Archival Report

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α-Ketoglutarate Is a Circulatory Exercise Factor That Promotes Learning and Memory Recall and Has Antidepressant Properties

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ABSTRACT

BACKGROUND: Depression poses a significant societal burden, necessitating effective treatment options. Conventional approaches often fall short, highlighting the need for alternatives. Exercise has emerged as a promising nonpharmacological strategy for improving mental health outcomes. Exercise promotes memory recall and alleviates depression by modulating BDNF (brain-derived neurotrophic factor) expression. The effects of exercise on BDNF are influenced by circulatory metabolites known as exercise factors.

METHODS: Associative and spatial memory were evaluated in mice receiving α-ketoglutarate (aKG) and in exercise mice given a glutaminase inhibitor. To prevent and treat depression-like behaviors, male mice underwent daily defeat sessions by a CD1 aggressor for 10 days. Behavior was assessed on day 11 using social interaction and open-field tests. Mice received aKG for 5 days prior to the stress paradigm or as treatment for 14 days following the stress paradigm, after which social behavior was reassessed. BDNF signaling was examined via Western blots.

RESULTS: aKG was identified as a metabolite released into the bloodstream following exercise in male mice. aKG was shown to mediate the positive effects of exercise on spatial learning and memory formation. aKG was also shown to have prophylactic and antidepressant effects in a chronic social defeat stress model of depression.

CONCLUSIONS: aKG acts as a prophylactic and antidepressant to effectively counteract social avoidance behaviors by modulating BDNF levels in the hippocampus and nucleus accumbens.

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Mental health disorders, such as depression, have a profound impact on an individual's well-being and quality of life. The available treatment options often fall short of providing long-lasting relief or addressing the underlying causes of these disorders. The existing treatment options have limitations, including side effects and variable efficacy (1). Consequently, the search for alternative interventions has intensified recently, leading to an increased interest in trying to harness the therapeutic potential of exercise to improve mental health outcomes.

Exercise exerts important effects on cognitive function and mental well-being (2,3). Specifically, exercise has been associated with enhanced learning and memory formation, as well as alleviation of depression-like symptoms (4–10). Understanding the mechanisms that underlie these effects can pave the way for novel therapeutic strategies that target mental health disorders.

Several factors that underlie the positive effects of exercise on cognition and mental health have been identified. Firstly, exercise promotes neuroplasticity, leading to structural and functional changes in the brain that support learning and memory processes (11–13). Secondly, exercise influences the release of various neurotransmitters and growth factors, including serotonin (14,15), dopamine (16), and BDNF (brain-derived neurotrophic factor) (17), which are known to modulate mood and cognitive function. Finally, exercise induces changes in metabolic pathways. Recent studies have indicated that metabolic intermediates that play central roles in energy metabolism and cellular signaling act as exercise factors, contributing to the cognitive and mood-enhancing effects of physical activity (18).

Several metabolites have been implicated in the exercise-induced effects on depression and cognition. Beta-hydroxybutyrate (BHB), a ketone body produced during prolonged fasting or ketogenic diets, can mediate the cognitive and mood-enhancing effects of exercise. BHB levels increase following exercise (7), and BHB administration improves depression-like behaviors (19–21) and enhances cognitive function in rodents (7). BHB exerts its effects through multiple mechanisms, including modulation of neurotransmitter systems and activation of BDNF expression through epigenetic mechanisms (7,19–21). Lactate is another exercise-induced

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metabolite. It is generated during glycolysis and serves as an energy substrate for neurons. Studies have shown that lactate levels increase in response to exercise (22) and that lactate enhances cognitive function (22) and mood (23–25). For example, lactate promotes memory formation and synaptic plasticity in animal models (22). Moreover, lactate administration improves depression-like behaviors in rodents. In fact, it has both prophylactic and antidepressant-like effects (23–25).

Interestingly, exercise has also been found to modulate the levels of α-ketoglutarate (aKG), a key metabolite in the tricarboxylic acid cycle. Studies have shown that exercise increases aKG levels in the circulation (26–28). aKG supplementation extends lifespan in *Drosophila melanogaster* and *Caenorhabditis elegans* by inhibiting the activity of mTOR (mechanistic target of rapamycin) kinase, increasing autophagy, and inhibiting ATP synthase (29,30). In mice, aKG supplementation prolongs the lifespan and reduces aging-associated frailty through an unknown mechanism (31). aKG supplementation also reverses aging in humans as measured by DNA methylation patterns (32).

In this study, we tested whether aKG mediates the positive effects of exercise on learning, memory recall, and mental health. We showed that both voluntary and resistance exercise promote the release of aKG into the circulation in male mice. In addition, we showed that aKG can mediate the positive effects of exercise on spatial learning and memory formation. aKG pretreatment alone is sufficient to promote spatial learning and associative memory formation. Finally, we showed that aKG has both prophylactic and antidepressant effects in mice subjected to chronic social defeat stress. These effects were achieved via modulating BDNF levels in the hippocampus and nucleus accumbens (NAc).

METHODS AND MATERIALS

Animal Housing

For all experimental paradigms, adult male C57BL/6J mice were individually housed, provided with food and water ad libitum, and kept on a 12-hour light/dark cycle. All animal work was approved by the Lebanese American University Institutional Animal Care and Use Committee.

Exercise Paradigms

Voluntary Running Wheel Exercise. Male C57BL/6J mice (10 weeks old) were individually housed and received free access to a running wheel (33).

