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ORIGINAL ARTICLE

Identification of a novel, fast-acting GABAergic antidepressant

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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, 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.

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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, 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.

Several previous studies have identified correlations between depression-like behavior and both *Glo1* expression and GLO1 protein levels.[7,8,9] Previous studies have also shown that increased expression of *Glo1* increases anxiety-like behavior in mice.[6,10,11] In addition, 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.[12–14] 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), chronic 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 knockdown (KD) mice on a C57BL/6J (B6) background (Dr Michael Brownlee, Albert Einstein College of Medicine, Bronx, NY, USA) have a 45–65% reduction in GLO1 enzymatic activity.[15] Hemizygous male KD mice were bred to wild-type (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 (Bar Harbor, ME, USA) and tested at ages 8–15 weeks of age. Multiple strains were tested to rule out strain-specific

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effects. All mice were group-housed on a standard 12/12 h light/dark cycle unless otherwise noted (for example, during CMS) and underwent behavioral testing in the second half of their light cycle (1200–1700 hour). 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[16] and MeGFN (see Supplementary Materials) based on previously described methods.[5,17,18] For the tail suspension test (TST) and acute FST mice received pBBG (50 mg kg^{-1} in 8% DMSO/18% Tween-80 in H_2O), MeGFN (12.5, 25 or 50 mg kg^{-1} in 4% DMSO/9% Tween-80 in H_2O) or their corresponding vehicle by intraperitoneal (i.p.) injection 2 h 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.[19] Fluoxetine hydrochloride (FLX; Sigma-Aldrich, St Louis, MO, USA) was delivered via the drinking water in opaque water bottles at a concentration of 160 mg l^{-1} to achieve a dose of 18 mg kg^{-1} per day.[20]

Behavioral studies

TST. Male and female B6, FVB, Glo1KD and their WT littermates were assessed in the TST as described previously.[21] B6 and Glo1KD mice were scored using an observer blind to treatment/genotype. FVB mice were scored using EthoVision (Noldus Information Technology, Leesburg, VA, USA); scoring from this system was strongly correlated with scores from human observers (data not shown).

Acute FST. FST procedures were performed as previously described.[22] 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. 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 selective serotonin reuptake inhibitors;[20] therefore, the cFST can be used to determine the timing of antidepressant onset. BALB mice were implanted with minipumps delivering pBBG (5, 10 or 15 mg kg^{-1} per day), MeGFN (5, 10, 15 mg kg^{-1} per 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. Female BALB mice were exposed to a series of stressors that varied daily and repeated weekly, as described previously.[19] Following 6 weeks of stress, mice were surgically implanted with minipumps delivering vehicle, 10 mg kg^{-1} per day pBBG or 10 mg kg^{-1} per day MeGFN. Mice continued to receive stressors following surgery. After 5 days of drug treatment, coat state (see Supplementary Methods) was evaluated, followed by the sucrose preference test and finally the splash test (see Supplementary 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 four 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 (10 mg kg^{-1} per day) or MeGFN (10 mg kg^{-1} per 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 confounding effects on motor behaviors, these mice were also evaluated with the open field test (OFT), balance beam and grip strength tests on the day following FST (see Supplementary Methods).

Olfactory bulbectomy. Male B6 or female BALB mice underwent OBX or sham surgery as described previously.[19] Following surgery, mice were allowed to recover for 14 days after which minipumps containing 0 or 10 mg kg^{-1} per day pBBG or 0 or 10 mg kg^{-1} per day MeGFN were implanted. Five days after minipump implantation, mice were placed into OFT for 30 min to assess locomotor hyperactivity.

Western blots

Western blots were performed as previously described.[6,19] Briefly, 1.5 mm tissue punches were taken from mPFC or hippocampus. Membranes were probed with primary antibodies against phosphorylated CREB (pCREB), CREB, BDNF and α-tubulin, and then labeled with peroxidase-conjugated secondary antibodies (Cell Signaling Technology, Danvers, MA, USA). Additional details can be found in Supplementary Methods.

