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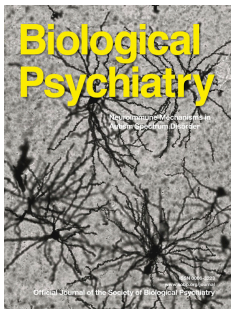

Glucocorticoid receptor-dependent astrocytes mediate stress vulnerability

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PII: S0006-3223(21)01833-3

DOI: https://doi.org/10.1016/j.biopsych.2021.11.022

Reference: BPS 14730

To appear in: *Biological Psychiatry*

Received Date: 9 July 2021

Revised Date: 4 November 2021

Accepted Date: 28 November 2021

Please cite this article as: Lu C.-L., Ren J., Mo J.-W., Fan J., Guo F., Chen L.-Y., Wen Y.-L., Li S.-J., Fang Y.-Y., Wu Z.-F., Li Y.-L., Gao T.-M. & Cao X., Glucocorticoid receptor-dependent astrocytes mediate stress vulnerability, *Biological Psychiatry* (2022), doi: https://doi.org/10.1016/j.biopsych.2021.11.022.

Glucocorticoid receptor-dependent astrocytes mediate stress vulnerability

Short Title: Astrocytes regulate depressive-like behaviors via GRs

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ABSTRACT

BACKGROUND: Major depressive disorder (MDD) is a devastating psychiatric illness that affects approximately 17% of the population worldwide. Astrocyte dysfunction has been implicated in the pathophysiology of MDD. Traumatic experiences and stress contribute to the onset of MDD, but how astrocytes respond to stress is poorly understood.

METHODS: Using western blot analysis, we identified stress vulnerability was associated with reduced astrocytic glucocorticoid receptor (GR) expression in mouse models of depression. We further investigated the functions of astrocytic GRs in regulating depression and the underlying mechanisms by using a combination of behavioral studies, fiber photometry, biochemical experiments, and RNA-sequencing methods.

RESULTS: GRs in astrocytes were more sensitive to stress than those in neurons. The GR absence in astrocytes induced depressive-like behaviors, whereas restoring astrocytic GR expression in the medial prefrontal cortex (mPFC) prevented the depressive-like phenotype. Furthermore, we found that GRs in the mPFC affected astrocytic Ca^{2+} activity and dynamic adenosine 5'-triphosphate (ATP) release in response to stress. RNA-sequencing of astrocytes isolated from GR deletion mice identified the PI3K-AKT signaling pathway, which was required for astrocytic GR-mediated ATP release.

CONCLUSIONS: These findings reveal that astrocytic GRs play an important role in the stress response and that the reduced astrocytic GR expression in the stressed subject decreases ATP release to mediate stress vulnerability.

Keywords: Depression; Astrocyte; GR; ATP; Stress vulnerability; PI3K

BACKGROUND

Major depressive disorder (MDD) is the most prevalent psychiatric disease, with approximately 17% of the world's population affected by MDD in their lifetime (1). The morbidity, mortality, and socioeconomic burden associated with MDD make it a devastating psychiatric illness. Astrocytes, the majority cell type among glial cells, execute a variety of essential functions, including their contribution to the blood-brain barrier, synaptogenesis, ion homeostasis, neurotransmitter buffering, and the secretion of neuroactive agents (2, 3). Accumulated evidence indicates that dysfunction of astrocytes is involved in the pathophysiology of MDD (4, 5). Evidence from human subjects and animal models of depression has shown that morphological and numerical changes in glia in the prefrontal area (6, 7) and pharmacologic glial ablation in the mPFC induce depressive-like behaviors (8). Astrocytes are known to release ATP in the mPFC, which are vital in the induction of depressive symptoms and antidepressant responses (9, 10). However, how astrocytes respond to stress and contribute to the pathogenesis of depression remains unclear.

Traumatic experiences and social stress, contributing to the onset of MDD, trigger stress responses including activation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of glucocorticoid hormones (11-13). Glucocorticoid hormones activate GRs, which are expressed ubiquitously in the brain, during the stress response (11, 12, 14). GRs in neurons have been shown to modulate depressive-like behaviors in animal studies (15-19). However, the GR expression is markedly higher in astrocytes than in neurons in the brain (20). Astrocytes are the key components of the brain-blood barrier (21), and

glucocorticoids already in the blood activate astrocytic GRs earlier than neuronal GRs in the brain. However, whether astrocytic GRs respond to stress and modulate depressive-like behaviors remains largely unexplored.

Here, we showed that stress vulnerability was associated with reduced astrocytic GR expression. Astrocyte-specific knockout of *Nr3c1* (encoding GRs) was sufficient to induce depressive-like behaviors, which was rescued by restoring GRs in astrocytes in the mPFC. We then showed that astrocytic GRs in the mPFC modulated depressive-like phenotypes. Moreover, the GR absence in astrocytes decreased the Ca^{2+} activity and ATP release in the mPFC. RNA sequencing indicated that the GR regulated ATP release from astrocytes via the PI3K-AKT signaling pathway. Our study suggests that astrocytic GRs buffer the consequences of stress and that the activation of astrocytic GRs in the stressed subject seems to release a putative messenger to alert the body to stress.

