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Branched-chain amino acids mediate resilience to chronic social defeat stress by activating BDNF/TRKB signaling

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ABSTRACT

How individuals respond to chronic stress varies. Susceptible individuals ultimately develop depression; whereas resilient individuals live normally. In this study, our objective was to examine the effect of branched-chain amino acids (BCAA), commonly used by athletes, on susceptibility to stress. Male C57BL/6 mice were subjected to daily defeat sessions by a CD1 aggressor, for 10 days. On day11, the behavior of mice was assessed using the social interaction test, elevated plus maze and open field. Mice received the BCAA leucine, isoleucine or valine before each defeat session. Furthermore, we examined whether BCAA regulate brain derived neurotrophic factor (BDNF) signaling by using a brain-permeable tropomyosin receptor kinase B (TRKB) inhibitor, ANA-12. We also tested the effect of voluntary exercise and high protein diets on susceptibility to stress. Mice exposed to chronic stress displayed increased susceptibility and social avoidance. BCAA promoted resilience to chronic stress, rescued social avoidance behaviors and increased hippocampal BDNF levels and TRKB activation. Inhibition of TRKB signaling abolished the ability of BCAA to promote resilience to stress and to rescue social avoidance. Interestingly, we found that BCAA activate the exercise-regulated PGC1a/FNDC5 pathway known to induce hippocampal BDNF signaling. Although both voluntary exercise and BCAA promoted resilience to stress, combining them did not yield synergistic effects confirming that they affect similar pathways. We also discovered that high protein diets mimic the effect of BCAA by rescuing social deficits induced by chronic stress and increase *Bdnf* expression in the hippocampus. Our data indicate that BCAA, exercise and high protein diets rescue susceptibility to stress by activating the hippocampal BDNF/TRKB signaling.

1. Introduction

Depression is one of the leading causes of disability worldwide (Smith, 2014). Although multiple antidepressants are available, less than half of depressed patients achieve complete remission (Block and Nemeroff, 2014). To develop novel therapies, recent work has focused on identifying cellular pathways that regulate susceptibility and resilience to stress. Some individuals appear to be more susceptible to stress, a major cause of depression. The gene expression networks that promote susceptibility to stress and depression have been analyzed (Bagot et al., 2016, 2017; Benatti et al., 2012; Krishnan et al., 2007). Interestingly, brain derived neurotrophic factor (*Bdnf*) expression is reduced in the hippocampi of rats that are susceptible to stress as compared to resilient rats (Duclot and Kabbaj, 2013). Indeed, chronic stress decreases hippocampal *Bdnf* expression (Jiang et al., 2014; Tsankova et al., 2006) and antidepressant treatment reverses this downregulation (Tsankova et al., 2006). The therapeutic effect of antidepressants is lost in the hippocampal *Bdnf* knockouts (Adachi et al., 2008; Bjorkholm and Monteggia, 2016; Monteggia et al., 2004, 2007) and chronic stress-induced memory deficits are reversed by regular exercise via induction of BDNF signaling (Kim and Leem, 2016). BDNF levels are also reduced in patients with depression and antidepressant treatment restores normal levels. A human BDNF polymorphism that changes valine (Val) to methionine (Met) at codon 66 (Val66Met), and

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that causes a 30% reduction in BDNF levels is associated with increased risk of depression and anxiety disorders (Notaras et al., 2015; Soliman et al., 2010; Yu et al., 2012).

In addition to altered gene expression profiles in the brain of depressed patients, fluctuations in the levels of a wide range of metabolites have been detected. For example, the levels of branched-chain amino acids (BCAA), leucine (Leu), isoleucine (Ile) and Val are decreased in patients suffering from psychiatric disorders such as major depressive disorder (Baranyi et al., 2016), immune-related major depression (Baranyi et al., 2018) and bipolar disorder (Fellendorf et al., 2018). BCAA were identified among twenty-four metabolites whose hippocampal levels were increased after antidepressant treatment and Leu was proposed to serve as a biomarker that links the responses to antidepressant treatment in the serum to the hippocampus (Webhofer et al., 2011). Even though, Leu exhibits antidepressant properties in an inflammation-induced depression model (Walker et al., 2018), the relevance of BCAA in the etiology of depression remains unclear.