Resistance Exercise. Male C57BL/6 mice (10 weeks old) were subjected to 1 session of ladder climbing (27). The mice climbed a 1-m long ladder, with 2-cm grids, at an 85° inclination with a resting chamber at the top. Each mouse climbed the ladder for 40 minutes, carrying increasing weight loads. The mice were first trained by climbing with no load; after 4 successful climbs, they carried a load equivalent to 10% of their body weight. After every 4 successful climbs, 2 g of load was added. The mice were given 1 minute of rest in between climbs.

aKG Measurement

Serum levels of aKG were measured using the α-Ketoglutarate Assay Kit according to the manufacturer's protocol (#MET-5131; Cell Biolabs, Inc.).

Intraperitoneal Injections

Male C57BL/6J mice received daily intraperitoneal (i.p.) injections of either saline or aKG (300 mg/kg) for 5 days (34). The mice were tested using the Morris water maze (MWM) after 5 days of injections. Alternatively, mice subjected to a voluntary running wheel exercise received either vehicle (10% dimethyl sulfoxide and 90% phosphate-buffered saline) or a brain-permeable glutaminase antagonist (35,36), JHU-083 dissolved in vehicle (1.82 mg/kg) (36) on alternating days starting on day 8 of the voluntary running wheel exercise. The mice were tested using MWM at the end of the exercise paradigm.

Morris Water Maze

The MWM assesses spatial learning and memory recall (37). It tests the ability of the mice to use visual cues placed on the borders of a pool to escape the water and reach a hidden platform (22,33).

Fear Conditioning Protocol

Associative memory was assessed using fear conditioning testing for 3 consecutive days as described in the Supplement.

Chronic Social Defeat Stress Model

The chronic social defeat stress (CSDS) model induces social avoidance behavior in male C57BL/6J mice. Experimental C57BL/6J mice were subjected to social defeat stress for 10 consecutive days. Each defeat session consisted of direct physical contact between the aggressor mouse and the experimental mouse for 7 minutes followed by sensory interaction for the next 24 hours. On day 11, behavioral testing was performed (25,38,39).

Social Interaction Test

The social interaction (SI) test was conducted 1 day after the last CSDS as previously described (40). To establish whether the mouse was susceptible or resilient to stress, the SI ratio was calculated by dividing the time spent in the interaction zone by the time spent in the no-interaction zone. The mouse was considered susceptible if the ratio was <1 and resilient to stress if the ratio was >1 (41).

Elevated Plus Maze

The elevated plus maze (EPM) is a validated test for anxiety. The time spent in closed and open arms was recorded.

aKG Injections in Stress Models

In pretreatment experiments, mice either received daily i.p. injections of saline or aKG for 5 days prior to the start of CSDS. For the posttreatment paradigm, mice were subjected to CSDS. Mice were split into susceptible or resilient groups. Susceptible mice received i.p. injections of either saline or aKG for 14 days, after which the SI test was performed again.

Immunoblot Analysis

To determine BDNF, ACTIN, and GAPDH relative protein levels, total cellular proteins were extracted from the hippocampi and NAc of mice, and Western blots were performed.

Statistical Analysis

Two-way analysis of variance (ANOVA) followed by Tukey post hoc tests were used to measure statistical significance. All error bars are presented as averages and standard errors except in Figure 1C and D, where they are presented as averages and standard deviations.

RESULTS

Exercise Induces an Increase in Serum aKG Levels

To assess whether exercise induces increases in serum aKG levels, 10-week-old male mice were subjected to resistance exercise or voluntary running wheel exercise. Mice subjected to either type of exercise showed a significant increase in serum aKG levels compared with sedentary mice ($p < .0001$ for sedentary vs. resistance exercise, $p = .0261$ for sedentary vs. running wheel exercise; 1-way ANOVA followed by Tukey's post hoc test: $F_{2,9} = 30.83$, $p < .001$) (Figure 1A). These results suggest that aKG is released into the circulation following exercise.

aKG Pretreatment Enhances Spatial Learning and Associative Memory Formation

To determine whether aKG promotes learning and memory formation, we first assessed the effects of systemic aKG on spatial learning using the MWM paradigm. Male mice were tested using MWM after receiving daily i.p. injections of either saline or aKG (300 mg/kg) for 5 days. MWM is a spatial learning task that requires mice to locate a hidden platform in an opaque pool of water using visual cues. Acquisition of spatial learning in mice receiving saline or aKG was observed as

