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Acute hippocampal BDNF restores motivational and forced swim performance after corticosterone

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Abstract

Background—Alterations in cellular survival and plasticity are implicated in the neurobiology of depression, based primarily on the characterization of antidepressant efficacy in naïve rodents, rather than on models that capture the debilitating and protracted feelings of anhedonia and loss of motivation that are core features of depression.

Methods—In adult male mice, we evaluated persistent effects of oral corticosterone (CORT) exposure on anhedonic-like behavior, immobility in the forced swim test (FST), motivational performance in the progressive ratio task, and later endogenous CORT secretion. After verifying long-term decreases in hippocampal Brain-derived Neurotrophic Factor (BDNF) and cAMP Response Element Binding protein phosphorylation (pCREB), the ability of direct hippocampal BDNF microinfusion after CORT exposure to reverse deficits was investigated.

Results—Prior CORT exposure decreased sucrose consumption, appetitive responding, and FST mobility without long-term effects on water:quinine discrimination and endogenous CORT secretion. Critically, BDNF replacement mimicked chronic antidepressant treatment (ADT) by reversing CORT-induced reductions in instrumental performance and FST mobility.

Conclusions—Together these findings link persistent alterations in hippocampal BDNF expression and CREB transcriptional activity with a persistent depressive-like state—as opposed to ADT efficacy. These results identify hippocampal BDNF as an essential molecular substrate that *bidirectionally* regulates appetitive instrumental behavior. Additionally, we suggest this CORT model may provide a powerful tool for future investigation into the neurobiology of complex stress-associated depressive symptoms that persist long after stress exposure itself.

Keywords

glucocorticoid; stress; BDNF; CREB; hippocampus; anhedonia; progressive ratio; motivation

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Introduction

Stressful experiences have a profound impact on neural survival and plasticity, notably in the hippocampus, and regionally-selective morphological stress-related alterations are implicated in the pathophysiology of MDD(1). In rodents, both environmental stress and oral CORT exposure produce hippocampal dendritic retraction(2). These and other alterations may contribute to decreased hippocampal volume in MDD(3,4), though CORT concentrations in previous experiments mimic extreme stress, and functional relationships have yet to be established.

Chronic stress exposure decreases hippocampal BDNF(5–7) and pCREB(8). CREB, in turn, regulates several targets involved in activity-dependent neuronal plasticity, including BDNF. Nevertheless, much of what is believed to be known about neurobiological factors of MDD, including the involvement of hippocampal neurotrophin-regulated signaling, is based upon understanding the actions of standard ADTs in naïve rodents. For example, although enhanced hippocampal pCREB may mediate ADT efficacy(9–12), whether identical mechanisms reverse a depression-like phenotype is not known.

We previously showed that non-invasive CORT exposure induces a persistent depression-like state including chronic ADT-sensitive helplessness-like behavior and decreased instrumental responding for appetitive reinforcement(13). These behavioral changes correlate with a down-regulation of BDNF and its targets in the hippocampus, yet the ability of a *direct* infusion of BDNF to reverse these deficits was not evaluated. Lesion studies implicate the hippocampus in appetitive instrumental behavior(14,15), but putative molecular substrates are largely not identified, despite strong evidence for hippocampal dysfunction in MDD(16). Here, we show direct acute hippocampal BDNF infusion mimics chronic ADT by *reversing* motivational loss and *restoring* FST mobility after prior CORT exposure.

Methods

Subjects and drugs

Male C57BL/6 mice (4–5/cage; Charles River Laboratories, Kingston, NY) were given CORT in place of drinking water for 14 days and then weaned over 6 days to allow for recovery of normal levels of endogenous CORT secretion (for further details, see Supplementary Methods). Mice were experimentally naïve, maintained on a 12-hr light cycle (0700 on), and 12 weeks old at the start of experimentation. They were provided food and water *ad libitum* unless noted. Procedures were Yale University Animal Care and Use Committee approved.