Brain concentration of MeGFN

We have shown previously that i.p. injection with pBBG increases MG levels in the brain.[6] To confirm that MeGFN also acts centrally, we assayed MeGFN concentration in brain tissue at 5, 10, 30, 60, 120 and 240 min after i.p. injection (see Supplementary Methods).

Statistical analysis

Data were analyzed using analysis of variance 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 s.d.'s from the mean; this standard was applied to all studies and was predetermined (see Supplementary 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 ± s.e. 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) nor the doses of pBBG and MeGFN used in this study altered behavior to an extent that they would have unblinded the observer.

RESULTS

TST

In the TST, Glo1KD mice showed significantly less immobility than their WT littermates ($F(1,44) = 7.447$, $P < 0.01$; Figure 1a). There was no significant effect of sex on immobility nor was there a significant interaction between sex and genotype. I.p. injection of pBBG 50 mg kg^{-1} significantly reduced immobility in B6 ($F(1,20) = 12.022$, $P < 0.01$; Figure 1b) and FVB mice (FVB $F(1,39) = 4.642$, $P < 0.05$; Figure 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 (Supplementary Figure 7). A second GLO1 inhibitor, MeGFN, also reduced immobility in the TST in male B6 mice ($F(3, 51) = 3.186$, $P < 0.05$; Figure 1d and Supplementary Figure 1). *Post hoc* tests revealed that MeGFN significantly reduced immobility at 12.5 and

