; CR, corticosterone responder; Ctr, control; Ext., extinction; Veh, vehicle.

fear extinction. This is consistent with the well-known facilitatory role of glucocorticoids on memory consolidation (81–83), including fear extinction memory (84–87).

We found that low-CR rats displayed higher MR levels (significant in the CA1 region and a strong trend in the DG) than Ctr rats. Importantly, given the lack of significant differences for the GR, low-CR animals showed a considerably higher MR/GR ratio in these hippocampal subregions than Ctr animals.

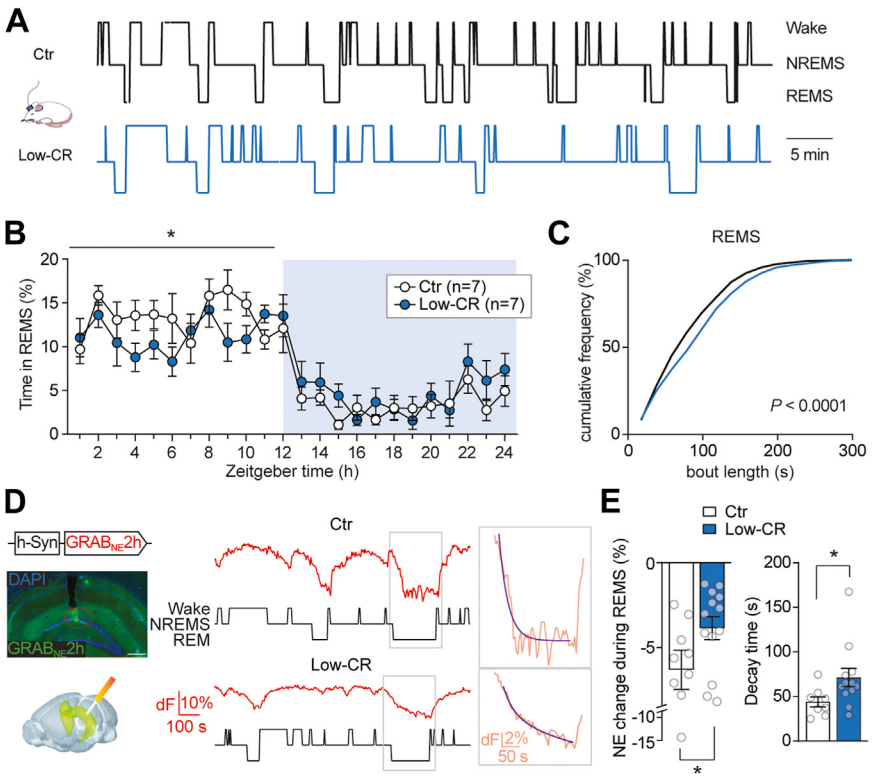

Figure 5. Blunted glucocorticoid responsiveness to stress is associated with REMS alterations. **(A)** Example hypnograms from a Ctr rat and a low-CR rat that had received implants for electroencephalography/electromyography recordings. **(B)** Percentage time Ctr and low-CR rats spent in REMS across the sleep-wake cycle. **(C)** Cumulative distribution of REMS bout lengths across the sleep-wake cycle. **(D)** Schematic representation of photometric recordings in the hippocampus using the NE sensor GRAB$_{NE}$2h, with the micrograph showing an example post hoc verification of the viral infection and recording site. Example traces show hippocampal NE levels (red) measured in a Ctr rat and a low-CR rat and corresponding hypnogram (black), with enlarged portions showing biexponential fit (purple) of the photometric signal during REM episodes. **(E)** Mean values of the change in NE levels and decay times of NE signals (weighted τ from biexponential fit) measured during REMS in Ctr and low-CR rats. The numerical data in **(B, E)** are means ± SEMs; the number of observations is indicated by data points in the bar graphs. Statistical significance was assessed by unpaired *t* test **(E)**, Kolmogorov-Smirnov test **(C)**, repeated-measures two-way analysis of variance **(B)**, with $^*p < .05$. Nonsignificant comparisons are not indicated. CR, corticosterone responder; Ctr, control; NE, norepinephrine; NREMS, non-REMS; REMS, rapid-eye-movement sleep.