METHODS AND MATERIALS

Animals

The *Nr3c1*$^{loxP/loxP}$ mice were purchased from the Jackson Laboratory (cat# 021021). The *Fgfr3-iCreER*^{T2} mice (C57BL/6J background) were generously provided by William D. Richardson (University College London, London, UK). All animal experiments were approved by the Southern Medical University Animal Ethics Committee.

Detailed methods and materials regarding additional animals, reagents, virus injections, behavioral tests, and biochemical experiments are provided in the Supplement Information.

RESULTS

The GR in astrocytes is more sensitive to stress than that in neurons

To confirm that the aberrant expression of GRs is universal in depression (22, 23), we first used chronic social defeat stress (CSDS), a well-established model of depression that mimics several psychopathological dimensions of depression (24). In the 10-days CSDS protocol, eight-week-old male C57BL/6J mice were exposed to a physically aggressive CD-1 mouse (10 min d^{-1}) for social defeat (Figure 1A). CSDS induced a social avoidance behavior in a subset of mice as assessed with the social interaction (SI), termed stress-susceptible (Sus). Defeated mice that did not display social avoidance were considered resilient (Res; Figure 1B). Then, we detected the GR protein level in the mPFC, a candidate site for impaired function in depression (9, 10). Western blot analysis showed that the GR protein was significantly reduced in the mPFC of Sus mice compared to control mice (Figure 1C). Additionally, the GR protein level was positively correlated with the social interaction (SI) ratio in the social interaction test (Figure 1D). Meanwhile, the adult mice were subjected to 3-days subthreshold social defeat stress (SSDS), which did not induce social avoidance behaviors and a reduction of total GR expression in the mPFC (Figure S2A, B). We next evaluated whether significant changes in GR expression were present in which depression was induced by treatment with lipopolysaccharide (LPS) (25). Ten days of LPS injection (0.5 mg kg^{-1} per day, intraperitoneal (i.p.)) in eight-week-old C57BL/6J mice was sufficient to cause depressive-like behaviors in the forced swim test (FST; Figure 1 E, F) and sucrose preference test (25). Mice with LPS-induced depression also showed a significant decrease in the GR protein level in the

mPFC (Figure 1G), and the GR protein level was negatively correlated with immobility time in the FST (Figure 1H).

Neurons and astrocytes are two major cell types in the adult brain. To determine which subtype of cells is more sensitive to stress, we first detected GR expression in neurons and astrocytes. We found that GRs were present in most astrocytes and neurons (Figure S1A-E). Western blot analysis showed that the GR protein level was higher in cultured astrocytes than in neurons (Figure S1F), consistent with previous reports (20). We then detected the GR protein level in cultured astrocytes and neurons during the stress response. A reduction of GR protein level was observed in cultured astrocytes treated with LPS (1 μg ml^{-1}) at 8 d in vitro (DIV8), and neurons (DIV14) showed no difference in the GR protein level upon LPS (1 μg ml^{-1}) treatment (Figure 1I). Stress activates the HPA axis and enhances the release of glucocorticoid hormones, which activate GRs in the brain (13). Then, we directly treated cultured astrocytes (DIV8) and neurons (DIV14) with GR agonist dexamethasone (DXMS) to mimic the stress condition. The results showed that the GR protein level was downregulated in astrocytes exposed to 1 μM and 5 μM DXMS for 6 h, but no difference was observed in neurons (Figure 1J). We also found that the GR protein level was downregulated in astrocytes exposed to DXMS (1 μM) for 24 h and 48 h. Meanwhile, the treatment of neurons with DXMS (1 μM) for 48 h induced the downregulation of the GR protein (Figure 1K). To determine the levels of astrocytic GRs of mice under stress, adult mice were subjected to SSDS or CSDS. After SSDS or CSDS, the mPFC slices of mice were stained to visualize GRs with S100β (a marker of astrocytes) and NeuN (a marker of neurons). Immunostaining and quantification revealed

that the GR protein level was markedly decreased in astrocytes in the mPFC after SSDS (Figure 1L), but no difference was observed in neurons (Figure 1M). After CSDS, GRs were decreased in both astrocytes and neurons in the mPFC of Sus mice (Figure S2C, D). Then, we isolated astrocytes from the mPFC by fluorescence-activated cell sorting (FACS) after the CSDS paradigm. Simple western blot analysis also showed that astrocytic GRs were decreased in the mPFC of Sus mice compared with control mice (Figure S2E). Taken together, these results suggest that astrocytes contribute to the stress-induced reduction of GRs, and GRs in astrocytes are more sensitive to stress than those in neurons in the mPFC.

Astrocyte-specific knockout of *Nr3c1* induces depressive- and anxiety-like behaviors

To evaluate the role of astrocytic GRs in depression, we generated an astrocyte-specific GR deletion mouse line, *Fgfr3-iCreER^{T2}; Nr3c1$^{loxP/loxP}$*, for behavioral studies (Figure 2A). To obtain this model, we crossed mice with the floxed *Nr3c1* allele with the *Fgfr3-iCreER^{T2}* mouse line, allowing selective *Nr3c1* deletion in astrocytes via tamoxifen-inducible Cre recombination (Figure 2A and Figure S3A). To examine the efficiency and specificity of GR deletion in astrocytes, brain tissues were collected from adult mice and studied 28 days after the first tamoxifen injection. *Fgfr3-iCreER^{T2}; Nr3c1$^{loxP/loxP}$* (cKO) and littermate control (Ctrl) mice exhibited normal growth rates, body weights and brain sizes (Figure S3 B-D). No apparent differences in brain structure, astrocytes, and neurons density were observed between the cKO and Ctrl mice (Figure S3 E-G). Immunostaining revealed that GRs were attenuated in GFAP-positive astrocytes (Figure S3H) and not changed in

NeuN-positive neurons (Figure S3I) in the mPFC of cKO mice compared to Ctrl mice. In addition, flow cytometry analysis showed a more than 50% reduction of astrocytic GR expression in cKO mice compared to Ctrl mice (Figure 2 B, C, and Figure S4A).