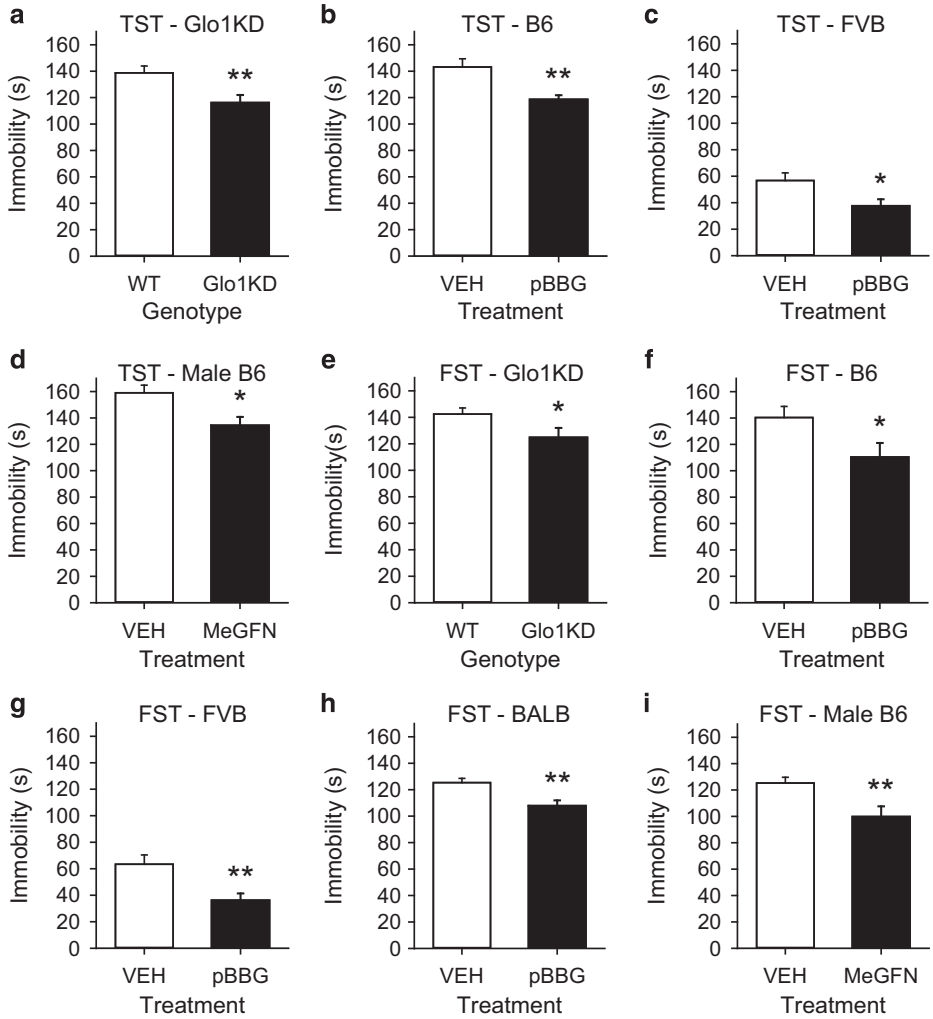

Figure 1. Reductions in GLO1 reduce depression-like behavior acutely in the TST and FST. In the TST, immobility is reduced in (**a**) Glo1KD ($n = 20$) mice compared to their WT ($n = 25$) littermates or (**b**) after i.p. pBBG (50 mg kg^{-1}) in B6 ($n = 9$ VEH, $n = 12$ pBBG), (**c**) FVB ($n = 20$ VEH, $n = 20$ pBBG) mice. (**d**) A pharmacologically distinct GLO1 inhibitor, MeGFN (12.5 mg kg^{-1}), 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). *$P < 0.05$, **$P < 0.01$. FST, forced swim test; i.p., intraperitoneal; KD, knockdown; MeGFN, methyl-gerfelin; pBBG, S-bromobenzylglutathione cyclopentyl diester; TST, tail suspension test; WT, wild type.

25 mg kg^{-1} ($P < 0.05$), while there was a nonsignificant trend at 50 mg kg^{-1} ($P = 0.067$).

Acute FST

Glo1KD mice also showed significantly less immobility than littermate WT mice in the FST ($F(1,48) = 4.15$, $P < 0.05$; Figure 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 three inbred mouse strains (B6: $F(1,29) = 3.681$, $P < 0.05$, Figure 1f; FVB: $F(1, 43) = 10.105$, $P < 0.01$, Figure 1g; BALB: $F(1,58) = 10.989$, $P < 0.01$, Figure 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 ($F(1,43) = 7.895$, $P < 0.01$), the interaction between sex and treatment was not significant. MeGFN also reduced immobility in FST ($F(1,36) = 7.803$, $P < 0.01$; Figure 1i) in male B6 mice.

cFST

After 14 days of treatment with pBBG (0, 5, 10 or 15 mg kg^{-1} per day), there was a significant effect of treatment on the cFST in male BALB mice ($F(3,93) = 3.926$, $P < 0.05$, Figure 2a). *Post hoc* testing indicated that the 5 and 10 mg kg^{-1} per day doses significantly reduced immobility ($P < 0.05$), while 15 mg kg^{-1} per day showed a nearly significant trend toward 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, Figure 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 ($F(3, 90) = 4.267$ $P < 0.01$, Supplementary Figure 2A). *Post hoc* tests revealed that 10 mg kg^{-1} per day significantly increased center duration compared to VEH