Increases in MR expression as well as MR/GR balance are known to be crucial in determining glucocorticoid regulation, particularly leading to enhanced negative feedback on the hypothalamic-pituitary-adrenal axis. Accordingly, these findings may underlie—at least to some extent—the blunted glucocorticoid responsiveness in these animals. Importantly, several studies have suggested that fear extinction may rely on forebrain MRs during periods of low circulating CORT levels at the time of extinction training (88,89). Moreover, the balance between MR and GR activation seems to be crucial for adequately processing aversive memories (90).

We used 3 CORT rat lines to parametrically investigate how variations in glucocorticoid function during the postextinction period and hippocampal size relate to the efficiency of fear extinction consolidation. First, our data indicated that there was an inverted-U-shape relationship between constitutional glucocorticoid responsiveness and hippocampal size, consistent with previous observations in humans (91). Next, we found that 1) in low-CR rats, which showed impaired extinction and reduced hippocampal volume, increasing systemic CORT levels rescued extinction memory; 2) in Ctr rats, which showed normative levels of fear extinction, CORT responsiveness, and hippocampal size, blocking CORT synthesis led to increased freezing levels at subsequent extinction retrieval; 3) in high-CR rats, which did not show impairments in fear extinction or fear relapse at reinstatement but displayed reduced hippocampal volume, antagonizing GRs impaired the consolidation of fear extinction. Accordingly, blunted, but not high, glucocorticoid responsiveness was associated with impaired fear extinction memory consolidation. These findings strongly implicate CORT signaling in proper consolidation of the extinction memory and suggest that hippocampal volume may not relate to fear extinction deficits per se but rather through its capacity to regulate hypothalamic-pituitary-adrenal axis functioning and glucocorticoid signaling (34,37).

Sleep disturbances, in particular disturbances in REMS, constitute a major symptom of PTSD (38–40) that is linked to negative outcomes (92–94) and which has been hypothesized to play an etiological role in PTSD (41) and to be predictive of worse disease symptomatology (62,63,93). Consistent with these data, low-CR rats spent less time in REMS during their inactive phase. Postextinction REMS is required for the successful consolidation of extinction memory (74,75). In our study, the failure of low-CR rats to achieve sufficient REMS after extinction is consistent with data showing that REMS deprivation immediately after extinction training impairs fear extinction in rats (74). Furthermore, in humans, glucocorticoids have been demonstrated to decrease REMS (95). However, a natural rise in cortisol during the latter part of the night is associated with REMS and facilitates memory consolidation (45). The effects of exogenously administered glucocorticoids on REMS may be influenced by endogenous glucocorticoid levels. Specifically, in Addison disease patients with low cortisol levels, exogenous cortisol supports REMS regulation and sleep consolidation (96). Similarly, our results with low-CR rats, which display diminished glucocorticoid responsiveness and disrupted REMS, suggest

