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Identification of a novel, fast acting GABAergic anti-depressant

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Abstract

Current pharmacotherapies for depression exhibit slow onset, side effects and limited efficacy. Therefore, identification of novel fast-onset antidepressants is desirable. GLO1 is a ubiquitous cellular enzyme responsible for the detoxification of the glycolytic byproduct methylglyoxal (**MG**). We have previously shown that MG is a competitive partial agonist at GABA-A receptors. We examined the effects of genetic and pharmacological inhibition of GLO1 in two antidepressant assay models: the tail suspension test (**TST**) and the forced swim test (**FST**). We also examined the effects of GLO1 inhibition in three models of antidepressant onset: the chronic FST (**cFST**), chronic mild stress (**CMS**) paradigm, and olfactory bulbectomy (**OBX**). Genetic knockdown of *Glo1* or pharmacological inhibition using two structurally distinct GLO1 inhibitors (S-bromobenzylglutathione cyclopentyl diester (**pBBG**) or methyl gerfelin (**MeGFN**)) reduced immobility in the TST and acute FST. Both GLO1 inhibitors also reduced immobility in the cFST after 5 days of treatment. In contrast, the serotonin reuptake inhibitor fluoxetine (**FLX**) reduced immobility after 14, but not 5 days of treatment. Furthermore, 5 days of treatment with either GLO1 inhibitor blocked the depression-like effects induced by CMS on the FST and coat state,

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Drs. Palmer and McMurray have applied for a patent related the manipulation of GLO1 to treat various neurological and psychiatric disorders; beyond this, the authors have no conflicts of interest.

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and attenuated OBX-induced locomotor hyperactivity. Finally, 5 days of treatment with a GLO1 inhibitor (**pBBG**), but not FLX, induced molecular markers of the antidepressant response including brain-derived neurotrophic factor (**BDNF**) induction and increased phosphorylated cyclic-AMP response binding protein (**pCREB**) to CREB ratio in the hippocampus and medial prefrontal cortex (**mPFC**). Our findings indicate that GLO1 inhibitors may provide a novel and fast-acting pharmacotherapy for depression.

Introduction

Depression affects at least one in six adults at some point in their lifetime[1,2]. Current pharmaceutical treatments for depression are limited by slow onset of therapeutic effects (2–4 weeks), side effects and limited efficacy[3,4]. Thus, identification of novel targets for antidepressant drug development is urgently needed.

GLO1 is a ubiquitous cytosolic enzyme that catalyzes the reduction of methylglyoxal (**MG**), which is a non-enzymatic side product of glycolysis[5]. Therefore, MG concentrations are inversely proportional to GLO1 enzymatic activity. Electrophysiological recordings from primary neuronal cultures demonstrated that MG is a competitive partial agonist at GABA-A receptors[6], suggesting that GLO1 inhibitors and direct administration of MG could act to increase GABA-A receptor activity.

A previous study reported increased depression-like behavior in mice overexpressing *Glo1* in the tail suspension test (**TST**)[7], a highly reliable screen for antidepressant drug activity[8]. Previous studies have also shown that increased expression of *Glo1* also increases anxiety-like behavior in mice[6,9,10]. Additionally, administration of MG or a GLO1 inhibitor, S-bromobenzylglutathione cyclopentyl diester (**pBBG**), decreased anxiety-like behavior in mice[6]. Anxiety and depression are highly comorbid, show shared genetic liability, and can both be treated with antidepressants[11–13]. However, no studies have examined the potential antidepressant effects of GLO1 inhibition.

Therefore, we investigated the effect of genetic and pharmacological GLO1 inhibition in acute preclinical screens for antidepressant efficacy using *Glo1* knockdown mice and two structurally distinct GLO1 inhibitors. We then assessed the time-course of antidepressant action of the two GLO1 inhibitors using the chronic forced swim test (**cFST**), chromic mild stress (**CMS**), and olfactory bulbectomy (**OBX**) models of antidepressant onset. Finally, we assessed whether 5 days of treatment with GLO1 inhibitors induced molecular markers of the antidepressant response, including Brain-Derived Neurotrophic Factor (**BDNF**) induction and cyclic-AMP response binding protein (**CREB**) phosphorylation in hippocampus and medial prefrontal cortex (**mPFC**).

Materials and Methods

Mice

Glo1 knock-down (**KD**) mice on a C57BL/6J (**B6**) background (Dr. Michael Brownlee, Albert Einstein College of Medicine, Bronx, NY) have a 45–65% reduction in GLO1 enzymatic activity[14]. Hemizygous male knockdown mice were bred to WT females all on a

B6 background. Resulting offspring (KDs and WT littermates) were tested at ages 8–14 weeks.

For studies using the GLO1 inhibitors pBBG or methyl-gerfelin (**MeGFN**), male and female B6, BALB/cJ (**BALB**) or FVB/NJ (**FVB**) mice were purchased from The Jackson Laboratory (**JAX**) and tested at ages 8–15 weeks of age. Multiple strains were tested to rule out strain-specific effects. All mice were group housed on a standard 12/12 hour light/dark cycle unless otherwise noted (e.g. during CMS) and underwent behavioral testing in the second half of their light cycle (12–5pm). Separate cohorts were used in each behavioral study unless otherwise noted. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Chicago or at the University of California and performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Drugs

We synthesized pBBG (see McMurray et al. 2015)[15] and MeGFN (see supplemental materials) based on previously described methods (see Thornalley et al. 1996; Kawatani et al. 2008; Kanoh et al. 2013)[5,16,17]. For the TST and acute FST mice received pBBG (50 mg/kg in 8% DMSO/18% Tween80 in H_2O), MeGFN (12.5, 25 or 50 mg/kg in 4%DMSO/ 9%Tween80 in H_2O) or their corresponding vehicle by I.P. injection 2 hours before testing. For the cFST, CMS and OBX, minipumps were filled with pBBG, MeGFN, or vehicle (50% DMSO, 50% PEG400) and inserted into a small subcutaneous incision made on the back[18]. Fluoxetine hydrochloride (FLX; Sigma-Aldrich, St. Louis, MO) was delivered via the drinking water in opaque water bottles at a concentration of 160mg/L to achieve a dose of 18 mg/kg/day[19].