For behavioral studies, all mice were treated with tamoxifen for 5 continuous days. Allowed 28 days recovery to induced downregulation of GRs, mice were then subjected to a battery of depression-related behavioral tests. In the FST, the duration of immobility was increased in cKO mice compared with Ctrl mice (Figure 2D). To assess whether a loss of GRs increases stress vulnerability, we then subjected mice to the SSDS experiment (26), in which no difference in interaction zone time when the social target was present was observed between defeated and undefeated Ctrl mice (Figure 2E). However, defeated cKO mice displayed greater social avoidance than undefeated cKO mice (Figure 2E). No difference was observed in sucrose preference (Figure S5A) and tail suspension test (TST; Figure S5B). Next, mice were subjected to anxiety-related behavioral tests. The results showed that cKO mice spent less time in the open arm in the elevated-plus maze (EPM; Figure 2F) and stayed longer in the dark box in the light-dark box (LD box; Figure 2G) compared to Ctrl mice. cKO mice also displayed reduced general locomotion in the open field test (OFT; Figure 2 H, I), but no difference in motor coordination was observed in the rotarod test (Figure 2J). These observations indicate that the GR absence in astrocytes induces depressive- and anxiety-like behaviors in adult mice.

Astrocytic GR dysfunction in the mPFC displays depressive-like phenotypes

To determine whether the selective knockout of astrocytic GRs in the mPFC would induce

depressive-like behaviors, we bilaterally injected AAV-GFAP-Cre or AAV-GFAP-EYFP viruses into the mPFC of *Nr3c1*$^{loxP/loxP}$ mice for behavioral studies (Figure 3A). Confocal imaging showed that the GFAP-Cre virus was specifically expressed on astrocytes in the mPFC (Figure 3B and Figure S6A), and western blot analysis showed that the GR protein level was decreased in the knockout mice (Figure 3C). We next assayed depressive-like phenotypes and found that mice infected with the AAV-GFAP-Cre virus displayed depressive-like behaviors, including increased immobility time in the FST (Figure 3D) and social avoidance after the SSDS (Figure 3E and Figure S6B). However, the AAV-GFAP-Cre virus had no effect on anxiety-related behaviors or general locomotion (Figure 3F-H).

To determine whether the restoration of astrocytic GRs in the mPFC would be sufficient to reverse the depressive-like behaviors caused by astrocytic GR knockout, we bilaterally injected AAV-DIO-Nr3c1 or control viruses into the mPFC of cKO and Ctrl mice. Confocal imaging showed that the virus was expressed on mPFC astrocytes (Figure 3I) and western blot analysis showed that the GR was overexpressed in the mPFC in the mice infected with the AAV-DIO-Nr3c1 virus (Figure S6C). Then, all mice were subjected to the SI test to assess the social avoidance behavior before and after the SSDS. After the SSDS paradigm, cKO mice infected with the control virus also displayed social avoidance behaviors in the SI test (Figure 3J and Figure S6D). Interestingly, the social avoidance behavior in cKO mice was reversed by the restoration of GRs in astrocytes in the mPFC (Figure 3J and Figure S6D). In parallel experiments, the GR overexpression had no detectable effect on the social avoidance behavior of control mice before and after the

197 SSDS (Figure 3J and Figure S6D). Additionally, no difference in locomotion activity in the
198 OFT was observed (Figure 3 K, L). Taken together, these results indicate that the
199 impairment of astrocytic GRs in the mPFC is sufficient to induce depressive-like
200 behaviors.
201

202 **The GR deletion causes dysfunction of astrocytic Ca^{2+} activity in the mPFC**
203 Astrocyte Ca^{2+} signaling is important for intercellular communication to regulate
204 physiological function (27-29). Therefore, we assessed astrocytic function in the mPFC of
205 cKO and Ctrl mice. We injected the AAV-GFAP-GCaMP6s virus, which preferentially
206 targets astrocytes due to the GFAP promoter, into the mPFC, and optical fibers were
207 implanted above the infected cells to carry out fiber photometry recordings (Figure 4A, B).
208 Confocal images and quantification showed that more than 90% of Gcamp6s were
209 expressed in GFAP-positive astrocytes (Figure S7A-C). Fluorescence signals from
210 astrocytes in the mPFC were measured two weeks after injection while mice in a
211 protective wire-mesh enclosure were attacked by an aggressor mouse in the forced
212 interaction test (FIT; Figure 4A, B) (30, 31). Before the SSDS paradigm, the astrocytic
213 Ca^{2+} signals of cKO mice were increased during attack periods compared to non-attack
214 periods and similar to those of control mice (Figure 4 C-E). After 3 days of SSDS, control
215 mice also showed a large increase in Ca^{2+} signaling during an attack. Notably, the
216 magnitude of this increase was attenuated in cKO mice (Figure 4 C-E). These results
217 suggest that Ca^{2+} activity is reduced in response to stress after the deletion of astrocytic
218 GRs and that GRs in astrocytes may affect the Ca^{2+}-evoked release of neurotransmitters.