In this work, we decided to determine whether BCAA protect against stress. We tested the hypothesis that BCAA promote resilience to stress in a chronic social defeat stress (CSDS) model, a validated model of depression (Berton et al., 2006; Krishnan et al., 2007). Our results suggest that BCAA promote resilience to stress and rescue social avoidance behavior by increasing hippocampal BDNF levels through the activation of the exercise-regulated PGC1a/FNDC5 pathway. Indeed, BCAA induce the activation of the BDNF receptor, tropomyosin receptor kinase B (TRKB), and inhibition of TRKB signaling abolishes the ability of BCAA to promote resilience to stress. Although both voluntary exercise and BCAA promote resilience to stress, combining them does not yield synergistic effects confirming that they affect similar pathways. We also show that a high protein diet (HPD) can mimic the effects of BCAA on social behavior potentially through activation of hippocampal *Bdnf* expression. Our results suggest that BCAA, HPD and exercise promote resilience to stress by activating hippocampal BDNF signaling.

2. Methods

2.1. Animal housing and BCAA injections

Adult male C57BL/6 mice received daily intraperitoneal injections of saline, Leu (32.5 mg/kg), Ile (32.5 mg/kg), Val (28.5 mg/kg), and ANA-12 (0.5 mg/kg). The doses of the BCAA were selected based on the observation that the used concentration of Leu activates the mammalian target of rapamycin (mTOR) and protein synthesis (Poncet et al., 2014). Animal care and use was in accordance with the guidelines and as approved by the ACUC.

Exercise paradigm: Male C57BL/6 mice were individually housed. They were divided into 2 groups: sedentary animals or exercising animals. The exercising animals were housed with free access to a running wheel.

Diets: Male C57BL/6 mice were divided into 2 groups: a group that received a standard diet (STD) and a group that received a high protein diet (HPD). The composition of the diet is included in Table 1. The diets were designed to be isocaloric.

2.2. RNA extraction and Real Time PCR

Total RNA was prepared from hippocampi using the Rneasy Plus Mini RNA extraction kit (Qiagen) and reverse transcription was performed using QuantiTect reverse transcription kit (Qiagen) according to the manufacturer's protocol. Real-time PCR was performed using a standard PCR protocol, with Sybr green dye (BioRad).

The primer sequences used are *Bdnfl:* (CAGGACAGCAAAGCCAC AAT and GCCTTCATGCAACCGAAGTA) and *Gapdh:* (CTCTCTGCTCCT CCCTGTTC and CCGACCTTCACCATTTTGTC).

Table 1
Composition of the STD and HPD.

Ingredients	Standard Diet	High Protein Diet
Casein (in g)	85	262
Corn Starch (in g)	374	134
Sucrose (in g)	60	99
Cellulose (in g)	30	31
Oil (in g)	24	31
Fat (in g)	–	–
Vitamin mix (in g)	6	12
Mineral mix (in g)	21	31
Total weight (in g)	600	600

2.3. Chronic social defeat stress (CSDS) model

We used the CSDS model in mice to mimic the symptoms of depression. The CSDS paradigm was performed as previously described (Golden et al., 2011). Cages were divided into two compartments separated by a perforated plexiglass. First, we screened and selected the aggressive CD-1 mice. Subsequently, experimental mice were subjected to ten days of CSDS sessions. During these ten days, experimental mice were introduced into the compartment of the aggressor mouse for 7 min. After this timed direct physical contact, mice were placed in the adjacent compartment allowing only sensory interaction with the aggressor. On the eleventh day, social behavioral testing was performed followed by animal sacrifice and brain tissue harvesting.

2.4. Social interaction test

The social interaction test was performed one day after the last defeat session as previously described (Kaidanovich-Beilin et al., 2011). Mice were habituated for 5 min in a cage containing three compartments with two compartments having a circular wire enclosure. After the habituation phase, a social stimulus C57BL/6J mouse (ten weeks) was confined to one of the circular enclosures and the experimental mouse was reintroduced to the cage in the central chamber. The movement of the experimental mouse was monitored using a camera for 10 min. The time spent in each compartment (central, social and non-social) was measured by the ANY-maze program. The total time spent by the mouse in the social compartment was divided by the total time spent in the non-social compartment and the mouse was considered susceptible if the ratio is < 1 and resilient if the ratio is > 1 (Henriques-Alves and Queiroz, 2015).

2.5. Elevated plus maze

The elevated plus maze (EPM) is a validated test for anxiety (File, 2001). Mice were allowed to navigate the maze for 5 min. The time spent in the closed arms was recorded with a camera and measured by the ANY-maze program.

2.6. Open field

Each mouse was allowed to freely explore the open field for 5 min. The average distance travelled was recorded with a camera and measured by the ANY-maze program.