Behavioral Studies

TST—Male and female B6, FVB, Glo1KD and their WT littermates were assessed in the TST as described previously[8]. B6 and Glo1KD mice were scored using an observer blind to treatment/genotype. FVB mice were scored using EthoVision (Noldus Information Technology, Leesburg, VA); scoring from this system was strongly correlated with scores from human observers (data not shown).

Acute FST—FST procedures were performed as previously described[20]. Briefly, male and female B6, FVB, BALB, Glo1KD and their WT littermates were placed into round buckets 22 cm across and 20 cm deep that were filled with water (23–25°C; 16 cm deep) for 10 min. On day 2, mice were placed in the same buckets for 6 min. The final 4 min on the second day were scored for immobility by a trained observer.

Chronic Forced Swim Test (cFST)—When treated chronically rather than by acute bolus injection, BALB mice show reduced immobility in the FST. In particular, this response is observed in response to chronic (14 days), but not subchronic (5 days) treatment with SSRIs[19]; therefore the cFST can be used to determine the timing of antidepressant onset. BALB mice were implanted with minipumps delivering pBBG (5, 10 or 15mg/kg/day), MeGFN (5, 10, 15 mg/kg/day) or vehicle for either 5 days (male and female) or 14 days

(males) and then underwent the FST as described above (the design was between subjects; mice tested at 5 days were never retested at 14 days).

Chronic Mild Stress (CMS)—Female BALB mice were exposed to a series of stressors that varied daily and repeated weekly, as described previously (Opal et al. 2013). Following 6 weeks of stress, mice were surgically implanted with minipumps delivering vehicle, 10mg/kg/day pBBG, or 10mg/kg/day MeGFN. Mice continued to receive stressors following surgery. After 5 days of drug treatment, coat state (see supplemental methods) was evaluated, followed by the sucrose preference test and finally the splash test (see supplemental methods). The next day, mice underwent the FST. Non-stressed control animals were housed in a separate room under standard housing conditions and received only vehicle treatment. Thus, there were 4 groups: unstressed + VEH, stressed + VEH, stressed + pBBG, and stressed + MeGFN.

In a follow up control experiment, mice were subjected to the conditions of the unstressed VEH treated mice (described above), but then received minipumps delivering VEH, pBBG (10mg/kg/day) or MeGFN (10mg/kg/day) for 5 days. After 5 days of treatment, coat state was evaluated, followed by the sucrose preference test, splash test and FST using identical procedures to the previous groups. (above) To determine whether chronic inhibitor treatment produces any possibly confounding effects on motor behaviors, these mice were also evaluated with the OFT, balance beam, and grip strength tests on the day following FST (see supplemental methods).

Olfactory Bulbectomy (OBX)—Male B6 or female BALB mice underwent OBX or sham surgery as described previously[18]. Following surgery, mice were allowed to recover for 14 days after which minipumps containing 0 or 10 mg/kg/day pBBG or 0 or 10 mg/kg/day MeGFN were implanted. Five days after minipump implantation, mice were placed into OFT for 30 minutes to assess locomotor hyperactivity.

Western Blots

Westerns were performed as previously described[6,18]. Briefly, 1.5mm tissue punches were taken from mPFC or hippocampus. Membranes were probed with primary antibodies against pCREB, CREB, BDNF, and α-tubulin, and then labeled with peroxidase-conjugated secondary antibodies (Cell Signaling Technology). Additional details can be found in supplemental methods.

Brain concentration of MeGFN

We have shown previously that i.p. injection with pBBG increases MG levels in the brain (Distler et al., 2012). To confirm that MeGFN also acts centrally, we assayed MeGFN concentration in brain tissue at 5, 10, 30, 60, 120, and 240 minutes after i.p. injection (see supplemental methods).

Statistical Analysis

Data were analyzed using ANOVA or Student's *t*-test. Holm-Sidak post hoc tests (Glantz 2005) were used to determine which doses yielded significantly different responses. All data appeared to be normally distributed and variance across groups appeared to be similar.

Methodological details

Outliers were removed if they were greater than two standard deviations from the mean, this standard was applied to all studies and was predetermined (see Supplemental Methods for an exhaustive list of outliers that were removed). *p*-values <0.05 were considered significant and are for two-sided tests unless specifically indicated. With the exception of the Holm-Sidak post hoc tests, we did not correct for multiple testing. For figures that use histograms, data represent the mean ± standard error. Sample sizes were determined based on the availability of mice of the appropriate strain, genotype, sex and age. Beyond this we used our experience with similar studies to determine the sample sizes. In some cases, data from multiple iterations of the same study were combined to obtain the final sample size, in those instances additional animals were sometimes added only if analyses of preliminary data were not significant. No specific procedure was used to assign mice to different pharmacological treatments, however mice were inbred and should have been equivalent in every way, we did counterbalance known differences such as age, sex and genotype (KD or TG); for studies comparing mice of different genotypes randomization of genotype was inherent to the design. Some behavioral studies were scored by a computer, for studies scored by human observers, the observer was blind to the genotype and/or treatment of the mice. Neither the genetic manipulations (KD, TG) not the doses of pBBG and MeGFN used in this study altered behavior to an extent that they would have unblended the observer.

Results

TST

In the TST, *Glo1*KD mice showed significantly less immobility than their WT littermates ($F(1,44)=7.447$, $p<0.01$; Fig. 1A). There was no significant effect of sex on immobility nor was there a significant interaction between sex and genotype. IP injection of pBBG 50 mg/kg significantly reduced immobility in B6 ($F(1,20)=12.022$, $p<0.01$; Fig. 1B) and FVB mice (FVB $F(1,39)=4.642$, $p<0.05$; Fig. 1C). There was no effect of sex on immobility nor was there an interaction between treatment and sex in either B6 or FVB mice. In contrast, the same dose of pBBG was not sufficient to reduce immobility in the TST in transgenic *Glo1* overexpressing mice on a B6 background, presumably because of their increased enzymatic capacity (Supplemental Fig. 7). A second GLO1 inhibitor, MeGFN also reduced immobility in the TST in male B6 mice ($F(3, 51)=3.186$, $p<0.05$; Fig. 1D and Supplemental Fig. 1). Posthoc tests revealed that MeGFN significantly reduced immobility at 12.5 and 25 mg/kg ($p<0.05$), while there was a non-significant trend at 50 mg/kg ($p=0.067$).