GRs modulate astrocytic ATP release in the mPFC

To explore how astrocytic GRs in the mPFC modulate depressive-like behaviors, we first detected plasma corticosterone levels. No difference in plasma corticosterone was observed between cKO and Ctrl mice under basal conditions or after an additional acute social defeat episode (Figure 5A). We next analyzed the amounts of neurotransmitters and inflammatory cytokines, known to be secreted by astrocytes (10, 32, 33), in the PFC of cKO and Ctrl mice (Figure 5B and Figure S8A). Notably, the concentration of ATP was lower in the artificial cerebral spinal fluid (ACSF) from PFC slices derived from cKO mice (Figure 5B). Furthermore, in vivo microdialysis in freely moving mice demonstrated that ATP concentrations in the interstitial fluid of the mPFC were significantly decreased in cKO mice compared to Ctrl mice (Figure 5C), indicating that GRs seem to regulate astrocytic ATP release.

To determine whether GRs regulate ATP release from astrocytes, we first employed pharmacological approaches. The application of DXMS increased ATP accumulation in the medium collected from cultured astrocytes 4 h after the treatment (Figure 5D). The ability of GRs to enhance ATP release from astrocytes was further confirmed using virus-mediated conditional knockout of GRs in cultured astrocytes isolated from *Nr3c1*$^{loxP/loxP}$ mice. The results showed that cultured astrocytes infected with pLenti-EGFP-Cre induced downregulation of the GR protein (Figure S8B-C). DXMS treatment also increased ATP release from astrocytes infected with the control virus, and the effect of DXMS in enhancing ATP levels was blocked by the knockout of GRs (Figure 5E).

Furthermore, to detect extracellular ATP dynamics in the mPFC in vivo at a high temporal resolution and with high specificity and sensitivity, we virally expressed an ATP sensor (AAV-GfaABC1D-ATP1.0) in astrocytes of the mPFC of cKO and Ctrl mice, and the amount of extracellular ATP was indicated by the intensity of fluorescence produced by green fluorescent protein (GFP). Two weeks after injection of the virus, fluorescence signals from astrocytes while mice were attacked by an aggressor mouse during the FIT were recorded using fiber photometry (Figure 5F, G). Before SSDS, fluorescence signals in both cKO and Ctrl mice increased when the mice were attacked by a CD1 aggressor (Figure 5 H-I). No significant difference in peak fluorescence signal was observed between the two groups (Figure 5J). After 3 days of SSDS, control mice also displayed a large increase in fluorescence during attack periods (Figure 5 H-I). However, this effect was reduced in cKO mice (Figure 5J).

Next, to define the role of ATP in the depressive-like behaviors caused by astrocytic GR deletion, we directly administered ATP to cKO mice (Figure 5K). Following the i.p. injection of ATP (125 mg per kg body weight) into the cKO mice, we observed that ATP treatment reduced the immobility time of the cKO mice in the FST (Figure 5L). No difference in locomotion activity in the OFT was observed (Figure S8D, E). Taken together, these results demonstrate that ATP release is impaired after the deletion of astrocytic GRs and that impairment of ATP release contributes to the depressive-like behaviors induced by astrocyte-specific loss of GRs.

GRs regulate ATP release from astrocytes mediated by the PI3K-AKT signaling

pathway

The GR is a constitutively expressed transcriptional regulatory factor (TRF) that controls many distinct gene networks (34). To explore how GRs modulate astrocytic ATP release, we examined the mRNA expression profiles of astrocytes isolated from the mPFC of cKO and Ctrl mice with FACS by RNA sequencing (RNA-seq) analysis. A volcano plot showed that 389 genes were upregulated, while 89 genes were downregulated in cKO mice (Figure 6A). The overall expression profiles of the Ctrl and cKO groups obtained after hierarchical cluster analysis showed clear separation of the differentially expressed genes (DEGs; Figure 6B). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that the PI3K-AKT signaling pathway was significantly enriched within the dataset (Figure 6C).

To directly test whether the PI3K-AKT signaling pathway is involved in GR-dependent ATP release from astrocytes, we employed pharmacological approaches. Western blot analysis showed that DXMS significantly increased the phosphorylation of PI3K and AKT in cultured astrocytes (Figure 6D). LY29004, a selective PI3K inhibitor, attenuated astrocytic ATP release caused by DXMS (Figure 6E). These results indicate that GR-dependent ATP release from astrocytes was mediated by the PI3K-AKT signaling pathway. PI3K has been reported to regulate intracellular vesicular traffic and homeostasis of the lysosome (35). Lysosome exocytosis is shown to be responsible for ATP release in astrocytes (36). Therefore, we then investigated the effects of glycylphenylalanine 2-naphthylamide (GPN) that selectively induces lysosome osmodialysis on the ATP release in cultured astrocytes (36). The results revealed that the