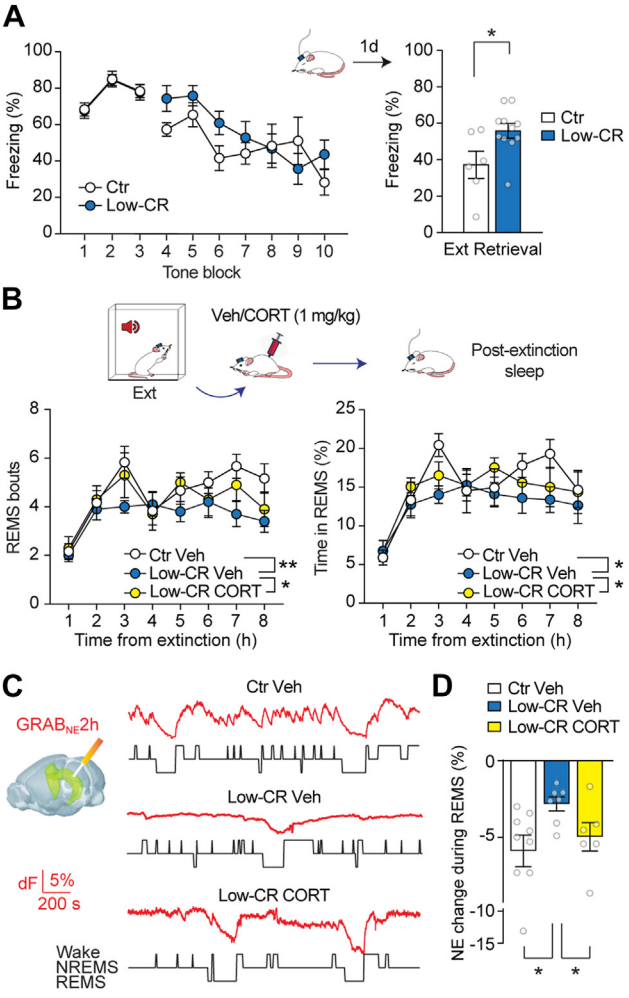

Figure 6. Postextinction REMS alterations are normalized by CORT administration. **(A)** Freezing levels during extinction training and extinction retrieval of Ctr and low-CR rats that had received implants for electroencephalography/electromyography recordings. **(B)** Density of REMS bouts and the percentage of time spent in REMS in the postextinction period for Ctr and low-CR rats injected with vehicle and low-CR rats injected with CORT (1 mg/kg) after the extinction training session. **(C)** Schematic representation of photometric recordings in the hippocampus using the NE sensor $GRAB_{NE}2h$, with example NE signals (red) and corresponding hypnograms (black) measured in a vehicle-treated Ctr and low-CR rat and a low-CR rat injected with CORT. **(D)** Mean values of the change in NE levels measured during REMS in Ctr and low-CR rats. The numerical data are means ± SEMs; the number of observations is indicated by data points in the bar graphs. Statistical significance was assessed by unpaired *t* test [**(A)** right], Mann-Whitney test **(D)**, repeated-measures two-way analysis of variance [**(A)**, **(B)** left], with $^{*}p < .05$, $^{**}p < .01$. Nonsignificant comparisons are not indicated. CORT, corticosterone; CR, CORT responder; Ctr, control; NE, norepinephrine; NREMS, non-REMS; REMS, rapid-eye-movement sleep; Veh, vehicle.

that exogenous CORT can enhance REMS, potentially aiding in sleep and memory consolidation.

During REMS, the brain experiences a characteristic neuromodulatory profile that involves high glucocorticoid and low catecholamine levels, including NE (43). The LC decreases its activity and shuts down immediately before and during REMS (73), and these silent LC periods during REMS may be crucial for memory consolidation (64,68). Heightened LC-NE activity is also a recognized alteration associated with PTSD and has been implicated in hyperarousal during sleep (69,70,97). Our observations suggest that low-CR rats exhibit excessive hippocampal NE levels during REMS. These findings provide the first experimental evidence in support of a recent hypothesis positing that a high NE tone during REMS is causally involved in the failure to consolidate extinction memory (71). Ultimately, during NE-enriched REMS, also referred to as "restless REMS" (98), a failure of the hippocampus–prefrontal cortex–amygdala network may be responsible for impaired consolidation of extinction memory. Therefore, in our study, REMS emerged as an important physiological factor that is highly modulated by variations in glucocorticoid responsiveness and, in parallel, strongly involved in the adaptive consolidation of cued fear extinction memory.

Our data identify the postextinction training period as the crucial time period during which a glucocorticoid deficiency should be targeted by treatments and identify the restoration of sleep architecture as a likely mechanism of action implicated in glucocorticoid effects. Previous studies have shown that glucocorticoids facilitate fear extinction (85,99–102). However, only a limited number of studies have tried this intervention with patients with PTSD (103). Our findings underscore the importance of considering interindividual differences in hypothalamic-pituitary-adrenal axis responsiveness when evaluating a clinical strategy and highlight the postextinction phase as a critical time for glucocorticoid administration.