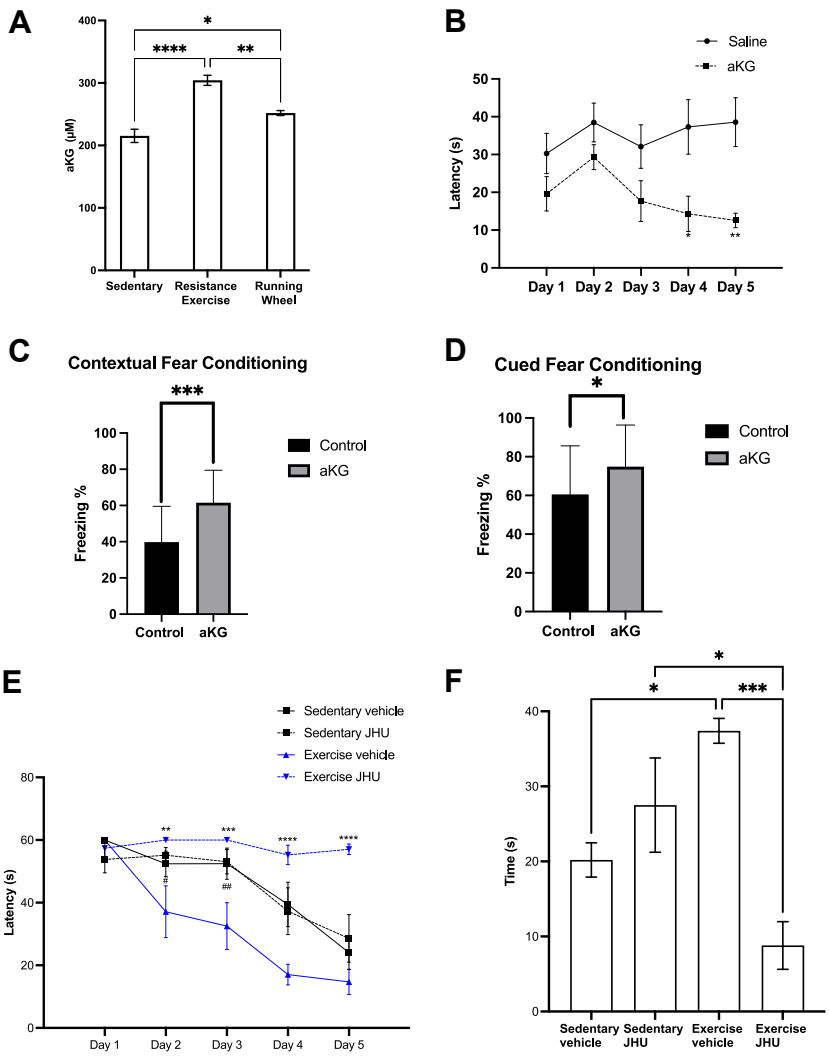

Figure 1. aKG mediates exercise and memory recall in male mice. **(A)** Voluntary running wheel exercise and resistance exercise significantly increased serum levels of aKG compared with controls ($n = 4$). **(B)** aKG enhanced spatial learning by significantly decreasing the escape latency in the Morris water maze ($n = 10$). **(C)** aKG enhanced contextual memory by significantly increasing the freezing percentage ($n = 25$). **(D)** aKG enhanced cued memory by significantly increasing the freezing percentage ($n = 25$). **(E)** Inhibition of aKG production abolished the positive effects of exercise on spatial learning as observed by the significant increase in escape latency in exercise mice that received JHU-083 ($n = 6–7$) compared with exercise mice that received vehicle. **(F)** Inhibition of aKG production abolished the positive effects of exercise on spatial memory recall as observed by the significant decrease in the time spent in the target quadrant by exercise mice receiving JHU-083 ($n = 6–7$) compared with exercise mice receiving vehicle. Stars (*) indicate significant differences between the exercise+vehicle and exercise+JHU groups, while number signs (#) indicate significant differences between the exercise+vehicle and sedentary+vehicle groups. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$, #$p < .05$, ##$p < .01$. aKG, α-ketoglutarate.

reduced latency to reach the hidden platform by day 5. Mice that received saline did not show a significant enhancement in spatial learning acquisition over the 5 days of the experiment. Mice that received aKG significantly outperformed mice that received saline ($p = .0117$ on day 4 and $p = .0032$ on day 5 for aKG- vs. saline-treated mice; 2-way ANOVA followed by Tukey's post hoc test: treatment $F_{1,90} = 25.73$, $p < .0001$) (Figure 1B). These results suggest that systemic delivery of aKG promotes spatial learning. While we found that aKG has a significant effect on spatial learning, we did not observe any effects on spatial memory recall during the probe test (data not shown). To further evaluate the effects of aKG on hippocampal-dependent memory formation, 10-week-old male mice were trained using a fear conditioning paradigm prior to a memory test 24 hours later. Mice that received aKG showed markedly increased freezing behavior in both context- and tone-dependent fear learning (unpaired t test: tone, $p = .0002$; context, $p = .0343$) compared with control mice that received saline (Figure 1C, D). These observations suggest that systemic treatment with aKG results in enhancement of associative learning. Taken together, our results are consistent with the hypothesis that systemic delivery of aKG significantly enhances hippocampal-dependent spatial learning and associative memory in male mice.

Inhibition of aKG Production Abolishes Exercise-Induced Spatial Learning and Memory Recall

It is well established that exercise induces spatial learning and memory formation (6,7,22,33,42,43). To test whether the exercise-dependent increase in aKG can mediate the positive effects of exercise on spatial learning and memory recall, exercise mice received either a vehicle or JHU-083. JHU-083, a proagent of 6-diazo-5-oxo-L-norleucine, is an orally active and selective glutaminase antagonist. JHU-083 inhibits glutaminase-mediated glutaminolysis and its downstream production of aKG (35,36). As expected, exercise mice that received vehicle exhibited significantly enhanced learning curves compared with sedentary mice injected with vehicle ($p = .0159$ on day 3 and $p = .005$ on day 4 for exercise vs. sedentary; 2-way ANOVA followed by Tukey's multiple comparison test: day $F_{4,110} = 21.96$, $p < .0001$; treatment $F_{3,110} = 27.53$, $p < .0001$; interaction $F_{12,110} = 3.378$, $p = .0003$) (Figure 1E) and showed significant enhancement of memory recall, as indicated by the increased time spent in the target quadrant ($p = .0323$ for exercise vs. sedentary; 1-way ANOVA followed by Tukey's multiple comparison test: treatment $F_{3,19} = 9.9853$, $p = .0004$) (Figure 1F). Interestingly, JHU-083 treatment had significant effects on learning acquisition and memory recall in exercise mice (Figure 1E, F). Exercise mice that received JHU-083 showed worsened learning curves ($p = .0024$ on day 2, $p = .0002$ on day 3, and $p < .0001$ on days 4–5 for exercise+JHU-083 vs. exercise; 2-way ANOVA followed by Tukey's multiple comparison test: day $F_{4,110} = 21.96$, $p < .0001$; treatment $F_{3,110} = 27.53$, $p < .0001$; interaction $F_{12,110} = 3.378$, $p = .0003$) and impaired memory recall ($p = .0002$; 1-way ANOVA followed by Tukey's multiple comparison test: treatment $F_{3,19} = 9.9853$, $p = .0004$) (Figure 1E, F). The treatments did not affect the swim speed of the animals. Our results suggest that inhibition of glutaminase-mediated glutaminolysis, possibly through its downstream production of aKG, may abolish exercise-induced spatial learning and memory formation. Our results are consistent with a model in which exercise induces aKG, which in turn promotes both spatial and associative memory formation.