2.7. Immunoblot analyses

To determine TRKB, pTRKB, PGC1a, FNDC5, GAPDH and ACTIN protein levels, total cellular proteins were extracted by lysing the hippocampi in RIPA-B (1% Triton X-100, 1% SDS, 50 mm Tris-Cl, pH 7.4, 500 mm NaCl and 1 mm EDTA) in the presence of the protease inhibitors cocktail (SIGMA) and MG132 (SIGMA), followed by Benzonase nuclease (SIGMA) digestion for 15 min. Antibodies against TrkB and

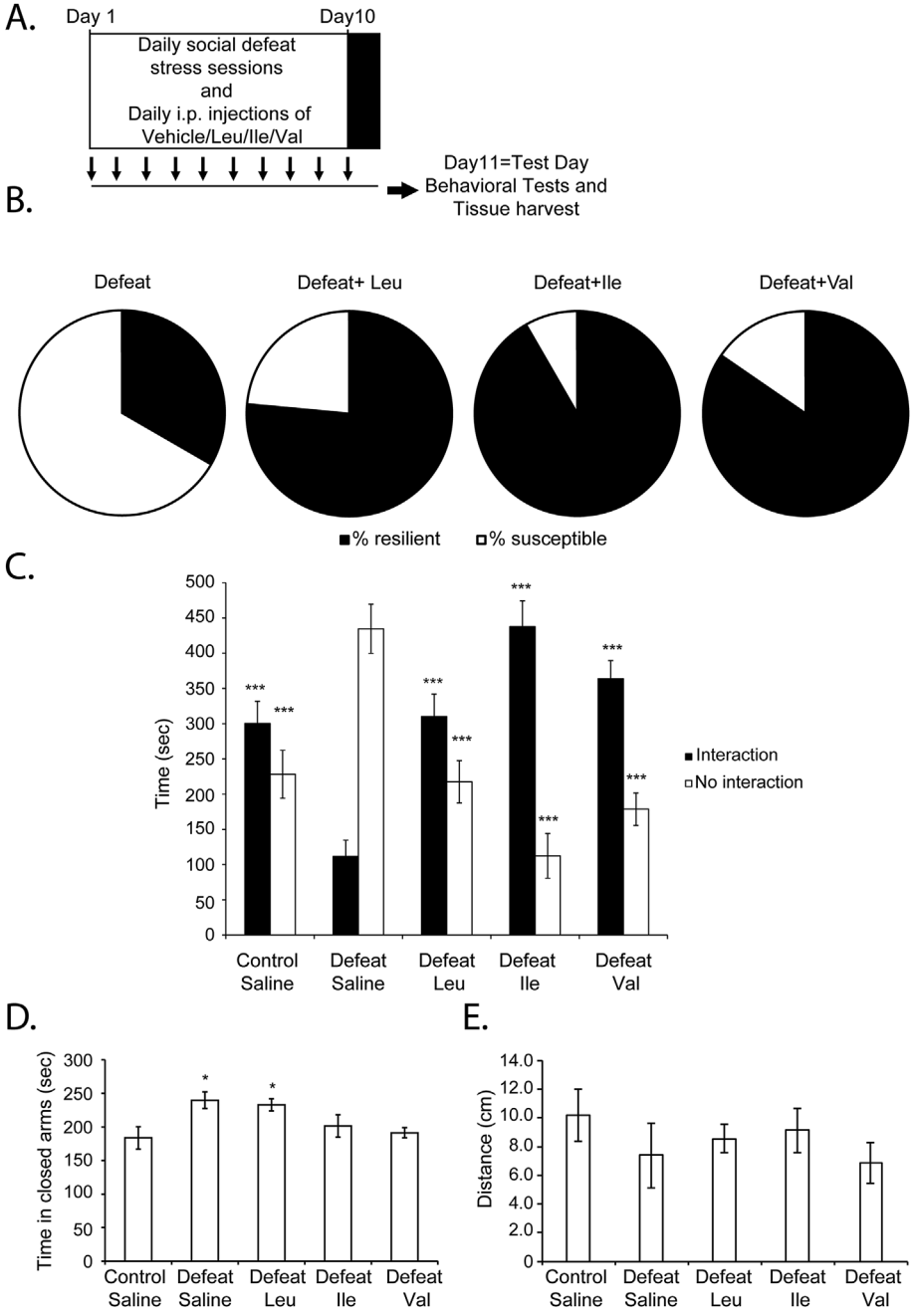

Fig. 1. BCAA mediate resilience to chronic social defeat stress and rescue social avoidance behavior. **(A)** The CSDS paradigm consists of ten days of daily defeat sessions. Each day, the experimental mouse is subjected to direct physical contact with an aggressor mouse for 7 min. On the eleventh day, behavioral tests and brain tissue collection are conducted. Mice receive daily intraperitoneal injections of either saline, Leu, Ile, or Val 15 min before each defeat session. **(B)** Leu, Ile and Val increase resilience to stress. In the group of mice (n = 24) receiving saline and subjected to CSDS, 33.3% are resilient to stress, whereas 66.7% are susceptible to stress. In the group of mice (n = 17) receiving Leu (32.5 mg/kg, daily for ten days) and subjected to CSDS, 76.5% are resilient to stress, whereas 23.5% are susceptible to stress. In the group of mice (n = 12) receiving Ile (32.5 mg/kg, daily for ten days) and subjected to CSDS, 91.7% are resilient to stress, whereas 8.3% are susceptible to stress. In the group of mice (n = 13) receiving Val (28.5 mg/kg, daily for ten days) and subjected to CSDS, 84.6% are resilient to stress, whereas 15.4% are susceptible to stress. **(C)** Intraperitoneal injections of Leu, Ile and Val reverse the social avoidance phenotype induced by CSDS as shown by the increase in the time spent in interaction zone of the social interaction test. Statistical significance was measured by 2way Anova followed by Bonferroni posttests. Significance was measured versus the defeat groups. ***p < 0.001. The n numbers for the control, defeat, defeat + Leu, defeat + Ile and defeat + Val are 16, 16, 17,12 and 13 respectively. **(D)** Intraperitoneal injections of Ile and Val, but not Leu decrease anxiety. defeat + saline and defeat + Leu animals exhibit anxiety-like behavior as measured by the significant increase in the time spent in