Sucrose consumption

A sucrose consumption protocol modeling anhedonia was developed to evaluate anhedonic-like responding to a palatable fluid. Testing was conducted 25 days after CORT (25–100 µg/ml) exposure to evaluate the long-term consequences of oral CORT. Briefly, mice were habituated to a 1% w/v sucrose (Sigma Aldrich, St. Louis, MO) solution and modest (4–19 hrs) water restriction. On the test day, mice were allowed access to sucrose in the home cage in the absence of cagemates. Body weights did not differ at test. See Supplementary Methods for additional details.

Western blotting

Forty-eight hours after the sucrose test, mice were sacrificed; brains were frozen and cut into 1 mm coronal slices using a chilled brain matrix (Plastics One, Roanoke, VA). Bilateral samples were collected from the dorsal hippocampus(18). Standard Western blotting techniques (see Supplementary Methods) were used to quantify pCREB(ser133), CREB, and GluR2/3, an

AMPA receptor subunit heterodimer constitutively cycled at the cellular membrane and not expected to change(19).

BDNF immunoassay

BDNF was quantified in the same tissue using a 2-site BDNF ELISA in accordance with manufacturer's instructions (Promega, Madison, WI). Homogenates from individual mice were diluted 3-fold and analyzed in duplicate.

Instrumental behavior

Instrumental training and PR experiments were conducted as described previously(13). Briefly, a new cohort of mice was trained to perform the operant response (nose poke) over several sessions during which 1, 2, or 3 responses yielded food reinforcement. After acquisition, mice were fed *ad libitum* and exposed to CORT. After CORT, daily "re-acquisition" sessions, identical to training, restored responding and verified CORT did not affect responding on this highly reinforcing schedule. The PR task was then conducted using a linearly increasing response:reinforcement requirement (*i.e.*, 1, 5, x+4 responses/reinforcement). Mice were tested every 3 days for 3 total sessions starting 2 weeks after CORT(13). The highest response:reinforcement ratios achieved—termed "break point ratios"—were analyzed as described below.

FST

Two weeks after CORT in another group of mice, FSTs were conducted using standard techniques (see Supplementary Methods).

BDNF microinfusion

Naïve mice were first trained to perform the instrumental task, exposed to CORT, and then subjected to "re-acquisition" sessions as above. Mice were anaesthetized with 1:1 2-methyl-2-butanol and tribromoethanol diluted 40-fold with saline. Standard surgical techniques and a stereotaxic frame (Kopf Instruments, Tujunga, CA) were used with bregma and lamda were identified on a flat skull. Recombinant human BDNF (Chemicon, Temecula, CA) dissolved in sterile saline was bilaterally infused 0.1 µl/site over 1 min at AP-1.3 mm, ML±1.0, DV-2.0 and AP-2.1, ML ±1.5, DV -2.2 (18,20), with the needle left in place for 1 min. The BDNF concentration was 0.4 µg/µl (adapted from 21). Prolonged behavioral testing prohibited histological verification, but 2 mice were subjected to excitotoxic lesion to confirm site specificity and provide approximate representations of affected hippocampal regions (see Supplementary Methods; Supplementary Fig. 1).

Mice were returned to food restriction 3 days after surgery at which point they appeared to have recovered and were tested the following day on the PR task. Two mice are not represented due to equipment error. After the session, ambulation was monitored in a clean cage with the Omnitech Digiscan Micromonitor system (Columbus, OH) equipped with 16 photocells. Data are represented as photobeams broken. Next, mice were tested in the FST. These mice were maintained on food restriction and tested in the PR task again 3 days later to confirm responding returned to predicted levels after expected BDNF wash-out(21). Mice were sacrificed; adrenal and thymus glands were excised (in accordance with 22) (Supplementary Fig. 1), as were glands from an independent group of age-matched mice immediately after 2 wks CORT exposure to verify gland weights decreased while CORT was on-board (Supplementary Fig. 1).