aKG Pretreatment Promotes Resilience to CSDS

It is well established that exercise alleviates depression-like symptoms (4,5,10,44,45). It has been established that circulatory exercise factors such as irisin, DBHB, and lactate promote resilience to chronic stress and have antidepressant-like effects (19,22,25,46). For this reason, we assessed whether aKG can also promote resilience to chronic stress and prevent social avoidance behavior. To determine whether aKG promotes resilience to stress, we subjected C57BL/6J male mice to a CSDS paradigm (47,48). The CSDS paradigm yields a depression-like phenotype among the defeat group that can be reversed by the administration of antidepressants (49). For 5 days, mice received i.p. injections of either saline or aKG (Figure 2A). On day 6, the CSDS sessions started, and aKG treatment was stopped. From day 6 to 16, mice were exposed to CSDS sessions followed by sensory contact with the aggressor, while control mice were only exposed to sensory contact with the resident mouse. In total, this experiment yielded 4 groups of mice: control mice that received saline, control mice that received aKG, defeat mice that received saline, and defeat mice that received aKG. After the final defeat session, mice underwent SI testing to screen for susceptibility versus resilience to stress. The SI test directly assesses susceptibility versus resilience to chronic stress by calculating the SI ratio. An SI ratio >1 indicates that the mice were resilient to stress, whereas a ratio <1 indicates that the mice were susceptible to stress. Next, we assessed the distribution of the SI ratios of the different groups (Figure 2B). As expected, the SI ratio of defeat mice that received saline was significantly lower than that of controls ($p = .0008$ for defeat+saline vs. control+saline; 2-way ANOVA followed by Tukey's multiple comparison test: defeat $F_{1,29} = 17.18$, $p = .0003$; treatment $F_{1,29} = 11.50$, $p = .0020$; interaction $F_{1,29} = 3.584$, $p = .0684$). In contrast, the SI ratio of defeat mice that received aKG was significantly higher than that of defeat mice that received saline ($p = .0051$ for defeat+saline vs. defeat+aKG) (Figure 2B). Accordingly, defeat mice that received saline spent significantly less time interacting with the social stimulus than control animals ($p = .006$ for defeat+saline vs. control+saline; 2-way ANOVA followed by Tukey's multiple comparison test: defeat $F_{1,36} = 11.49$, $p = .0017$; interaction $F_{1,36} = 10.21$, $p = .0029$) (Figure 2C). This social avoidance phenotype was prevented by aKG pretreatment because the average time spent interacting with the social stimulus was significantly higher in the defeat aKG group than the defeat saline group ($p = .0094$ for defeat+saline vs. defeat+aKG) (Figure 2C). The opposite results were observed when we assessed the no interaction time ($p < .0001$ for defeat+saline vs. control+saline and for defeat+saline vs. defeat+aKG; 2-way ANOVA followed by Tukey's multiple comparison test: defeat $F_{1,36} = 21.10$, $p < .0001$; treatment $F_{1,36} = 28.95$, $p < .0001$; interaction $F_{1,36} = 30.12$, $p < .0001$) (Figure 2D). Together, our results suggest that aKG serves as a protective and prophylactic treatment