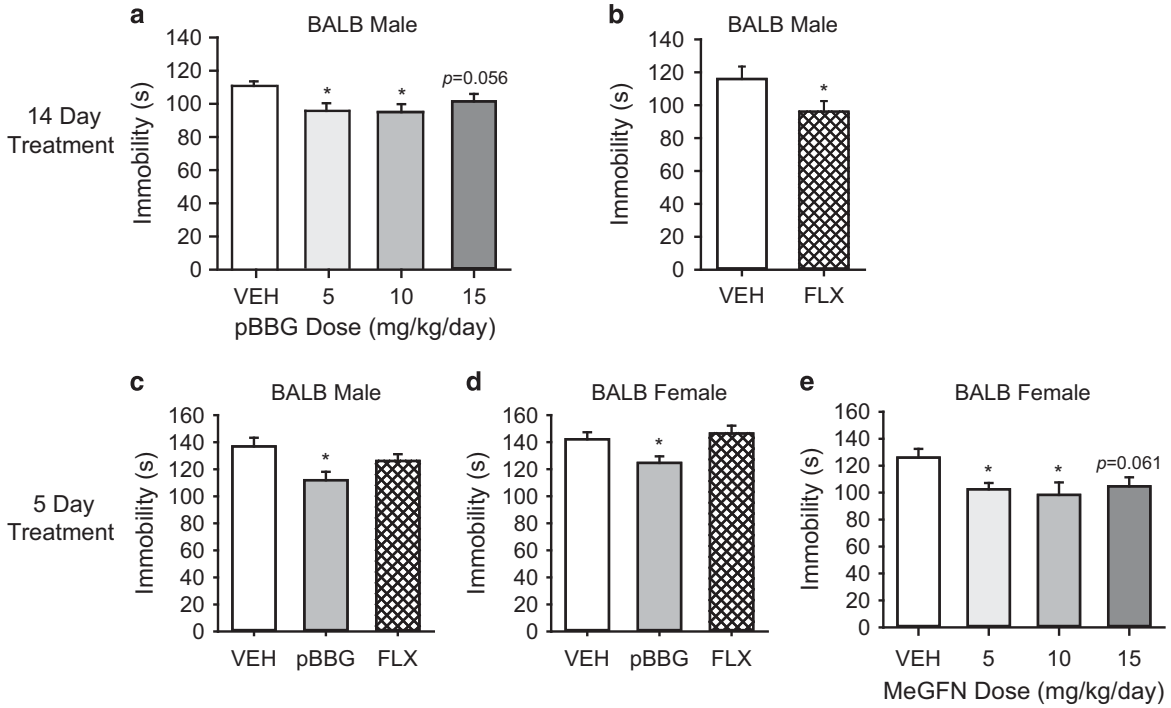

Figure 2. The GLO1 inhibitor pBBG reduces immobility in the cFST. (**a**) Chronic (14 days) treatment with pBBG reduced immobility in the cFST in male BALB mice. (**b**) Chronic (14 days) FLX (18 mg kg^{-1} per day) treatment reduced immobility in the cFST in male BALB mice. Following subchronic (5 days) treatment in a separate cohort, pBBG (10 mg kg^{-1} per day), but not FLX (18 mg kg^{-1} per 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 (**a**) VEH ($n = 42$) and 15 mg kg^{-1} per day ($n = 23$); *$P < 0.05$. cFST, chronic forced swim test; FLX, fluoxetine; MeGFN, methylgerfelin; pBBG, S-bromobenzylglutathione cyclopentyl diester.

treatment ($P < 0.01$); no other doses showed significant effects. None of the doses of pBBG altered general locomotor activity in the OFT ($F(3, 94) = 0.334$ $P > 0.05$, Supplementary Figure 2B). On the basis of 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^{-1} per 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 ($F(2,38) = 4.526$, $P < 0.05$, Figure 2c) and female ($F(2,41) = 4.775$, $P < 0.05$, Figure 2d) mice. *Post hoc* tests confirmed that 5 days of treatment with pBBG ($P < 0.05$) but not 5 days of treatment with FLX ($P > 0.05$) reduced immobility compared to the vehicle treatment. There was also a trend toward an increase in center duration in the OFT following subchronic treatment with pBBG and FLX ($F(2,42) = 3.107$, $P = 0.056$, Supplementary Figure 3A and C). 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^{-1} per day) on immobility in the cFST-used female BALB mice. There was significant effect of treatment on immobility ($F(3,58) = 2.794$, $P < 0.05$; Figure 2e). *Post hoc* tests revealed 10 and 15 mg kg^{-1} per day MeGFN-reduced immobility in the cFST ($P < 0.05$); there was a nonsignificant trend of reduced immobility following 5 mg kg^{-1} per day ($P = 0.061$). There were no significant effects of 4 days of MeGFN treatment on center duration or locomotor activity in the OFT ($F(3,56) = 1.568$, $P > 0.05$; $F(3,57) = 0.24$, $P > 0.05$, Supplementary Figures 3B & 3D). We chose the 10 mg kg^{-1} per 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.[23] Following CMS and treatment with VEH, pBBG (10 mg kg^{-1} per day) or MeGFN (10 mg kg^{-1} per 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 ($F(3,47) = 0.546$ $P > 0.05$, Figure 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 is difficult to interpret.