Strikingly, our genetic selection scheme, while being highly effective in attribute selection (i.e., achieving a specific range of CORT responsiveness within each line), concurrently leads to small hippocampal volume, impaired fear extinction, and REMS disturbances, each of which is an important PTSD vulnerability trait. Unlike previous PTSD studies that focused on 1—or a maximum of 2—traits, our genetic selection approach revealed the interconnectedness of these traits. The reasons for their interconnectivity can be multifactorial, including both genetic (e.g., pleiotropy, genetic correlation, and linkage disequilibrium) and nongenetic (e.g., mechanistic effects, where one trait affects the physiological or developmental processes that lead to changes in the other traits) factors. Glucocorticoids—our targeted trait for selection—through their genomic and nongenomic effects can regulate hippocampal structure, memory consolidation, and sleep architecture (82,103–107). Our own findings support a causal role of glucocorticoids in extinction memory and REMS alterations. In turn, changes in some of these traits (e.g., sleep disturbances, particularly in REMS processes) can also influence the expression of other traits (e.g., the consolidation of extinction memory) (44,78). Importantly, while we focus here on a genetic selection angle to PTSD vulnerability, it is important to acknowledge that the same—and additional—mediating factors (e.g., glucocorticoids) can affect the traits investigated here (i.e., brain structure, sleep, behavior) as a consequence of previous stress exposure, particularly during early life (108).

There are several reasons to believe that the multitrait interconnectedness found following selection for blunted

glucocorticoid responsiveness is representative of their relationship in nature. First, we followed gold standard genetic selection approaches to maximize genetic variance while facilitating selection response (109). The parental group was a large sample of outbred Wistar rats with minimum coancestry. Selection criteria over generations allocated animals to the different CORT groups according to fixed measures—as opposed to relative percentile cutoffs for each cohort—which ensured a robust selection response. Second, a crucial aspect of our nonrandom mating procedures for line generation (sibling mating was excluded, and a refreshing of the selection introduced in F12) was inbreeding avoidance, which favors genetic diversity (110) and polygenic inheritance (111). The latter is crucial to align with the polygenic nature of both variation in plasma cortisol levels (51,52) and genetic risk for PTSD (53) in humans. Third, for genetic selection schemes that avoid inbreeding, as in our case, the risk for genetic drift—and the associated instability of correlational traits—is minor.

Strikingly, despite the higher prevalence of PTSD in women (112), we did not find fear extinction impairment in female low-CR rats. However, the type of trauma and the age at the time of the traumatic event affect PTSD development in a sex-dependent manner (1,112). For example, Delahanty *et al.* (113) showed a sex-specific relationship between increased peritrauma cortisol levels and increased PTSD symptoms in male children but not female children. These observations suggest the presence of potential sex differences in biological predictors and PTSD vulnerability. Accordingly, a limitation of our study is the absence of a comprehensive exploration of whether alternative training protocols might reveal differences in glucocorticoid-related susceptibility to fear extinction deficits in females.

Conclusions

In conclusion, utilizing a rodent model with rats that were selectively bred for glucocorticoid response to stress, we demonstrated that blunted glucocorticoids were associated with PTSD-related features such as impaired fear extinction, reduced hippocampal volume, and REMS disturbances. Our research illuminates previously elusive aspects of PTSD, revealing that blunted glucocorticoid responsiveness not only predicts but may also contribute causally to core PTSD symptoms. This suggests potential benefits of glucocorticoid treatments for patients with diminished glucocorticoid responsiveness. Our findings also underscore the specific role of REMS and glucocorticoids in emotional memory processing.

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CS, SA, and SM conceived of the study. SM, SA, IGdS, JG, OZ, SEW, MM, TCW, and DC acquired and analyzed the data. CS and DC acquired funding. CS, SA, and SM wrote the manuscript. SA and SM prepared manuscript figures. All authors read, edited, and approved the final manuscript.

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All data reported in this paper will be shared by the corresponding author upon request.

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