Statistical analyses

Sucrose consumption, break point ratios, and BDNF/pCREB/GluR2/3 fluorescence scores comprising dose-effect curves (*i.e.*, figs. 1–2) were analyzed by 1-factor (CORT) analysis of

variance (ANOVA) or 2-factor (CORT $\times$ instrumental conditioning session) ANOVA with repeated measures (RM). Fluorescence scores were square-root transformed to preserve required homogeneity of variance. Post-hoc tests were Tukey's pairwise comparisons. The 25 and 100 $\mu\text{g/ml}$ groups never differed from each other ($p>0.28$), and because mice appeared qualitatively healthier while consuming the lower CORT dose, which robustly increased blood serum CORT to stress-like levels (fig. 7), we used the lower dose only for further experiments.

FST immobility scores in intact mice were analyzed by 2-factor (CORT $\times$ time bin) ANOVA with RM, latency scores by t-test. Post-surgery break points, locomotive and immobility scores, and body and gland weights were analyzed by 2-factor (CORT $\times$ BDNF) ANOVA with RM and Tukey's post-hocs when appropriate, and Student's post-hoc pairwise comparisons for non-RM analyses. Glands from intact mice were analyzed by t-test (Supplementary Fig. 1).

Other measures

Quinine discrimination and blood serum CORT levels during and after CORT consumption were measured in 2 additional groups of mice. Methods are detailed in Supplementary Methods.

Results

Sucrose consumption, instrumental responding

Despite equivalent body weights and habituation, CORT-exposed mice consumed less sucrose than control mice, indicating an anhedonic-like phenotype [$F_{(2,22)}=18.3$, $p<0.001$] (fig. 1b). (Post-hoc p values are provided in figure captions.) Analysis of hippocampal tissue revealed decreased BDNF [$F_{(2,22)}=4.0$, $p=0.03$] (fig. 1c) and pCREB [$F_{(2,22)}=6.3$, $p=0.007$] (fig. 1d), but no changes in GluR2/3 [$F_{(2,22)}<1$] (fig. 1e).

Next, naïve mice were trained to perform an instrumental response for food. Reinforcements earned increased across time [$F_{(11,352)}=56.5$, $p<0.001$], and task acquisition did not differ in mice designated to different doses [$F<1$] (data not shown). During "re-acquisition," we observed no main effect of dose [$F_{(2,32)}=1.4$, $p=0.25$] (fig. 2b), though a main effect of session indicated reinforcements earned progressively increased [$F_{(3,96)}=39.9$, $p<0.001$]. Non-reinforced responses did not differ by dose [$F<1$] (fig. 2c). Paired t-tests comparing earned reinforcements on the last day of the acquisition and "re-acquisition" periods demonstrated that CORT-exposed mice earned the same number of reinforcements as before CORT (all $p>0.5$), indicating responding on this schedule of reinforcement was unaltered. PR responding was, however, decreased at both doses [$F_{(2,32)}=8$, $p=0.002$] (fig. 2d), consistent with diminished sucrose consumption. Interaction effects were not observed (all $p>0.12$).

FST

Hippocampal BDNF is associated with mobility in the FST(21), designed to recapitulate feelings of helplessness in depression(23), and as predicted, prior CORT decreased mobility [$F_{(1,17)}=9.5$, $p=0.007$] (fig. 3a,b). Latency to become immobile was unaffected here and in the microinfusion study ($p>0.6$), and no interactions between time bin and CORT were detected [$F<1$].

Microinfusion

Because hippocampal BDNF was decreased long after CORT exposure, we investigated the consequences of locally manipulating BDNF by infusing BDNF in the hippocampus after CORT and evaluating PR responding and FST mobility.

Initially, mice designated to the 4 groups did not differ during training [$F_{(3,32)}=1.4$, $p=0.26$; interaction $F<1$], though a main effect of session was observed, confirming mice acquired the task [$F_{(7,224)}=49.0$, $p<0.001$]. Reinforcement earned and non-reinforced responses after CORT were also unchanged by designated group [$F_{(2,32)}=1.4$, $p=0.3$; $F_{(2,32)}=1.5$, $p=0.3$, respectively], with no interaction effects [$F_{(6,96)}=1.7$, $p=0.12$; $F<1$, respectively] (not shown). Break point ratios after CORT and BDNF microinfusion differed, however [interaction $F_{(1,30)}=7.3$, $p=0.01$] (fig. 4b): CORT-exposed saline-infused mice achieved lower ratios than controls, as predicted. BDNF infusion after CORT produced a partial recovery of responding, such that these mice did not differ from CORT-exposed saline-infused mice, or saline-infused controls.