the closed arm of the elevated plus maze (EPM), whereas defeat + Ile and defeat + Val behave similar to Control. Statistical significance was measured by one-way anova followed by Dunnett's multiple comparison test. Significance was measured versus the control groups *p < 0.05. The n numbers for control + saline, defeat + saline, defeat + Leu, defeat + Ile and defeat + Val are 16, 16, 17, 12 and 13 respectively. **(E)** There was no significant difference in the distance travelled in the open field between the different mice groups.

pTRKB were a kind gift from Dr. Moses V. Chao. These antibodies were used at 1:1000 and 1:500 dilutions respectively. Antibodies against PGC1a (ab54881), FNDC5 (ab1748333), GAPDH (ab8245, Abcam) and b-ACTIN (AC-74; Sigma Aldrich) were used at dilutions of 1:1000, 1:1000, 1:1000 and1:1000 respectively. Proteins were visualized using the ChemiDoc Imaging System (BioRad). Band quantification was analyzed with ImageJ software (Daaboul et al., 2017). The Western blot images shown in the different figures are representative images.

2.8. Statistical analysis

Unpaired *t*-test, 1way or 2way ANOVA followed by the Dunnett, Tukey or Bonferroni post tests were used to measure statistical significance. $p < 0.05$ was considered to be statistically significant. Data are represented as average ± standard error of the mean (SEM).

3. Results

3.1. BCAA promote resilience to chronic social defeat stress

To determine whether BCAA promote resilience to stress, we subjected C57BL/6J male mice (6–7weeks) to a CSDS paradigm (Berton et al., 2006; Krishnan et al., 2007). For ten days, mice received intraperitoneal injections of either saline, Leu, Ile or Val. We used a Leu concentration (32.5 mg/kg) that was previously reported to activate mTOR and protein synthesis (Poncet et al., 2014) and injected equivalent concentrations of Ile and Val. Fifteen minutes after each injection, mice were subjected to defeat sessions (Fig. 1A). Twenty-four hours after the final defeat session, mice underwent social interaction testing to screen for susceptibility versus resilience to stress. Mice exposed to CSDS and that exhibit social avoidance behaviors are classified as susceptible, whereas mice exposed to CSDS and that exhibit normal behavior are classified as resilient (Krishnan et al., 2007). We divided