DXMS-induced enhancement in astrocytic ATP release was decreased by the treatment with 200 μM GPN (Figure 6F). To evaluate the effect of PI3K activation on lysosomes, we treated astrocytes with 740 Y-P (PI3K agonist; 50 μg ml^{-1}) for 4 h (37, 38), and then analyzed the astrocytic lysosomal compartment with Lysotracker, a weakly basic amine fluorescent probe that accumulates in acidic compartments such as lysosomes. Astrocytes incubated with Lysotracker were imaged and the mean fluorescence intensity of fluorescence-positive lysosomes was quantified. We found that mean fluorescence intensity was decreased in astrocytes treated with 740 Y-P compared to control astrocytes (Figure 6G, H). To examine whether PI3K activation in astrocytes affects lysosome numbers or size, astrocytes were stained with Lamp1, a lysosomal marker, after treatment with 740 Y-P for 4 h. The results showed that PI3K activation significantly increased the number of lysosomes compared to control astrocytes (Figure 6I, J). No difference in distributions of the lysosomal size was observed between these two groups (Figure 6K). Taken together, these findings suggest that GR-dependent ATP release from astrocytes through lysosome exocytosis requires activation of the PI3K-AKT signaling pathway.

DISCUSSION

Overall, we found that chronic stress induced a reduction in GRs in astrocytes. Furthermore, astrocyte-specific knockout of GRs was found to be sufficient to induce depressive-like behaviors. The reduction in astrocytic GRs decreased ATP release, which was mediated by the PI3K-AKT signaling pathway. We previously reported that astrocyte-derived ATP modulated depressive-like behaviors and P2X2 receptors in the

mPFC were required for the antidepressant-like effect of ATP (10). Together, our study suggests that temporal elevations in glucocorticoid levels preferentially activated astrocytic GRs, thereby enhancing extracellular ATP release through lysosome exocytosis. ATP released from astrocytes binds to purinergic receptors on neurons. Chronic stress induced a reduction of astrocytic GRs in the mPFC, which consequently caused decreased ATP released from astrocytes to regulate depressive-like behaviors (Figure 6L).

Neurochemical evidence suggests that chronic stress enhances the excitability of the HPA, which drives the release of glucocorticoid hormones into the blood (16, 18, 39, 40). Glucocorticoid hormones in the blood can be transported into the brain through the blood-brain barrier. Previous studies have reported that hyperactivity of the HPA axis is observed in the majority of patients with depression (41), and up to 40–60% of depressed patients experience hypercortisolemia (17). Pathological activation of the prefrontal cortical GRs by chronic stress negatively impacts the GR expression, suggesting the loss of prefrontal feedback control (22, 42). Previous studies and our data indicated that the GR expression in astrocytes was higher than that in neurons in the brain (20). And chronic stress induced a reduction of astrocytic GRs in the mPFC. Therefore, astrocytic GRs may contribute to glucocorticoid hormones feedback control.

The GR expression is detected in most mouse cell subsets, including astrocytes, neurons, microglia, endothelial cells, and oligodendrocytes (20). Previous studies have shown that GRs in neurons modulate depressive-like behaviors (15-17, 43). However, the effects of GRs in other cell subtypes in depression, such as astrocytes, have been poorly

329 explored. In a previous study, astrocyte-specific elimination of GRs impaired contextual
330 fear memory but did not elicit depressive-like symptoms in TST and sucrose preference
331 (44). In our study, we found astrocyte-specific knockout of the GRs induced
332 depressive-like behaviors in FST and SSDS. Similarly, we also did not find cKO mice
333 displayed depressive-like behaviors in TST and sucrose preference (Figure S5A, B).
334 Given the multifactorial nature and heterogeneity of major depression, different animal
335 depression models can only mimic some of the characteristics of depression (45).
336 Besides, knockdown of the astrocytic GRs in the central nucleus of the amygdala
337 diminishes conditioned fear expression and anxiety (46). In our study, we found global
338 astrocyte-specific knockout of GRs induced anxiety-like behaviors. However, knockdown
339 of the astrocytic GRs in the mPFC did not induce anxiety-like behavior. Therefore,
340 astrocytic GRs in other brain regions may play a role in fear memory and anxiety. In
341 addition, our experiments indicated that chronic stress induced a reduction of the GR
342 expression in astrocytes and that astrocytes were more sensitive to the stress response
343 than neurons. In the present study, we provide direct evidence that astrocytes are likely to
344 respond to stress before neurons to protect neurons from high concentrations of
345 glucocorticoid hormones. Astrocytic GRs seem to be a necessary molecule to the onset of
346 depression. However, the role of GRs in other cell subtypes in response to stress and the
347 relationships among these cells in MDD require further determination.
348 Previous studies have shown that exposure to stress rapidly increases the release of
349 neurotransmitters from synapses, which leads to the Ca^{2+}-dependent exocytosis of ATP
350 from astrocytes (32, 33). ATP and its metabolite, adenosine, activate disparate

presynaptic purinergic receptor subtypes to regulate synaptic efficacy (32, 47). However, our results suggest that GRs in astrocytes activated by circulating corticosterone promote ATP release, indicating that stress-induced circulating corticosterone directly modulates astrocytic ATP release. Previous studies have also proven that glucocorticoid hormones regulate ATP release from spinal astrocytes (48). Several possible pathways have been shown to be responsible for ATP release in astrocytes including vesicular exocytosis and non-exocytosis (49). Here, we show that GR-dependent ATP release from astrocytes through lysosome exocytosis requires activation of the PI3K-AKT signaling pathway. Though the PI3K has been reported to regulate intracellular vesicular traffic and homeostasis of the lysosome (35), the relationship between lysosome exocytosis and the PI3K-AKT signaling pathway under stress needs to be further determined.