Acute FST

GLO1KD mice also showed significantly less immobility than littermate WT mice in the FST ($F(1,48)=4.15$, $p<0.05$; Fig. 1E). There was no significant effect of sex on immobility

nor was there a significant interaction between treatment and sex ($p>0.05$). pBBG significantly reduced immobility in all 3 inbred mouse strains (B6: $F(1,29)=3.681$, $p<0.05$, Fig. 1F; FVB: $F(1, 43)=10.105$, $p<0.01$, Fig. 1G; BALB: $F(1,58)=10.989$, $p<0.01$, Fig. 1H). There was no significant effect of sex on immobility nor was there a significant interaction between treatment and sex in B6 or BALB mice ($p>0.05$); although there was a significant effect of sex in FVB mice ($F(1,43)=7.895$, $p<0.01$), but the interaction between sex and treatment was not significant. MeGFN also reduced immobility in FST ($F(1,36)=7.803$, $p<0.01$; Fig. 1I) in male B6 mice.

cFST

After 14 days of treatment with pBBG (0, 5, 10 or 15mg/kg/day), there was a significant effect of treatment on the cFST in male BALB mice ($F(3,93)=3.926$, $p<0.05$, Fig. 2A). Post hoc testing indicated that the 5 and 10 mg/kg/day doses significantly reduced immobility (p's<0.05), while 15 mg/kg/day showed a nearly significant trend towards a reduction in immobility compared to vehicle ($p=0.056$). We did not observe any effect on body weight (data not shown; $F(3, 93)=1.095$, $p>0.05$) of mice treated for 14 days with pBBG. In a separate cohort of male BALB mice treated with FLX in the drinking water we confirmed that 14 days of treatment with FLX reduced immobility ($t=1.996$, $p<0.05$ by one-tailed t-test, Fig. 2B).

These same animals were also tested in the OFT prior to cFST to determine whether they showed anxiolytic or general locomotor effects after 12 days of treatment. There was a significant effect of treatment with pBBG on center duration, reflecting the expected anxiolytic effect ($F(3, 90)=4.267$ $p<0.01$, Supplemental Fig. 2A). Post hoc tests revealed that 10 mg/kg/day significantly increased center duration compared to VEH treatment ($p<0.01$); no other doses showed significant effects. None of the doses of pBBG altered general locomotor activity in the OFT ($F(3, 94)=0.334$ $p>0.05$, Supplemental Fig. 2B). Based on the significant reductions in depression-like and anxiety-like behaviors in the cFST and OFT studies, and the lack of any confounding differences in general locomotor behavior, we chose to treat mice with 10 mg/kg/day pBBG in subsequent studies.

Next, we investigated subchronic (5 day) treatment with pBBG in cFST to determine whether GLO1 inhibition might have a faster onset of antidepressant effects versus FLX. Because male and female BALB mice were tested separately, we performed separate analyses. There was a significant effect of treatment in both male ($F(2,38)=4.526$, $p<0.05$, Fig. 2C) and female ($F(2,41)=4.775$, $p<0.05$, Fig. 2D) mice. Post hoc tests confirmed that 5 days of treatment with pBBG (p's<0.05) but not 5 days of treatment with FLX (p's>0.05) reduced immobility compared to the vehicle treatment. There was also a trend towards an increase in center duration in the OFT following subchronic treatment with pBBG and FLX ($F(2,42)=3.107$, $p=0.056$, Supplemental Fig. 3A & 3C). In the OFT, FLX but not pBBG, significantly increased locomotor activity compared to VEH ($p<0.05$).

In a separate cohort of mice, we examined the effect of 5 days of MeGFN treatment (5, 10 and 15 mg/kg/day) on immobility in the cFST used female BALB mice. There was significant effect of treatment on immobility ($F(3,58) =2.794$, $p<0.05$; Fig. 2E). Post hoc tests revealed 10 and 15 mg/kg/day MeGFN reduced immobility in the cFST ($p<0.05$); there

was a non-significant trend of reduced immobility following 5 mg/kg/day (p=0.061). There were no significant effects of 4 days of MeGFN treatment on center duration or locomotor activity in the OFT (F(3,56)=1.568, p>0.05; F(3,57)=0.24, p>0.05, Supplemental Fig. 3B & 3D). We chose the 10 mg/kg/day dose of MeGFN for subsequent studies because it was effective in the cFST and had no confounding effects on general locomotor activity.

CMS

CMS is a commonly used model of depression-like behavior that responds to chronic but not subchronic treatment with classical antidepressants[21]. Following CMS and treatment with VEH, pBBG (10 mg/kg/day) or MeGFN (10 mg/kg/day) for 5 days, we performed the sucrose preference test, which is intended to model anhedonia, which is a common symptom of depression in humans. There was no significant effect of group on sucrose preference (F(3,47)=0.546 p>0.05, Fig. 3A) or total consumption (F(3,59)=1.858 p>0.05, not shown). Because stress did not have the expected effect on sucrose preference, the failure of pBBG or MeGFN to ameliorate the effects of stress on sucrose preference are difficult to interpret.

Following CMS and 5 days of treatment with pBBG (10 mg/kg/day) or MeGFN (10 mg/kg/day), there was a significant effect of group in the FST (F(3,52)=2.94 p<0.05, Fig. 3B). Post hoc tests revealed that stress increased immobility relative to unstressed mice (Stressed VEH vs unstressed VEH; p<0.05); no other between group comparisons were significant, which we interpreted as evidence that both GLO1 inhibitors blocked the effects of stress on the FST. There was also a significant effect of group on coat state (F(3, 59)=5.713 p<0.01, Fig. 3C). Stress led to a significantly deteriorated coat state (indicated by an increased score) compared to unstressed mice (p<0.001), which was improved by treatment with either pBBG (p<0.05 vs stressed VEH) or MeGFN (p<0.05 vs stressed VEH). We also performed the splash test and found a significant effect of group on the number of grooming bouts (F(3,59)=3.194 p<0.05, Supplemental Fig. 4A). Post hoc tests revealed that stressed mice had fewer bouts relative to unstressed mice (p<0.05), but there were no differences between stressed mice treated with vehicle and stressed mice treated with either pBBG or MeGFN (p>0.05). Finally, there were no group effects on the total duration of grooming (F(3,56)=0.396 p>0.05, Supplemental Fig. 4B) or the latency to begin grooming (F(3,55)=1.914 p=0.139, Supplemental Fig. 4C).