Interestingly, BDNF infusion *decreased* responding in control mice. While hippocampal BDNF infusion can be epileptogenic(24,25), which could decrease responding, we did not observe seizures.

Ambulation did not differ between groups [interaction $F<1$] (fig. 4c), suggesting that the patterns of operant responding could not be attributed to gross differences in locomotion. Body weights were also unchanged [interaction $F<1$] (fig. 4c). In the FST, however, an interaction between CORT and BDNF was found [$F_{(1,30)}=5.8$, $p=0.02$] (fig. 5). Saline-infused CORT-exposed mice were highly immobile, as predicted. Acute BDNF restored mobility to baseline; infusion in non-CORT mice had no effect.

After expected BDNF wash-out, mice were retested in the PR task. Responding returned to predicted levels, such that a main effect of CORT was detected [$F_{(1,32)}=4.2$, $p<0.05$], with no interactions ($p=0.98$) (fig. 4d).

Quinine discrimination

Because alterations in gustatory processes could influence animals' responses to food/sucrose, intact mice were exposed to CORT, and quinine aversion was evaluated. Consumption ratios did not differ by CORT across 3 concentrations [$F_{(1,36)}=1.1$, $p=0.3$] (fig. 6); a main effect of concentration confirmed animals detected these differences [$F_{(2,36)}=12.1$, $p<0.01$], with no interaction [$F<1$]. Mice displayed equal preference for 10% (w/v) sucrose ($t_2=0.5$, $p=0.6$). This concentration would not be expected to detect anhedonic-like responding(26).

Blood serum CORT

Blood serum CORT values were robustly increased 1–2 weeks into the exposure period [$H_{(5,60)}=38.4$, $p<0.001$] (fig. 7a), paralleling patterns of weight loss (Supplementary Fig. 2). Daytime blood serum levels 2 weeks *after* exposure did not differ based on CORT status or collection time, with no interaction effects [$F_s<1$] (fig. 7b). When compared to nighttime samples, a main effect of time was detected [$F_{(2,41)}=2.1$, $p=0.03$], consistent with the expected diurnal peak in endogenous CORT at the start of the dark cycle.

Discussion

These studies demonstrate that prior chronic oral CORT exposure induces a persistent anhedonic-like state in mice and provides new evidence for hippocampal BDNF as a molecular substrate regulating primary motivational processes. Major findings can be summarized as follows: 1) CORT resulted in an anhedonic-like decrease in sucrose consumption and diminished PR responding for food reinforcement, but not quinine:water discrimination. 2) CORT decreased hippocampal BDNF and pCREB expression long after exposure. 3) Reduced PR responding was rescued in CORT-exposed animals by replacing hippocampal BDNF, which also restored mobility in the FST. Using this novel, etiologically-relevant model of depression—which increased serum CORT without affecting later basal levels—our data

reveal major molecular substrates that have been identified as ADT targets in *naïve* animals to be persistently suppressed. Together with our previous study(13), these data indicate that direct acute hippocampal BDNF microinfusion mimics chronic ADT effects on motivational drive.

The hippocampus and subiculum constitute the hippocampal formation, with the subiculum providing topographically-organized projections to the nucleus accumbens (NAC), a primary “motivation center”(27). Subicular efferents regulate NAC dopamine neurotransmission(22, 28–31) and sensitivity to reward value(14). Further, dorsal hippocampal lesion/inactivation modulates operant responding for reward(14,15), in such a manner that suggests that the hippocampus influences motivational state, though molecular substrates are largely not identified. Using the well-established PR task, in which animals perform an increasing number of operant responses for each subsequent reinforcement(32), we provide novel evidence that BDNF aids in optimizing PR performance within a narrow dose window, with too little BDNF (due to CORT exposure) or too much (due to infusion in a drug-naïve rodent) decreasing responding.