Moreover, we found that a reduction in astrocytic GRs results in increased inflammatory factors (Figure S8A). Given that neuroinflammation is tightly associated with the onset of depression (25, 50, 51), it seems likely that astrocyte neuroinflammatory mechanisms also play a role in depression.

In summary, our findings demonstrate a critical role for astrocytic GRs as the regulator of depressive-like behaviors and establish the priority of astrocytes in response to stress.

ACKNOWLEDGMENTS AND DISCLOSURES

We thank William D. Richardson (University College London, London, UK) for providing the *Fgfr3-iCreER^{T2}* mice. We also thank Xiao-Wen Li, Li-Rong Sun, and Ting Guo

(Southern Medical University) for their technical support. This work was supported by the National Natural Science Foundation of China (31771187), the Guangzhou Science and Technology Project (201904020039, 202007030013), the National Program for Support of Top-notch Young Professionals, The Key Area Research and Development Program of Guangdong Province (2018B030334001, 2018B030340001), and the Program for Changjiang Scholars and Innovative Research Team in University (IRT_16R37).

The authors report no biomedical financial interests or potential conflicts of interest.

AUTHOR CONTRIBUTIONS

X.C. and C.-L.L. designed the study and wrote the paper. X.C. J.R. and C.-L.L. analyzed the data. J.R. and C.-L.L. performed most of the experiments. J.R. and C.-L.L. performed behavioral experiments and stereotactic injection with the help of Y.-Y.F. and F.G. performed the Western blotting. J.-W.M. and Y.-L.W. performed the FACS. J.F. performed the ATP assays. J.R. and S.-J. L. were responsible for cell culture. L.-Y.C. carried out genotyping. F.-Z. W. and Y.-L. L. provided the $GRAB_{ATP1.0}$ virus. T.-M. G. reviewed and edited the manuscript. X.C. supervised all phases of the project.

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Figure legends

Figure 1. The GR in astrocytes is more sensitive to stress than that in neurons. (A-B)

Schematic of CSDS paradigm (A), and social interaction ratio of susceptible (Sus), resilient (Res), and control (Ctrl) mice (B). **(C-D)** The GR protein in the mPFC of mice after the CSDS (C) and correlated with social avoidance (D; n = 7-8 mice per group). **(E-F)** Schematic representation of LPS-induced mouse model (E), and total immobility time of mice treated with LPS in the FST (F; n = 8 mice per group). **(G-H)** The GR protein level in the mPFC of adult C57BL/6J mice treated with LPS or saline (G) and correlated with immobility time in the FST (H, n = 6 mice per group). **(I),** Western blot analysis of the GR protein in primary cultured astrocytes (DIV8) and neurons (DIV14) treated with LPS (1μg ml^{-1}) or saline for 4 h (n = 5-6 per group). **(J-K)** Western blot analysis of the GR protein level in primary cultured astrocytes (DIV8) and neurons (DIV14) exposed to dexamethasone (DXMS; 0, 0.5, 1 and 5 μM) for 6 h (J; n = 4 per group) or DXMS (1 μM) for 0, 4, 24 and 48 h (K; n = 4 per group). **(L)**, Representative images and quantification of the co-expression of GR (red) and S100β (green) in the mPFC of adult C57BL/6J mice (n = 6 mice); Scale bars, 50 μm. **(M)** Representative images quantification of the co-expression of GR (red) and NeuN (green) in the mPFC of adult C57BL/6J mice (n = 6 mice); Scale bars, 50 μm. Data are the mean ± s.e.m. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Two-tailed unpaired *t*-test (**F, G, I, L, M**), one-way ANOVA followed by Bonferroni's multiple comparison test (**B**, **C, J, K**), and correlations were evaluated with Pearson's correlation coefficient (**D, H**).

Figure 2. Astrocyte-specific loss of GRs displays depressive- and anxiety-like behaviors. (A) Genetic crosses used to delete astrocytic GRs from the whole brain and experimental design of behavioral studies. PND, postnatal day. **(B)** Flow cytometry

549 analysis of the GR expression in astrocytes from cKO and Ctrl mice 28 days after
550 tamoxifen induction. The gray line depicts unstained control, black and red line GR
551 staining, and numbers indicate the percentage of GR-positive astrocytes. **(C)**
552 Quantification of GR-positive astrocytes of cKO and Ctrl mice **(**n = 3 mice per group). **(D)**
553 Total immobility time in the FST (n = 11,14 mice). **(E)** Representative heatmaps and
554 quantification of time spent in the interaction zone before and after SSDS (n = 9, 13 mice).
555 **(F)** Time spent in the open arms and closed arms in the EPM (n =11, 15 mice). **(G)** Time
556 spent in the dark box in the LD box (n = 12,15 mice). **(H-I)** Total distance (H) and center
557 time (I) for cKO and Ctrl mice in the open field test (n = 13, 15 mice). **(J)** Latency to fall of
558 cKO and Ctrl mice in the rotarod test (n = 11,13 mice). Data are the mean ± s.e.m. $^{*}P <$
559 0.05, $^{**}P <$ 0.01, $^{***}P<0.001$. Two-tailed unpaired *t*-test (**C, D, F, G, H, I**) and matching
560 two-way ANOVA followed by Bonferroni's multiple comparison test (**E, J**).