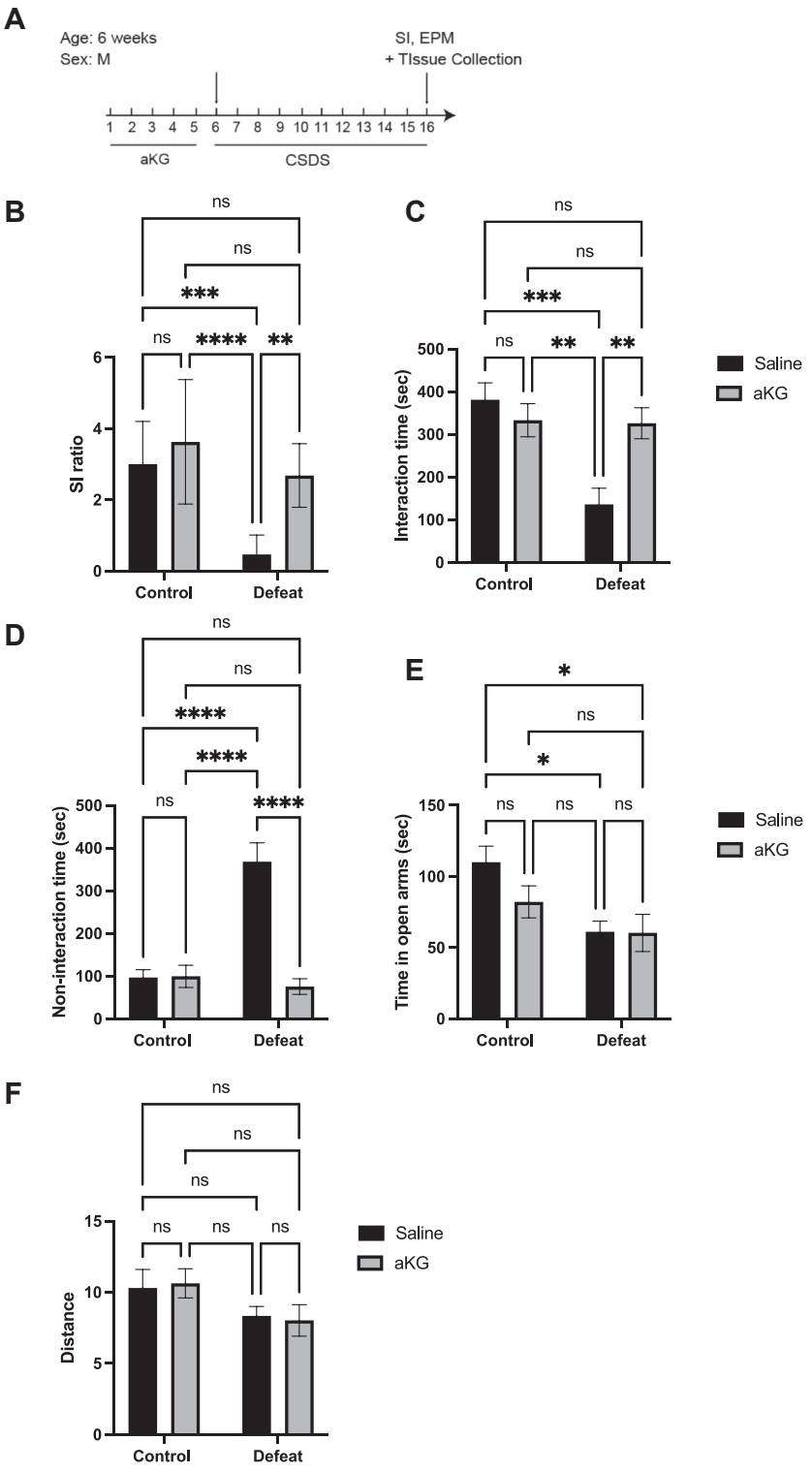

Figure 2. aKG promotes resilience to chronic stress and rescues social avoidance behavior but not anxiety-like behaviors. **(A)** Timeline depicting the experimental paradigm. Mice received aKG (300 mg/kg) 5 days prior to the start of CSDS. The CSDS paradigm consisted of 10 consecutive days of defeat sessions that involved direct contact between the resident (aggressor) mouse and the experimental mouse for 7 minutes. On day 11 of the CSDS paradigm, behavioral tests and brain tissue collection were conducted. **(B)** aKG treatment promoted resilience to stress. SI ratio distribution across the different mice groups. Defeat mice receiving saline had a significantly lower SI ratio than control mice. In contrast, defeat mice receiving aKG had a significantly higher SI ratio than defeat mice receiving saline (n = 7–10). **(C)** aKG pretreatment reversed the chronic social defeat phenotype as shown by the increase in the time spent in the interaction zone of the SI test. Statistical significance was measured by 2-way ANOVA followed by Tukey's multiple comparison test. **(D)** aKG pretreatment reversed the chronic social defeat phenotype as shown by the decrease in the time spent in the no-interaction zone of the SI test. Statistical significance was measured by 2-way ANOVA followed by Tukey's multiple comparison test. **(E)** aKG pretreatment did not affect anxiety given that no significant increase in the time spent in the open arms of the EPM was observed in defeat mice that received aKG vs. defeat mice that received saline. Statistical significance was measured by 2-way ANOVA followed by Tukey's multiple comparison test. **(F)** The locomotor activity of all mice groups was not affected by aKG or defeat as measured by the distance traveled in the EPM. Statistical significance was measured by 2-way ANOVA followed by Tukey's multiple comparison test. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$. aKG, α-ketoglutarate; ANOVA, analysis of variance; CSDS, chronic social defeat stress; EPM, elevated plus maze; M, male; ns, nonsignificant; SI, social interaction.

against the onset of depressive-like symptoms associated with this paradigm. Interestingly, aKG could not rescue anxiety symptoms associated with defeat. Defeat mice that received saline or aKG spent significantly less time in the open arms of the EPM than control mice (p = .0162 for defeat+saline vs. control+saline and p = .0142 for defeat+aKG vs.

control+saline; 2-way ANOVA followed by Tukey's multiple comparison test: defeat $F_{1,36}$ = 10.42, p = .0027; treatment $F_{1,36}$ = 1.717, p = .1984; interaction $F_{1,36}$ = 1.531, p = .224) (Figure 2E), even though defeat or treatment did not affect locomotor behavior in mice because all mice groups traveled similar distances in the EPM (2-way ANOVA followed by Tukey's multiple comparison test) (Figure 2F).

aKG Pretreatment Promotes Resilience to CSDS by Modulating BDNF Levels in the Hippocampus and NAc