The same inverted-U-like function was not observed in the FST, where BDNF restored mobility after CORT, but did not affect control animals. How might we reconcile these differences? BDNF is transported to the hypothalamus and perhaps the NAC after hippocampal infusion (33). Supra-physiological levels of hippocampal BDNF in CORT-naïve mice could therefore have been transported to the hypothalamus, where its expression reduces feeding(*e.g.*,34), but not necessarily swimming. CORT-naïve BDNF-infused animals did not weigh less as might be expected (fig. 4c), but all animals were food-restricted, masking any effects of BDNF infusion on free-feeding, but that were otherwise revealed in the instrumental conditioning experiment. Future studies will be aimed at evaluating BDNF transport to relevant feeding and reward “centers.”

Another implication of the BDNF PR data is that ADTs, which enhance hippocampal BDNF and PR responding(13), may restore sensitivity to reward after stress exposure not by simply increasing BDNF but by restoring optimal, homeostatic BDNF levels. Prior CORT may also have persistent consequences for adult neurogenesis(35) and/or long-term potentiation(36, 37), which could render the CORT-exposed animal less sensitive to “reinforcement” by motivational drive(38–40) and impair task performance(41). These hypotheses remain to be explored.

A MDD diagnosis requires multiple symptoms, but few established depression assays incorporate multiple behavioral features of depression, such as robust motivational dysfunction or symptom chronicity, in a single model(42). The consistency with which chronic mild stress decreases motivated behavior, in addition to hedonic responding, has been questioned(43, but see44). By contrast, prior CORT exposure selectively and robustly decreases PR responding for a desirable outcome, and models motivational loss, anhedonia, and “behavioral despair.” This behaviorally-comprehensive depression-like model is also highly persistent, allowing for repeated testing the same animals. In that vein, our data are the first to demonstrate long-lasting CORT-associated decreases in BDNF, as most published reports use tissue collected ≤ 1 day of exposure(45–47).

BDNF and CREB are broadly distributed throughout the brain; whether they play beneficial or detrimental roles for mood regulation likely depends on activity and expression within specific brain regions and circuits. Evidence posits opposing roles for hippocampal and NAC BDNF(48) in negative mood. Similarly, oral CORT *increases* NAC pCREB, and systemic administration of the phosphodiesterase inhibitor, rolipram, mimics—rather than reverses—CORT’s effects on PR responding (Gourley, Kiraly, Taylor, unpublished). Here, we provide strong evidence for an essential role of hippocampal BDNF in the regulation of motivational

state in CORT-exposed animals. Further elucidating the regionally-specific mechanisms by which CORT influences expression and activity of BDNF and related molecules may provide additional insight into stress-associated psychopathologies and ADT development(49,50).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Prior chronic CORT exposure decreased sucrose consumption and hippocampal BDNF and pCREB

(a) Representative CORT intake plots: During CORT exposure, consumption was monitored daily, and consumption values were converted to average doses (mg/kg) per mouse by normalizing daily consumption values to the total body weight of the animals in the cage. Bars represent average *daily* doses achieved during the first two weeks of CORT exposure (*i.e.*, before weaning). (b) When tested after CORT exposure, mice exposed to 25 and 100 µg/ml CORT consumed significantly less sucrose solution than non-exposed control mice of the same weight. (c) Hippocampal BDNF was also decreased in these animals, (d) as was pCREB, (e) while GluR2/3, an AMPA receptor subunit heterodimer constitutively cycled at the cellular membrane (19), was unchanged as expected. Representative bands loaded in the same order as the data presented are inset where appropriate. All bands fell at the expected molecular weights (pCREB/CREB=43 kD; GAPDH=37 kD; GluR2/3=100 kD), and each represents a single animal. Bars represent means (+SEM) per treatment group (* $p \leq 0.03$; ** $p < 0.001$ compared to control group), $n=8-9$ /group.