Following CMS and 5 days of treatment with pBBG (10 mg kg^{-1} per day) or MeGFN (10 mg kg^{-1} per day), there was a significant effect of group in the FST ($F(3,52) = 2.94$ $P < 0.05$, Figure 3b). *Post hoc* tests revealed that stress increased immobility relative to unstressed mice (Stressed VEH versus 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 ($F(3, 59) = 5.713$ $P < 0.01$, Figure 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 ($P < 0.05$ versus stressed VEH) or MeGFN ($P < 0.05$ versus stressed VEH). We also performed the splash test and found a significant effect of group on the

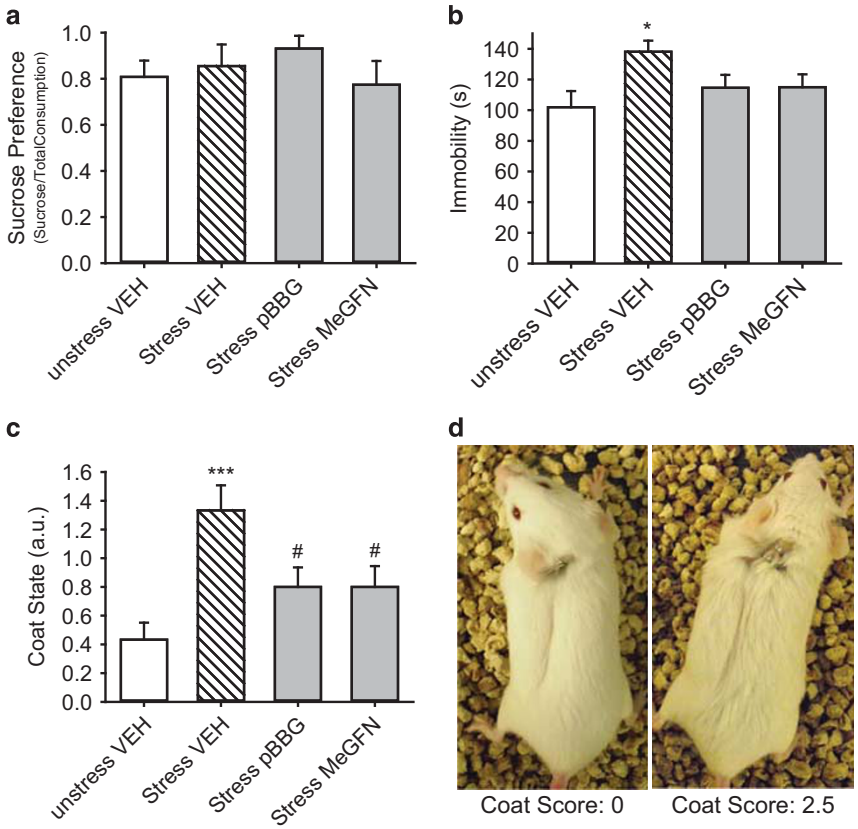

Figure 3. The effects of chronic mild stress are ameliorated by GLO1 inhibition. Following 6 weeks of CMS in female BALB mice, (**a**) there were no differences in sucrose preference between stressed and unstressed mice. However, stressed VEH mice did show (**b**) increased immobility in the FST relative 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. $^{*}P < 0.05$ versus unstressed VEH, $^{***}P < 0.001$ versus unstressed VEH, $^{\#}P < 0.05$ versus stress VEH. CMS, chronic mild stress; FST, forced swim test; MeGFN, methyl-gerfelin; pBBG, S-bromobenzylglutathione cyclopentyl diester.