Alterations in BDNF levels have been reported to occur in multiple brain regions in major depressive disorder (MDD), and these alterations have been associated with different outcomes. For example, BDNF expression is decreased in the hippocampus in response to chronic stress, and this decrease is associated with depression-like symptoms (39,49). Direct infusion of BDNF into the hippocampus of rodents has antidepressant effects (50). In contrast, in other brain regions such as the NAc, BDNF exerts a potent prodepressant effect. In fact, chronic stress increases BDNF expression within the NAc (47). Direct infusion of BDNF into the NAc enhances depression-like behaviors (48,51), whereas a selective BDNF knockout in the NAc has antidepressant-like effects (47). Because chronic stress modulates BDNF expression in these brain regions, we assessed whether aKG pretreatment can reverse the effects of chronic stress on BDNF expression in both the hippocampus and NAc. First, we tested whether aKG rescues BDNF expression in the hippocampi of mice subjected to CSDS. Western blot analysis revealed a significant decrease in BDNF levels in the hippocampi of defeat mice that received saline compared with controls (p = .0424 for defeat+saline vs. control+saline; 2-way ANOVA followed by Tukey's multiple comparison test: defeat $F_{1,28}$ = .03292, p = .8573; treatment $F_{1,28}$ = 2.051, p = .1632; interaction $F_{1,28}$ = 15.55, p = .0005). aKG pretreatment significantly increased the hippocampal BDNF protein levels in defeat mice back to control levels (p = .0055 for defeat+saline vs. defeat+aKG) (Figure 3A, B). Because PGC1a acts as an upstream activator to BDNF and is regulated by exercise (22,43), we assessed whether its levels are modulated by stress and aKG. Interestingly, while stress did not significantly modulate hippocampal PGC1a levels, aKG pretreatment significantly increased hippocampal PGC1a levels in defeat mice but not in control mice (p = .0072 for defeat+saline vs. defeat+aKG; 2-way ANOVA followed by Tukey's multiple comparison test: defeat $F_{1,22}$ = 1.667, p = .2101; treatment $F_{1,22}$ = 9.801, p = .0049; interaction $F_{1,22}$ = 6.956, p = .0150) (Figure 3C, D). Our results suggest that hippocampal BDNF is modulated by stress and that aKG pretreatment restores normal BDNF levels in part through increasing the levels of PGC1a. Next, we assessed whether aKG rescues BDNF expression in the NAc of defeat mice. Western blot analysis revealed that BDNF levels in the NAc were significantly increased in defeat mice that received saline (p = .0453; 2-way ANOVA followed by Tukey's multiple comparison test: defeat $F_{1,5}$ = 0.0646, p = .8155; treatment $F_{1,5}$ = 0.07421, p = .7962; interaction $F_{1,5}$ = 28.64, p = .0031), but this increase was prevented in the defeat mice that received aKG pretreatment (p = .0315) (Figure 4A, B). Interestingly, no

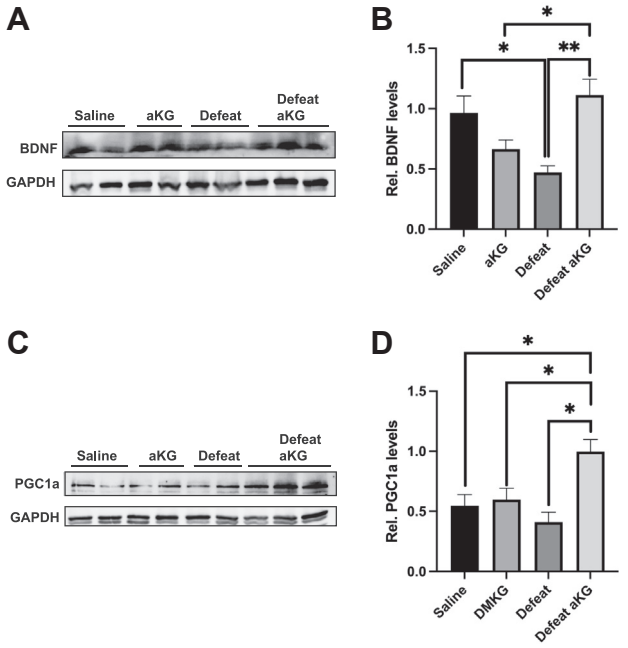

Figure 3. aKG pretreatment restores hippocampal BDNF protein levels in mice exposed to CSDS. **(A)** Representative Western blot images depicting hippocampal BDNF levels in control, aKG, defeat, and defeat+aKG mice. Mice exposed to CSDS had lower levels of hippocampal BDNF than control mice. aKG pretreatment restored BDNF protein levels. **(B)** Quantification of the BDNF Western blots. Statistical significance was measured by 2-way ANOVA followed by Tukey's multiple comparison test. The number of hippocampi analyzed was 9 for control, 8 for aKG, 6 for defeat, and 9 for the defeat+aKG groups, respectively. **(C)** Representative Western blot images depicting hippocampal PGC1a levels in control, aKG, defeat, and defeat-+aKG mice. aKG pretreatment increased hippocampal PGC1a protein levels in defeat mice compared to all other groups. **(D)** Quantification of the PGC1a Western blots. Statistical significance was measured by 2-way ANOVA followed by Tukey's multiple comparison test. The number of hippocampi analyzed was 8 for control, 8 for aKG, 4 for defeat, and 6 for the defeat+aKG groups, respectively. *$p < .05$, **$p < .01$. aKG, α-ketoglutarate; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CSDS, chronic social defeat stress; rel., relative.

significant changes were observed in its upstream activator, PGC1a (Figure 4C, D). Taken together, our results are consistent with aKG pretreatment promoting resilience to CSDS by modulating BDNF levels in specific brain regions. In the hippocampus, aKG pretreatment promotes resilience via the PGC1a-BDNF signaling pathway, whereas in the NAc, aKG pretreatment promotes resilience by decreasing BDNF levels independent of PGC1a regulation.

aKG Has Antidepressant Properties and Can Reverse Social Avoidance Behavior in Mice Subjected to CSDS

Next, we assessed whether aKG has antidepressant effects in addition to its prophylactic effects. To test whether aKG can be used as an antidepressant, we developed a posttreatment paradigm in male mice. Male mice were first subjected to CSDS. On the 11th day of the CSDS paradigm, the SI test was performed to classify the mice as susceptible or resilient to