561 **Figure 3. Astrocytic GRs in the mPFC modulate depressive-like behaviors. (A)**
562 Experimental design of behavioral studies of *Nr3c1*$^{loxP/loxP}$ mice infected with
563 AAV-GFAP-CRE virus. **(B)** Representative images of AAV-GFAP-CRE expression in the
564 mPFC of *Nr3c1*$^{loxP/loxP}$ mice. Scale bars, 500 μm (left), 50 μm (right). **(C)** Western blot
565 analysis of GRs in the mPFC in different groups (n = 5 mice per group). **(D)** Total
566 immobility time in the FST (n = 11, 12 mice). **E**, Social avoidance behaviors before and
567 after SSDS (n = 9 mice per group). **(F)** Time spent in the open and closed arms for the
568 control and Cre in the EPM test (n = 11, 12 mice). **(G-H)** Total distance (G) and center
569 time (H) for Cre and control mice in the open field test (n = 11, 12 mice). **(I)** Representative
570 images of AAV-Efα1-DIO-Nr3c1-3xFlag expression in the mPFC of cKO mice. Scale bars,

500 μm (left), 50 μm (right). **(J)** The social avoidance behaviors of cKO and Ctrl mice before and after SSDS (n = 10 mice for Ctrl+EYFP and Ctrl+GR; n = 11 mice for cKO+EYFP; n = 12 mice for cKO+GR). **(K-L)** Total distance (K) and center time (L) for Ctrl and cKO mice infected with AAV-Efα1-DIO-Nr3c1-3xFlag or control virus (n = 8 mice per group). Data are the mean ± s.e.m. **P* < 0.05, ***P*< 0.01, N.S. not significant. Two-tailed unpaired t-test (**C, D, F, G, H**), one-way (**K, L**), and matching two-way ANOVA followed by Bonferroni's multiple comparison test (**E, J**).

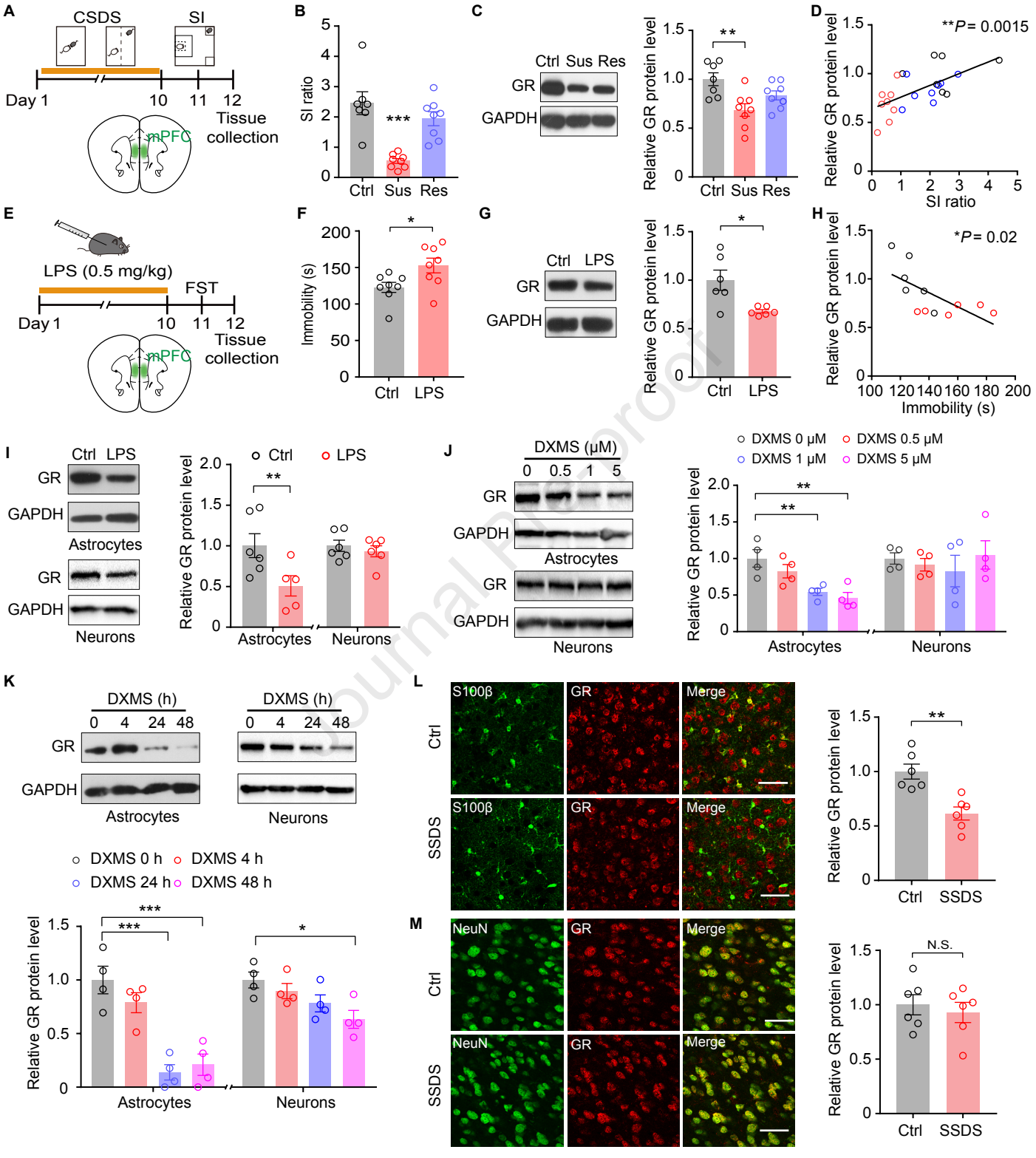
Fig. 4. The GR deletion decreases astrocytic Ca^{2+} activity in the mPFC. (A) Experimental design of fiber photometry. **(B)** Schematic illustrating fiber placement and representative images of GCamp6s expression. Scale bars, 500 μm (left), 50 μm (right). **(C)** Representative heatmaps of GCaMP6s transient z-scores event-locked to social interaction. Each row plots one trial and a total of 4 trials are illustrated. **(D-E)** Average (D) and peak (E) z-score changes during social interaction (n = 4 mice per group). Data are the mean ± s.e.m. **P* < 0.05. Matching two-way ANOVA followed by Bonferroni's multiple comparison test (**E)**.