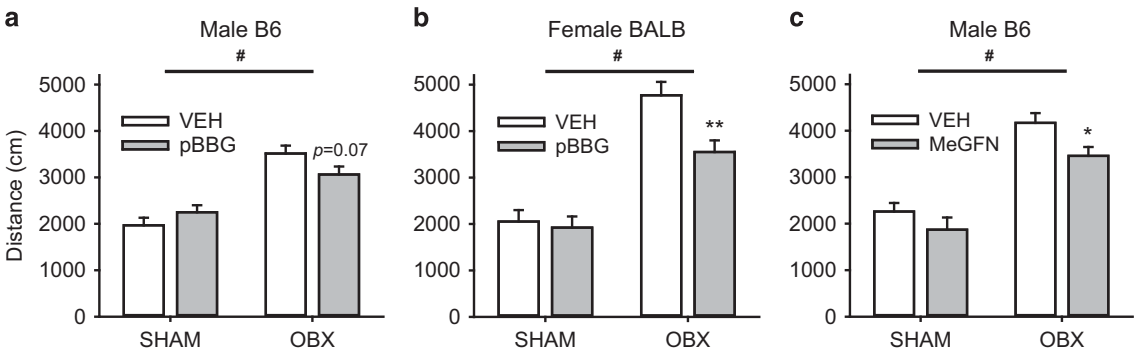

Figure 4. The effects of OBX are ameliorated by GLO1 inhibition. OBX induces hyperactivity that is reduced by 5 days of pBBG treatment in (**a**) male B6 mice ($P = 0.07$ OBX+VEH versus OBX+pBBG) and (**b**) female BALB mice. (**c**) OBX-induced hyperactivity is reduced by MeGFN in male B6 mice. $n = 11–14$ per group, $^{**}P < 0.01$ versus OBX+VEH; $^{*}P < 0.05$ versus OBX+VEH; $^{\#}P < 0.05$ main effect of SHAM versus OBX. MeGFN, methyl-gerfelin; OBX, olfactory bulbectomy; pBBG, S-bromobenzylglutathione cyclopentyl diester.

number of grooming bouts ($F(3,59) = 3.194$ $P < 0.05$, Supplementary Figure 4A). *Post hoc* tests revealed that stressed mice had fewer bouts relative to unstressed mice ($P < 0.05$); however, 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 ($F(3,56) = 0.396$ $P > 0.05$, Supplementary Figure 4B) or the latency to begin grooming ($F(3,55) = 1.914$; $P = 0.139$, Supplementary Figure 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^{-1} per day) or MeGFN (10 mg kg^{-1} per day) for 5 days. There was no effect of treatment on sucrose preference ($F(2,36) = 2.765$; $P > 0.05$; Supplementary Figure 5a) or coat state ($F(2, 38) = 0.0627$; $P > 0.05$; Supplementary Figure 5b). In the FST, there were no main effects of treatment, but there was a significant interaction between treatment and time ($F(6,155) = 2.402$; $P = 0.032$; Supplementary Figure 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: $F(2, 38) = 0.0592$; $P > 0.05$; Supplementary Figures 5d–f). We also tested the mice for ataxia and locomotor depression. There was no effect of treatment on the balance beam (Footslips: $F(2,38) = 0.027$; $P > 0.05$; Supplementary Figure 6a) or general locomotor behavior in the OFT ($F(2,38) = 0.834$; $P > 0.05$ Supplementary Figure 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 expected effect on the FST, but did not affect sucrose preference, coat state or the splash test. Furthermore, these 5 days of treatment with pBBG and MeGFN 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 (Figure 4a) and female BALB mice (Figure 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 toward 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 (Figure 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.[19,24,25] There was a significant effect of treatment on BDNF expression in the hippocampus ($F(2,27) = 3.87$, $P < 0.05$, Figure 5a) and mPFC ($F(2, 29) = 7.577$, $P < 0.01$, Figure 5b). *Post hoc* tests revealed that 5 days of pBBG treatment (10 mg kg^{-1} per 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 ($F(2,29) = 3.781$; $P < 0.05$, Figure 5c) and mPFC ($F(2,29) = 5.576$; $P < 0.01$, Figure 5d). In the hippocampus, pCREB/CREB levels were upregulated following pBBG treatment compared to VEH ($P < 0.05$), while there was a nonsignificant 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 ($P > 0.05$).

Brain concentration of MeGFN

We found that MeGFN was present in the brain within minutes of i.p. injection and showed a half-life of 90.8 min (Supplementary Figure 8).