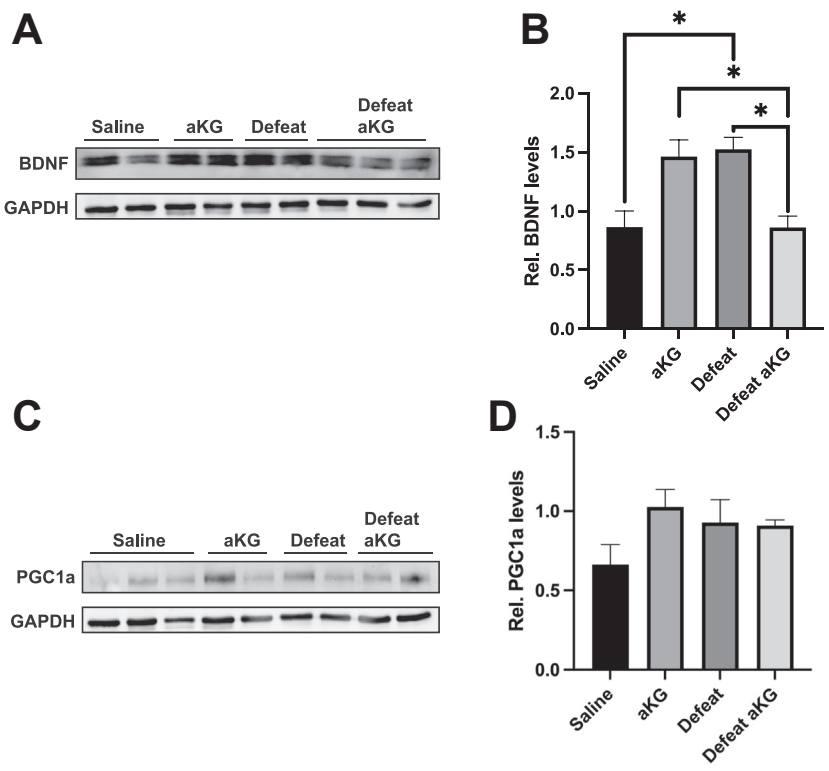

Figure 4. aKG pretreatment prevents increases in BDNF protein levels in the NAc of mice exposed to CSDS. **(A)** Representative Western blot images depicting BDNF levels in the NAc of control, aKG, defeat and defeat+aKG mice. Mice exposed to CSDS had higher levels of BDNF in the NAc than control mice. aKG pretreatment prevented the increase in the BDNF protein levels in the NAc. **(B)** Quantification of the BDNF Western blots. Statistical significance was measured by 2-way ANOVA followed by Tukey's multiple comparison test. The number of hippocampi analyzed was 2 for control, 2 for aKG, 3 for defeat, and 3 for the defeat+aKG groups, respectively. **(C)** Representative Western blot images depicting PGC1a levels in the NAc of control, aKG, defeat, and defeat+aKG mice. aKG pretreatment did not affect PGC1a protein levels in the NAc of defeat mice compared with all other groups. **(D)** Quantification of the PGC1a Western blots. Statistical significance was measured by 2-way ANOVA followed by Tukey's multiple comparison test. The number of hippocampi analyzed was 2 for control, 2 for aKG, 3 for defeat, and 3 for the defeat+aKG groups, respectively. *$p < .05$. aKG, α-ketoglutarate; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CSDS, chronic social defeat stress; NAc, nucleus accumbens; rel., relative.

stress. Only susceptible mice received either saline or aKG treatment for 14 days. After 14 days, the SI test was performed again (Figure 5A). Treatment with aKG significantly increased the SI ratio in defeat mice ($p = .0049$, unpaired t test) (Figure 5B) and reversed social avoidance behavior by increasing the interaction time (unpaired t test, $p = .0226$) (Figure 5C) and decreasing the noninteraction time (unpaired t test, $p = .0067$) (Figure 5D). Susceptible mice treated with

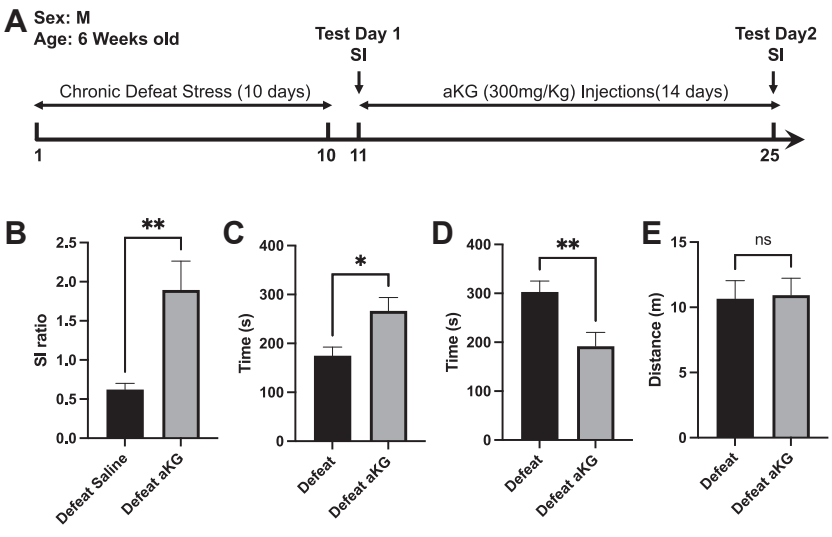

Figure 5. aKG is an antidepressant that can rescue social avoidance phenotypes after their establishment. **(A)** Modified CSDS paradigm to assess the therapeutic potential of aKG. This paradigm comprises 10 days of daily defeat sessions that involve direct physical contact with an aggressor mouse for 7 minutes. On day 11 (test day 1), behavioral tests were conducted to identify mice that were susceptible to stress. During the 10 days of CSDS, mice did not receive any aKG. From days 12 to 25, susceptible mice received daily intraperitoneal injections of either saline or aKG (300 mg/kg). On day 25 (test day 2), behavioral tests were conducted, and tissue was collected. **(B)** aKG rescues established defeat/depressed phenotype. Mice subjected to CSDS continued to exhibit social avoidance behavior because the SI ratio remained <1. Mice subjected to CSDS that received the aKG treatment had a significantly higher SI ratio than mice subjected to CSDS that received the saline treatment. **(C)** Intraperitoneal injections of aKG reversed the chronic social defeat phenotype as shown by the significant increase on test day 2 in the time spent in the interaction zone of the SI test. Statistical significance was measured by an unpaired t test. The numbers for defeat (test day 2) and defeat+aKG (test day 2) are 11 and 13, respectively. **(D)** Intraperitoneal injections of aKG reversed the chronic social defeat phenotype as shown by the significant decrease on test day 2 in the time spent in the no-interaction zone of the SI test. Statistical significance was measured by an unpaired t test. The numbers for defeat (test day 2) and defeat+aKG (test day 2) were 11 and 13, respectively. **(E)** aKG treatment did not affect the distance traveled by the animals. *$p < .05$, **$p < .01$. aKG, α-ketoglutarate; CSDS, chronic social defeat stress; M, male; ns, nonsignificant; SI, social interaction.