In a follow up study, mice were subjected to the conditions the unstressed VEH treated mice experienced in the CMS study and then treated with VEH, pBBG (10 mg/kg/day) or MeGFN (10 mg/kg/day) for 5 days. There was no effect of treatment on sucrose preference (F(2,36)=2.765; p>0.05; Supplemental Fig. 5a) or coat state (F(2, 38)=0.0627; p>0.05; Supplemental Fig. 5b). In the FST, there were no main effects of treatment, but there was a significant interaction between treatment and time (F(6,155)=2.402; p=0.032; Supplemental Fig. 5c). Post hoc tests revealed a significant effect of MeGFN and pBBG on immobility in the last minute of the FST (p<0.05, one-tailed). There were no effects of treatment on the splash test (bouts: F(2,38)=0.0246; p>0.05; latency: F(2,38)=0.0886; p>0.05; duration: F(2, 38)=0.0592; p>0.05; Supplemental Fig. 5d–f). We also tested the mice for ataxia and locomotor depression. There was no effect of treatment on the balance beam (Footslips: F(2,38)=0.027; p>0.05; Supplemental Fig. 6a) or general locomotor behavior in the OFT

(F(2,38)=0.834; p>0.05 Supplemental Fig. 6b), and no gross deficits in grip strength as measured by the vertical pole test (data not shown). Taken together, these data show that in the absence of stress, both GLO1 inhibitors had the expted effect on the FST, but did not affect sucrose preference, coat state or the splash test; furthermore, these 5 days of treatment with these GLO1 inhibitors had no more general effects on ataxia, general locomotor activity or grip strength.

OBX

We found that pBBG reversed OBX-induced hyperactivity in both male B6 (Fig. 4A) and female BALB mice (Fig. 4B). There was a significant interaction between OBX and treatment in male B6 mice ($F(1,47)=4.927$; $p<0.05$). Post hoc tests revealed a trend towards pBBG reducing locomotor hyperactivity in the OBX group ($p=0.07$), while there was no effect of pBBG in SHAM operated animals ($p>0.05$). There was also a significant interaction between OBX and pBBG treatment in female BALB mice ($F(1,45)=4.506$ $p<0.05$). Post hoc tests revealed that pBBG reduced hyperactivity in the OBX group ($p<0.01$) but not in the SHAM group ($p>0.05$). We also found that MeGFN attenuated OBX-induced hyperactivity in male B6 mice (Fig. 4C). There were significant main effects of OBX ($F(1,44)=66.985$ $p<0.001$) and treatment ($F(1,44)=6.624$ $p<0.05$), but not the interaction between OBX and treatment. However, when we performed *a priori* post hoc testing we found that MeGFN significantly reduced locomotor activity within OBX ($p<0.05$), but not SHAM mice ($p>0.05$).

Western Blots

Finally, we examined whether pBBG treatment could upregulate BDNF and the ratio of pCREB to CREB (pCREB/CREB) in the hippocampus and mPFC, which are associated with antidepressant onset (Duman and Voleti 2012; Browne and Lucki 2013; Opal et al. 2013). There was a significant effect of treatment on BDNF expression in the hippocampus ($F(2,27)=3.87$, $p<0.05$, Fig. 5A) and mPFC ($F(2, 29)=7.577$, $p<0.01$, Fig. 5B). Post hoc tests revealed that 5-days of pBBG treatment (10 mg/kg/day) significantly upregulated BDNF in both mPFC and hippocampus compared to VEH (mPFC: $p<0.01$; hippocampus: $p<0.05$). In contrast, BDNF levels following 5-days of FLX treatment were not different from vehicle for either brain region ($p>0.05$). There was also a significant effect of treatment on the ratio of pCREB/CREB in the hippocampus ($F(2,29)=3.781$; $p<0.05$, Fig. 5C) and mPFC ($F(2,29)=5.576$; $p<0.01$, Fig. 5D). In the hippocampus, pCREB/CREB levels were upregulated following pBBG treatment compared to VEH ($p<0.05$), while there was a non-significant trend of pBBG treatment compared to VEH in mPFC ($p=0.071$). Subchronic FLX treatment did not alter pCREB/CREB levels in either of these brain regions ($p's>0.05$).

Brain concentration of MeGFN

We found that MeGFN was present in brain within minutes of i.p. injection and showed a halflife of 90.8 minutes. (Supplemental Fig. 8).

Discussion

The present results show that inhibition of GLO1 has antidepressant-like effects in multiple acute and chronic preclinical paradigms. The use of both genetic and pharmacological tools to inhibit GLO1, including two chemically distinct GLO1 inhibitors, suggests that the effects are not due to non-specific effects of these treatments. Additionally, there were no locomotor effects in mice treated with pBBG or MeGFN, suggesting that these effects cannot be explained by hyper- or hypo-activity. In addition to the significance of identifying a novel mechanism for achieving anti-depressant effects, our results also indicate that manipulations of this system have the potential to produce fast acting antidepressant effects. Specifically, five days of GLO1 inhibition produced antidepressant effects in three tests sensitive to the timing of antidepressant onset: the cFST, CMS and OBX models. In contrast, our lab[18–20,24] and others[21,25,26] have shown tricyclic and SSRI (e.g. FLX) antidepressants, have consistently been shown to require 14 days of treatment before they become effective in these tests. Finally, we showed that two molecular markers of the antidepressant response changed after chronic treatment with Glo1 inhibitors.