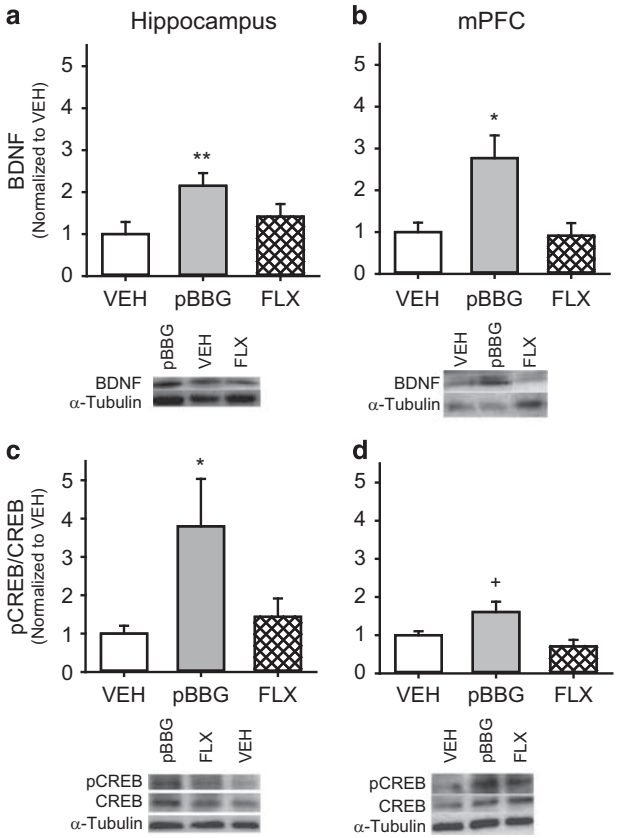

Figure 5. Five days of treatment with the GLO1 inhibitor, pBBG, but not FLX increased proteins associated with antidepressant onset. In male BALB mice, BDNF was upregulated in (**a**) hippocampus and (**b**) mPFC. pCREB/CREB was upregulated in (**c**) hippocampus, but not (**d**) in mPFC. $n = 9$–10 per group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{+}P < 0.10$ compared to VEH. BDNF, brain-derived neurotrophic factor; FLX, fluoxetine; mPFC, medial prefrontal cortex; pBBG, S-bromobenzyl-glutathione cyclopentyl diester; pCREB, phosphorylated cyclic-AMP response-binding protein.

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 nonspecific effects of these treatments. In addition, 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 antidepressant effects, our results also indicate that manipulations of this system have the potential to produce fast-acting antidepressant effects. Specifically, 5 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 laboratory[19,20,22,26] and others[23,27,28] have shown tricyclic and selective serotonin reuptake inhibitor (for example, FLX) antidepressants have consistently been shown to require 14 days of treatment before they become effective in these tests.[29,30] 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.[6] 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. In addition, chronic antidepressant treatment correlates with an increase in GABA,[31,32] supporting a potential role for GABAergic signaling in depression.[33,34] Although not typically used to treat depression, numerous reports indicate antidepressant effects in humans and/or animal models with the benzodiazepine, alprazolam[35,36] as well as both positive[37–39] and negative modulators of GABA-A receptors.[40] In addition, 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,[41] 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,[42,43] 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. In addition, because MG acts as a competitive partial agonist 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 potential inhibition of GABA-A signaling when local endogenous GABA concentrations are high.[44,45] Indeed, common side effects of GABA-A receptor agonists and modulators are sedation and locomotor ataxia; yet we previously showed that 50 mg kg^{-1} 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.[46,47] With traditional antidepressants, upregulation of BDNF requires 14–21 days of treatment, and occurs concurrently with the onset of antidepressant-like effects.[19,24,25,48] 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 is consistent with other putative fast-onset antidepressants, including short-term treatment with ketamine,[49,50] serotonin$_{2C}$ receptor antagonists[19] and a serotonin$_4$ receptor agonist.[51] 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.[52] 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 antiseizure effects[53] and reduce ethanol consumption.[16] 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.

CONFLICT OF INTEREST

Drs Palmer and McMurray have applied for a patent-related manipulation of GLO1 to treat various neurological and psychiatric disorders. The remaining authors declare no conflict of interest.

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