Figure 5. GRs modulate astrocytic ATP release in the mPFC. (A) The concentration of plasma corticosterone in cKO and Ctrl mice under basal conditions or after an additional acute social defeat episode (n = 6-8 mice per group). **(B)** Measurements of neurotransmitter levels (aspartate (Asp), glutamate (Glu), serine (Ser), glutamine (Gln), glycine (Gly), GABA, and ATP) in the medium of slices of the PFC (n = 5-8 mice). **(C)** In vivo microdialysis in freely moving mice showing ATP levels in the mPFC (n = 8 mice per group). **(D-E)** ATP levels in the medium of primary cultured astrocytes (D, n = 6) and

GR-knockdown astrocytes (E, n = 6) treated with DXMS or vehicle for 4 h. **(F)** Experimental design of fiber photometry. **(G)** Schematic illustrating fiber placement and representative images of AAV-GfaABC1D-ATP1.0 expression. Scale bars, 500 μm (left), 50 μm (right). **(H)** Representative heatmaps of ATP1.0 transient z-scores event-locked to social interaction. Each row plots one trial and a total of 4 trials are illustrated. **(I-J)** Average (I) and peak (J) z-score during social interaction (n = 4 mice per group). **(K)** Experimental design of ATP treatment. **(L)** Total immobility time in the FST (n = 9-12 mice). Data are the mean ± s.e.m. **P* < 0.05, ***P*< 0.01, ****P*< 0.01. Two-tailed unpaired t-test (**B**), two-tailed paired t-test (**C**), one-way ANOVA followed by Bonferroni's multiple comparison test (**D, L**), two-way ANOVA followed by Bonferroni's multiple comparison test (**A**, **E, J**).

Fig. 6. The PI3K-AKT signaling pathway mediates GR-dependent astrocytic ATP release. (A) Volcano plot of the differentially expressed genes (DEGs) between cKO and Ctrl astrocytes isolated from the mPFC (n = 3 independent biological samples per group). **(B)** Hierarchical clustering based on the expression profiles of DEGs. **(C)** Enriched KEGG pathways. **(D)** Western blot analysis of p-PI3K, PI3K, p-AKT, and AKT protein levels in cultured astrocytes after 4 h of DXMS (1 μM) or vehicle treatment (n = 5). **(E)** ATP levels in the medium of cultured astrocytes treated with DXMS (1 μM) or vehicle for 4 h in the presence or absence of the PI3K inhibitor LY29004 (40 μM) (n = 5-6). **(F)** Effects of GPN (200 μM) on the DXMS-induced release of ATP from astrocytes (n = 5-6). **(G)** Confocal fluorescent images of Lysotracker uptake in living astrocytes treated with 740-YP (50 μg ml^{-1}) and vehicle for 4 h. Scale bar, 20 μm. **(H)** The mean fluorescence intensity of

Lysotracker was quantified using ImageJ (n = 6 separate experiments). **(I)** Confocal fluorescent images of Lamp1 in astrocytes treated with 740-YP (50 μg ml^{-1}) or vehicle for 4 h. Scale bar,10 μm. **(J)** The number of lysosomes per 100 μm^2 in 19 and 18 astrocytes treated with vehicle or 740-YP, respectively. **(K)** Distribution of the lysosome surface (μm^2). 1136 and 1214 lysosomes were measured in astrocytes treated with vehicle or 740-YP, respectively. The lysosomal size was ranged from 0.01 to 1 μm^2. χ^2 test of independence: χ^2 = 2.419, df 10, *P* =0.992. **(L)** A model for mechanisms of depression indicated that the GR-dependent astrocytes mediated stress vulnerability in mice. Temporal elevations in glucocorticoid levels preferentially activated astrocytic GRs, thereby enhancing extracellular ATP release through lysosome exocytosis. ATP released from astrocytes bound to purinergic receptors on neurons. Chronic stress induced a reduction of astrocytic GRs in the mPFC, which consequently decreased ATP released from astrocytes to regulate depressive-like behaviors. Data are the mean ± s.e.m. **P* < 0.05, ***P*< 0.01, ****P*< 0.001, N.S., not significant. Two-tailed unpaired t-test (**D, H, J)** and one-way ANOVA followed by Bonferroni's multiple comparison test (**E, F**).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