The most straightforward explanation for the observed antidepressant-like effects is that GLO1 inhibition increases MG concentrations; we have previously shown that MG is a competitive partial agonist at GABA-A receptors (Distler et al. 2012). GABA has been implicated in depression and the antidepressant response in a variety of ways. For example, depression is associated with reductions in cerebrospinal fluid GABA levels and the number of GABA-A receptors in cortical regions. Additionally, chronic antidepressant treatment correlates with an increase in GABA[28,29], supporting a potential role for GABAergic signaling in depression[30,31]. Though not typically used to treat depression, numerous reports indicate antidepressant effects in humans and/or animal models with the benzodiazepine, alprazolam[32,33], as well as both positive[34–36] and negative modulators of GABA-A receptors[37]. Additionally, co-administration of eszopiclone (a preferential GABA-A partial agonist at α1, α2 and α3 subtypes) with FLX produced a greater antidepressant efficacy and a faster antidepressant onset in depressed patients than FLX alone[38], suggesting that GABA-A receptor activation may contribute to the rapid antidepressant response.

The effects of GLO1 inhibitors on GABAergic signaling is qualitatively distinct from any other GABA-A acting compounds. Inhibition of GLO1 will increase MG concentrations in proportion to local glycolytic activity[39,40], thus tying GABAergic neuronal inhibition to local energy utilization. We have previously shown that MG easily crosses cell membranes[6], indicating that it acts like a paracrine factor rather than as a neurotransmitter. Additionally, because MG acts as a competitive partial agonists at GABA-A receptors, modulation of MG concentrations by GLO1 inhibitors may have qualitatively different effects as compared to other GABA-A acting compounds, including potentially inhibition of GABA-A signaling when local endogenous GABA concentrations are high[41,42]. Indeed, common side effects of GABA-A receptor agonists and modulators are sedation and locomotor ataxia, yet we previously showed that 50mg/kg pBBG does not alter footslips on the balance beam test. Here, we also show that subchronic (5 day) treatment with GLO1 inhibitors (pBBG and MeGFN) has no effect on footslips on the balance beam, nor did either inhibitor decrease locomotor behavior in the OFT or affect grip strength in the vertical pole test.

Increased BDNF levels in hippocampus and mPFC are associated with an antidepressant-like response in behavioral models of depression, providing a biomarker that corroborates the onset of the behavioral effects (Duman and Monteggia, 2006; Polyakova et al., 2015). With traditional antidepressants, upregulation of BDNF requires 14–21 days of treatment, and occurs concurrently with the onset of antidepressant-like effects (Nubuya et al., 1995; Duman and Voleti 2012; Browne and Lucki 2013; Opal et al. 2013). In the present study, BDNF and pCREB/CREB were upregulated in the hippocampus and mPFC after just 5 days of pBBG treatment. This rapid elevation of BDNF and pCREB/CREB levels are consistent with other putative fast-onset antidepressants, including short-term treatment with ketamine[43,44], serotonin$_{2C}$ receptor antagonists[18], and a serotonin$_4$ receptor agonist[45]. An increase in BDNF levels following GLO1 inhibition is also consistent with previous reports of upregulated BDNF expression in rat hippocampal cultures following incubation with MG[46]. However, this correlation does not prove causality and further studies are necessary to determine whether upregulation of BDNF and pCREB/CREB are necessary for the rapid onset of GLO1 inhibitor antidepressant-like activity.

We have previously shown that genetic and pharmacological GLO1 inhibition, as well as MG administration, are anxiolytic[6], have anti-seizure effects[47] and reduce ethanol consumption[15]. The current results show that GLO1 inhibition might provide a unique strategy for treating depression with comorbid anxiety, epilepsy or alcohol use disorders, which would constitute a unique class of therapeutic compounds and would address an urgent need given the high comorbidity of these disorders. Future mechanistic studied are needed to fully dissect the precise mechanism by which GLO1 inhibition alters depression-like behavior. Finally, our findings suggest that modulation of GABA-A signaling may be a promising approach for the development of fast-acting, antidepressants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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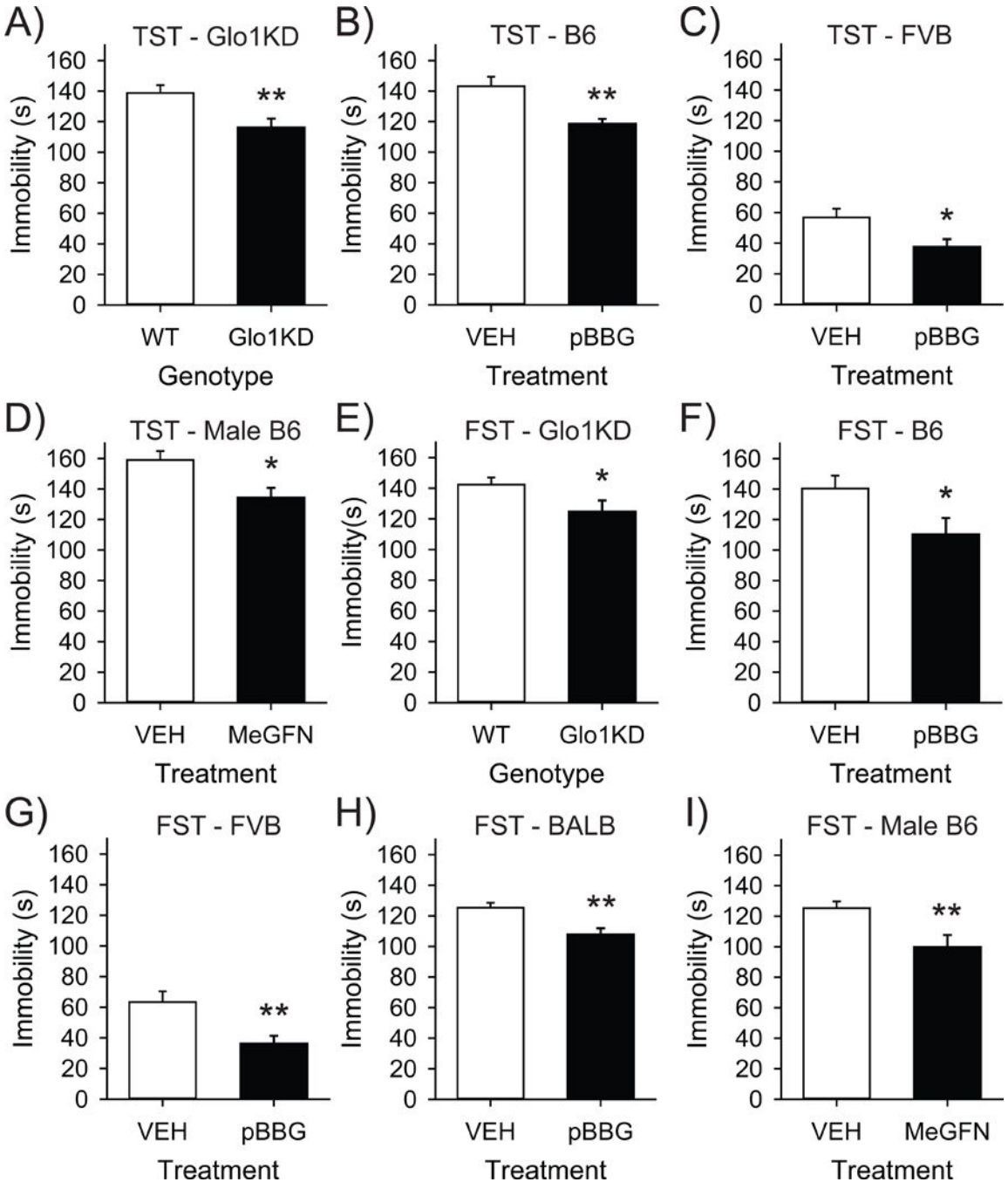