saline spent significantly less time interacting with the social stimulus than susceptible mice treated with aKG. No significant differences in the distance traveled were observed across groups (Figure 5E). Thus, we can rule out any effects caused by locomotor deficits due to aKG treatment. These results confirm that aKG is a potential antidepressant that reverses social avoidance behavior induced by CSDS in male mice.

DISCUSSION

Given the economic and health care burden that is created by depression, it is important to identify novel prophylactic and therapeutic strategies. Like genetic factors, environmental factors are involved in promoting susceptibility to depression. For this reason, lifestyle modifications such as regular exercise and a proper diet can help individuals to become resilient to stress. In this study, we showed that 1) aKG is an exercise factor that promotes learning and memory formation in male mice; 2) aKG acts as a protective factor against chronic stress; 3) aKG acts as antidepressant and thus can be developed as part of an exercise pill that can serve as a novel treatment for depression; and 4) aKG mediates antistress effects through differential restoration of normal BDNF levels in different brain regions. aKG emerges from our work as a pivotal factor in promoting brain health and mental well-being. Interestingly, recent work has uncovered its multifaceted roles, which can help explain its protective roles.

While we showed that aKG levels increase following exercise, the precise mechanisms by which aKG promotes memory recall remain unclear. Our data implicate the BDNF pathway in the protective effects of aKG within stress models, but it is uncertain whether other pathways are also involved in the typical memory formation process. Additionally, while our findings suggest that exercise may mediate its positive effects on learning and memory in part through aKG, additional experiments are necessary to confirm this pathway. Notably, data from the inhibitor JHU-083, which decreases aKG levels in different models, imply this connection; however, more direct experimental approaches are required to validate these findings comprehensively.

aKG emerges from our research as a critical factor in promoting brain health and mental well-being. Interestingly, recent studies have revealed its multifaceted roles. aKG's involvement as a Krebs cycle intermediate and a cofactor for crucial enzymatic reactions, including histone and DNA demethylation, underpins its potential anti-aging effects. Studies of model organisms such as flies and worms have demonstrated that aKG supplementation can extend the lifespan by modulating pathways like mTOR and AMPK (29,30). Dysregulation of aKG metabolism has also been linked to neurodegenerative diseases and stroke, highlighting its importance in neural protection and recovery (52). For example, dietary intake of aKG ameliorates α-synuclein pathology and rescues dopamine neuron degeneration in mouse models of Parkinson's disease (53). Moreover, aKG is neuroprotective in ischemia models, indicating its role in the response to glutamate excitotoxicity and mitochondrial dysfunction (54).

aKG functions as a cofactor for epigenetic enzymes. Changes in the intracellular aKG/succinate ratio regulate chromatin modifications, including H3K27me3 and ten-elevent translocation (Tet)-dependent DNA demethylation (55). The ability of aKG to influence the epigenetic status of cells may explain both its prophylactic and antidepressant effects because transcriptional dysregulation and aberrant epigenetic regulation are unifying themes in psychiatric disorders (56). This may also explain its ability to differentially regulate BDNF expression in the hippocampus and NAc. Further experiments are needed to identify whether aKG's role in regulating the enzymatic activity of chromatin-modifying enzymes is involved in its therapeutic effects.

aKG is also a cofactor for the FTO gene, which is an RNA demethylase (57). FTO levels are decreased in the hippocampus of patients with MDD and mouse models of depression, and overexpression of FTO rescues depression-like phenotype (58). FTO deregulation alters messenger RNA (mRNA) modifications that regulate transcript processing and translation that contribute to the pathophysiology of stress-related psychiatric disorders (59). Whether aKG-dependent regulation of mRNA modifications is necessary for its therapeutic effects remains to be determined.

Alternatively, aKG controls the posttranslational modification of hundreds of cellular proteins through succinylation in neurons (60). Interestingly, 624 succinylation sites in 494 proteins were identified in male mice that had received gut microbiota from fecal samples of patients with MDD compared with healthy control participants (61). Given the effects of aKG on protein succinylation, these observations may also link the antidepressant effects of aKG to its role in protein succinylation.

It is crucial to further dissect the underlying mechanisms by which aKG promotes resilience to chronic stress and reverses social avoidance phenotypes. In addition, more studies are needed to understand whether exercise mediates resilience to stress via aKG production. This can be achieved by blocking aKG production in exercising mice and testing whether exercise fails to mediate resilience to stress. If the effects of exercise are mediated through aKG, then aKG may be a pivotal component of an exercise pill together with lactate (22) and BHB (7) that can serve as both a prophylactic and an antidepressant treatment for depression.

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SFS conceived the study, analyzed the results, performed the experiments, and wrote the article. JSS helped conceive the study, analyzed the results, and wrote the article. FE performed the experiments and analyzed the data with the help of PEA, RK, DEM, YEZ, YS, JF, ZH, AM, and LMG.

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ARTICLE INFORMATION

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