Fig. 2. Prior CORT suppressed PR responding

(a) Experimental timeline: Naïve mice were first trained to perform an operant responding task for food. Animals were then exposed to the same doses of CORT used previously (0–100 $\mu\text{g}/\text{ml}$) and tested in the PR task to evaluate the long-term effects of CORT on effortful responding and outcome valuation. (b) Mice did not differ during “re-acquisition,” when the response contingency was highly reinforcing. (c) Responding on the inactive nose poke was low across all groups during this period. (d) As in the sucrose consumption experiment, however, mice previously exposed to CORT achieved lower break point ratios than control mice, and the effects of the two doses did not differ from each other. Symbols represent means ($\pm$ SEM) per treatment group (* $p \leq 0.02$ compared to control group), $n=9-17/\text{group}$.

Fig. 3. Prior CORT (25 µg/ml) increased “behavioral despair”

(a) Prior CORT robustly increased immobility in the FST; both total immobility (in b) and immobility in 2-min time bins (in a) are shown. Latency to assume the immobile posture was unchanged here and in the subsequent experiment. Bars and symbols represent means ($\pm$ SEM) per treatment group (* $p=0.007$), $n=9-10$ /group.

Fig. 4. Hippocampal BDNF infusion rescued PR responding after CORT (25 µg/ml)

(a) Experimental timeline: Naïve mice were first trained to perform an operant response for food. Animals were then exposed CORT (25 µg/ml). Mice were then infused into the dorsal hippocampus with BDNF, a major CREB target, and responding in the PR task was evaluated. (b) After CORT exposure and subsequent BDNF infusion, CORT mice with a saline infusion responded characteristically less than control saline-infused mice, while CORT-exposed mice infused with BDNF did not differ from either control saline-infused mice or CORT-exposed saline-infused mice, indicating BDNF infusion provided a partial rescue of responding. Interestingly, control BDNF-infused mice displayed decreased responding compared to control saline-infused mice. (c) Differences in responding could not be attributed to gross differences in locomotor activity or body weights at the time of test. (d) When re-tested upon expected BDNF wash-out (21), PR responding returned to previously-observed levels. Bars and symbols represent means ($\pm$ SEM) per treatment group (* p <0.05 compared to control group unless otherwise noted), n =8–10/group.

Fig. 5. Mobility in the FST was restored by acute BDNF infusion

Immobility in the FST was increased after CORT (25 µg/ml) plus saline infusion, as in intact mice (fig. 3), while BDNF infusion restored mobility to control levels. BDNF had no effects in control mice (but see ref. 21). Bars and symbols represent means (+SEM) per treatment group (* $p < 0.05$ compared to control group unless otherwise noted), $n = 8-10$ /group.

Fig. 6. Effects on quinine discrimination

Because alterations in basic gustatory discrimination could confound these experiments, we tested animals' ability to discriminate between quinine or a high concentration of sucrose and water after CORT (25 μ g/ml). Despite its effects on behavioral responding in other tasks, prior CORT did not influence responding here. Bars represent means (+SEM) per treatment group, $n=7$ /group.

Fig. 7. Serum CORT during and after oral CORT (25 µg/ml) exposure

(a) In a final experiment, the effect of oral CORT on blood CORT levels was quantified by collecting trunk blood at several points during and after the exposure period early in the dark cycle (10:00 p.m.) when mice would be expected to have been active and consuming CORT in the previous hours since lights off. The data reveal robustly increased levels at 1 and 2 weeks into exposure. CORT levels 1 day into exposure, 1 day after weaning, and 2 weeks after weaning were unchanged when analyzed by ANOVA. (b) Daytime CORT was also collected 2 weeks after exposure when behavioral testing began and at the times of day indicated, revealing no effects of prior CORT exposure, though these levels were significantly lower than nighttime control levels (in a), consistent with diurnal variation in endogenous CORT. During both the daytime and nighttime, control levels fell within the expected range for this strain of mice. Bars represent means (+SEM) per treatment group (* $p < 0.05$ compared to control samples), $n = 6-27$ /group.