Figure 1. Reductions in GLO1 reduce depression-like behavior acutely in the TST and FST

In the TST, immobility is reduced in (A) GLO1 knockdown (KD; n=20) mice compared to their wild-type (WT; n=25) littermates or (B) after I.P. pBBG (50 mg/kg) in B6 (n=9 VEH, n=12 pBBG), (C) FVB (n=20 VEH, n=20 pBBG) mice. (D) A pharmacologically distinct GLO1 inhibitor, MeGFN, (12.5 mg/kg) was also able to reduce immobility in male B6 mice in the TST (n=14 VEH, n=12 MeGFN). (E) In the FST, immobility was reduced in GLO1KD (n=29 WT, n=20 KD) mice. (F) pBBG also reduced immobility in B6 (n=14 VEH, n=16 pBBG), (G) FVB (n=22 VEH, n=22 pBBG) and (H) BALB/cJ mice (n=30

VEH, n=29 pBBG). (I) MeGFN reduced immobility in male B6 mice in the FST (n=18 VEH, n=19 MeGFN). **p<0.05, **p<0.01.*

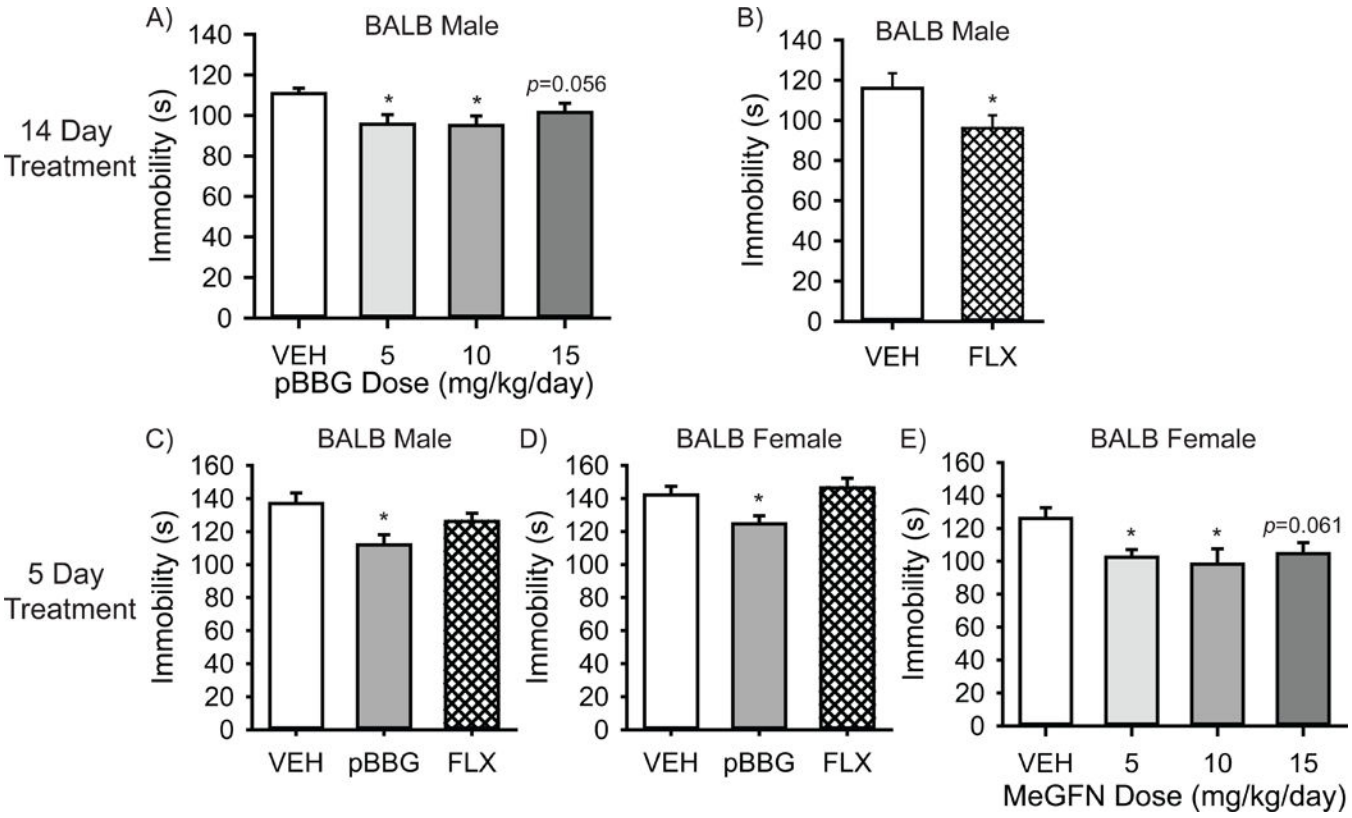

Figure 2. The GLO1 inhibitor pBBG reduces immobility at in the cFST

(A) Chronic (14 day) treatment with pBBG reduced immobility in the cFST in male BALB mice. (B) Chronic (14 day) FLX (18 mg/kg/day) treatment reduced immobility in the cFST in male BALB mice. Following subchronic (5 day) treatment in a separate cohort, pBBG (10 mg/kg/day), but not FLX (18 mg/kg/day), reduced immobility after 5 days in BALB (C) males and (D) females. (E) All three doses of MeGFN reduced immobility after 5 days in female BALB mice. *n=10–15 per group except in panel 'a' VEH (n=42) and 15 mg/kg/day (n=23); *p<0.05.*

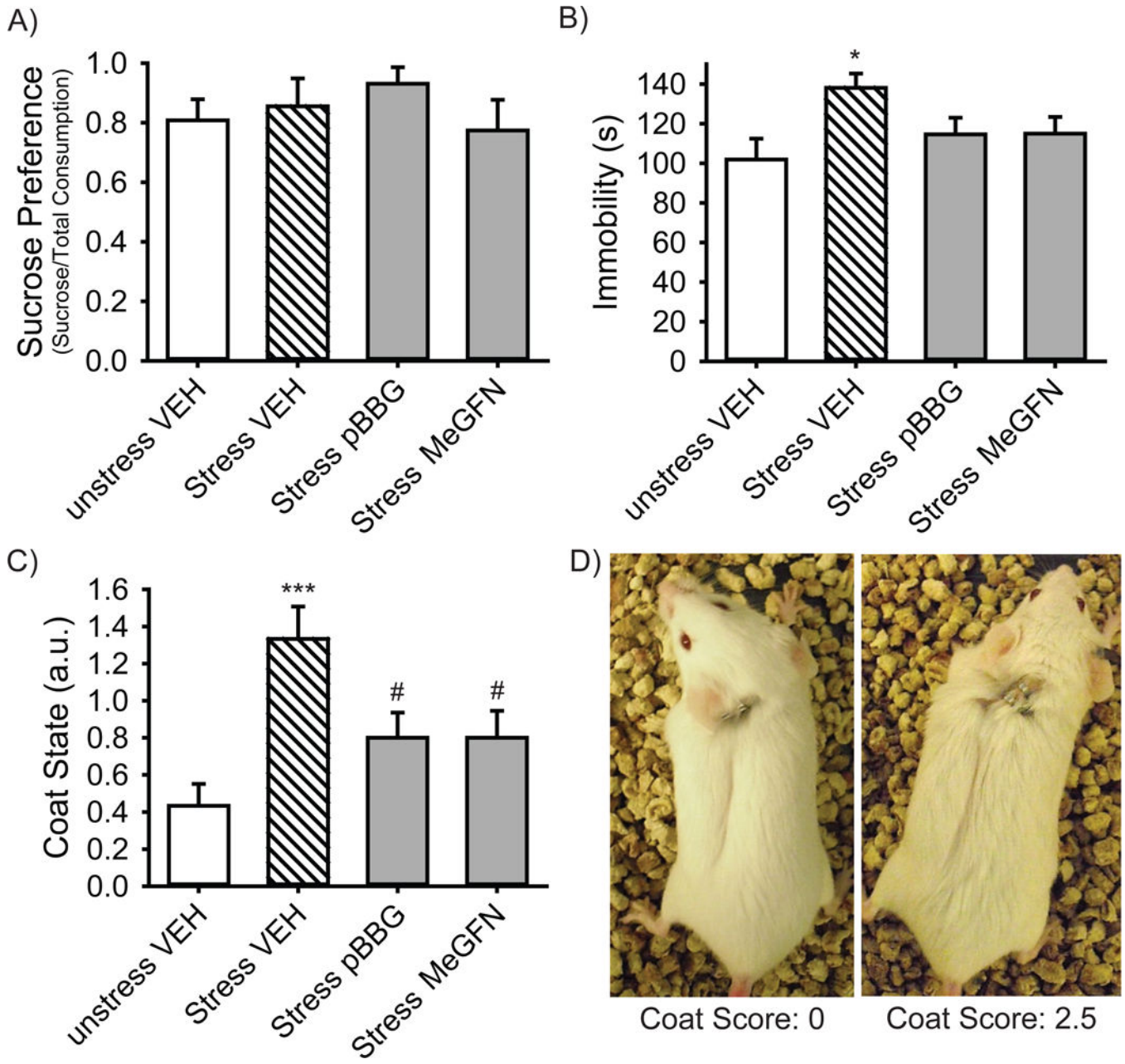

Figure 3. The effects of chronic mild stress and ameliorated by GLO1 inhibition

Following 6 weeks of CMS in female BALB mice, (A) there were no differences in sucrose preference between stressed and unstressed mice. However, stressed VEH mice did show (B) increased immobility in the FST relative to unstressed VEH. (C) CMS also led to a poor coat state in stressed VEH mice that was attenuated by 5 days of treatment with GLO1 inhibitors, pBBG and MeGFN; *n=13–15 per group.* (D) Representative unstressed and stressed vehicle-treated mice are shown. **p<0.05 vs unstressed VEH, ***p<0.001 vs unstressed VEH, # p<0.05 vs stress VEH.*

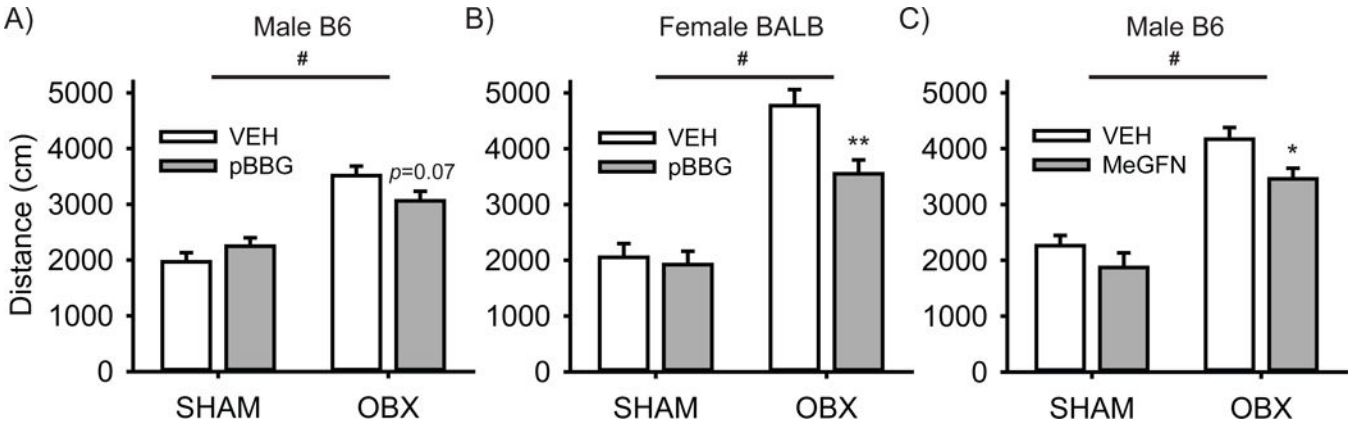

Figure 4. The effects of olfactory bulbectomy are ameliorated by GLO1 inhibition

OBX induces hyperactivity that is reduced by 5 days of pBBG treatment in (A) male B6 mice (p=0.07 OBX+ VEH vs OBX + pBBG) and (B) female BALB mice. (C) OBX-induced hyperactivity is reduced by MeGFN in male B6 mice. *n=11–14/group, **p<0.01 vs OBX + VEH; *p<0.05 vs OBX + VEH; # p<0.05 main effect of SHAM vs OBX.*

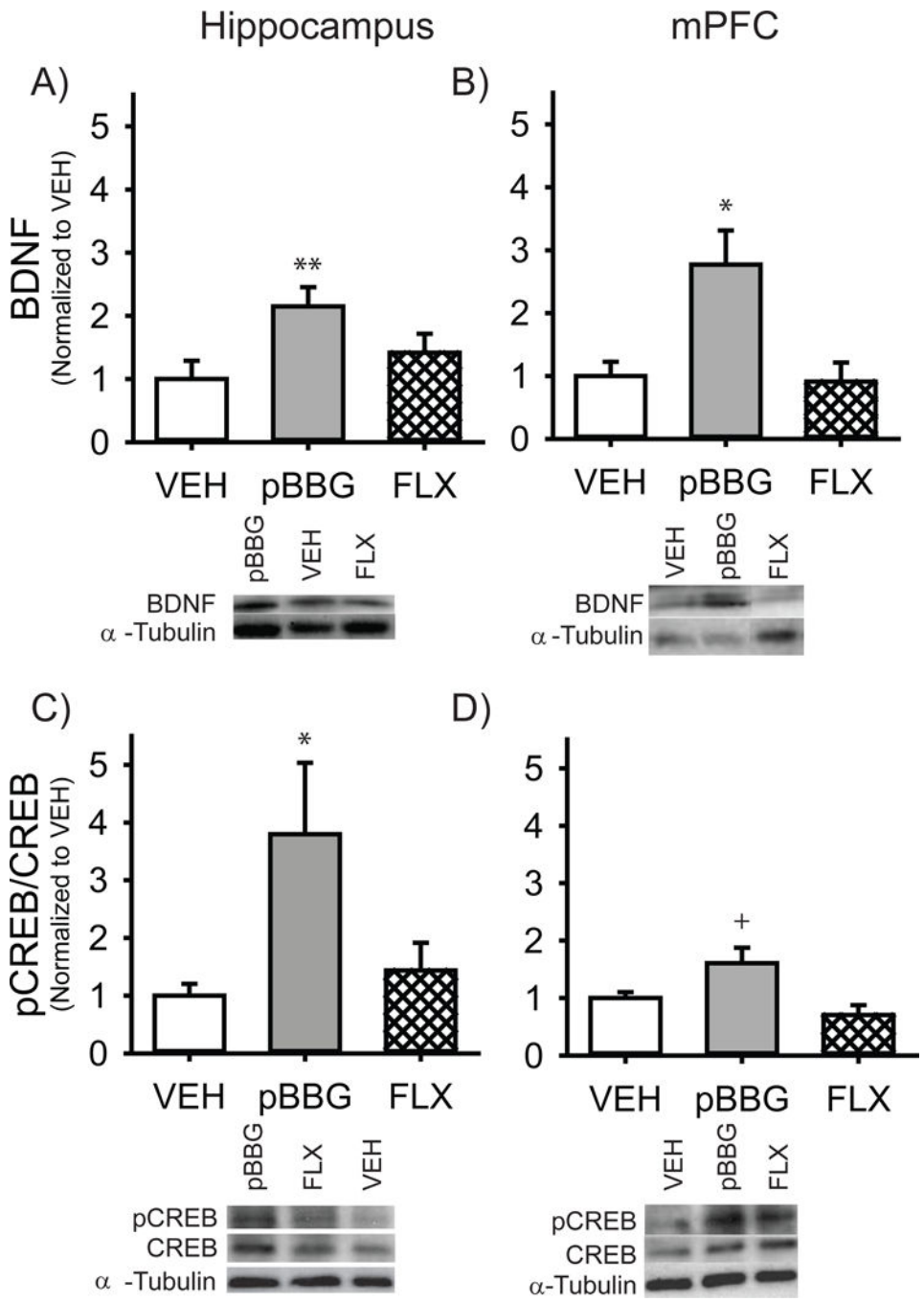

Figure 5. Five days of treatment with the GLO1 inhibitor, pBBG, but not FLX increased proteins associated with antidepressant onset

In male BALB mice, BDNF was upregulated in (A) hippocampus and (B) mPFC. pCREB/CREB was upregulated in (C) hippocampus, but not (D) mPFC. *n=9–10 per group; *p<0.05, **p<0.01, +p<0.10 compared to VEH.*