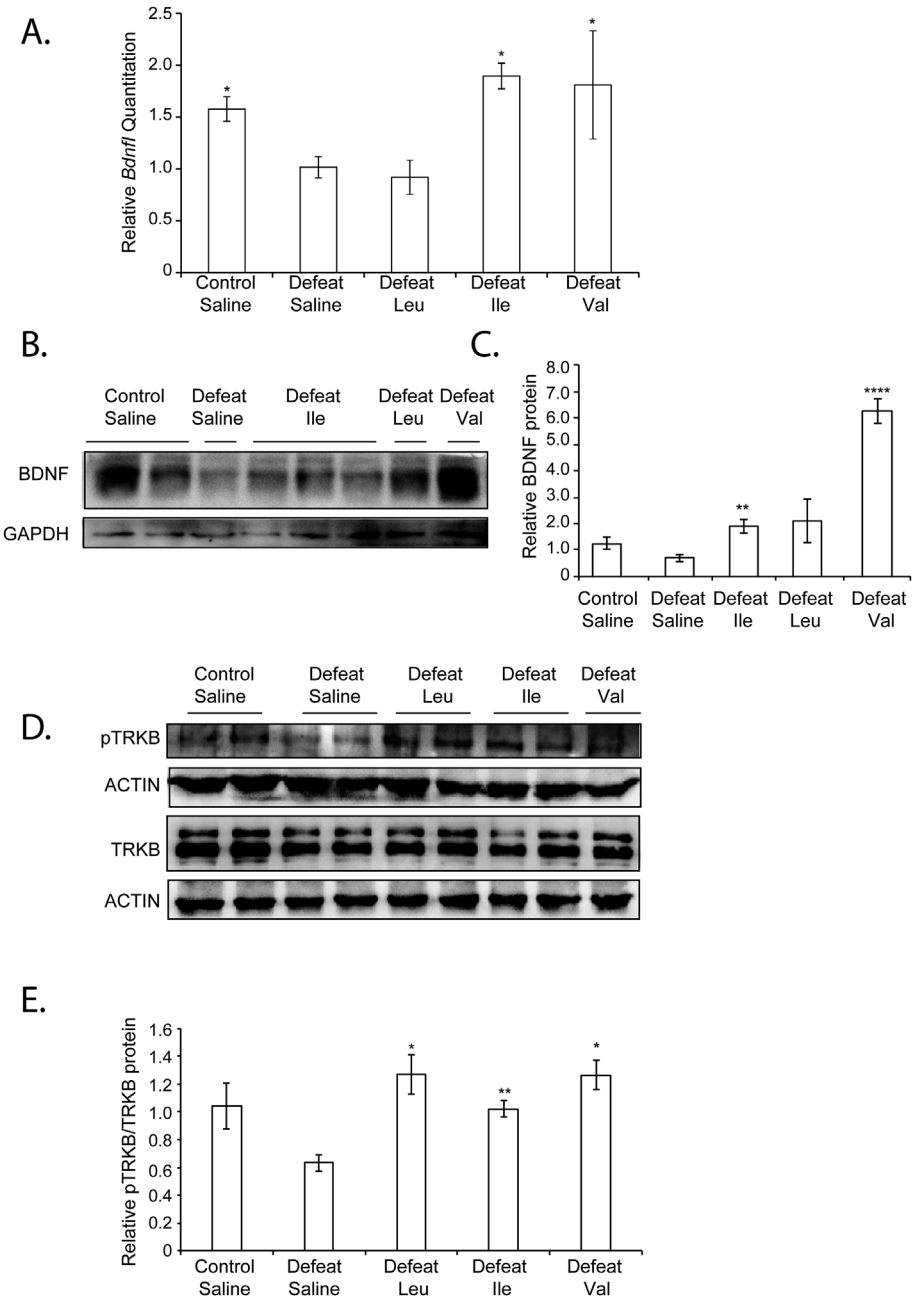

Fig. 2. BCAA restore control levels of BDNF protein and BDNF signaling in the hippocampus. **(A)** CSDS significantly decrease *BDNFI* expression in the hippocampus as measured by real-time RTPCR. Intraperitoneal injections of Ile and Val reverse this effect and restore control hippocampal *BDNFI* expression in animals subjected to CSDS. The number of animal used for control + saline, defeat = saline, defeat + Leu, defeat + Ile and defeat + Val are 13, 13 4, 3 and 3 respectively. Statistical significance was measured by one-way anova followed by Dunnett's multiple comparison test. *p < 0.05. Defeat vs control: p = 0.0228, defeat vs defeat + Leu: p = 0.9700, defeat vs defeat + Ile: p = 0.0264 and defeat vs defeat + Val: p = 0.0362. **(B)** Representative Western blot images depicting BDNF levels in hippocampus of control + saline, defeat + saline, defeat + Leu, defeat + Ile and defeat + Val animals. **(C)** Quantification of the BDNF western blots. Significance was measured versus defeat * p < 0.05. The n of hippocampi analyzed are 7,5,4,3, and 2 for control + saline, defeat + saline, defeat + Leu, defeat + Ile and defeat + Val groups respectively. Defeat vs control: p = 0.0940 and df = 10, defeat vs defeat + Leu: p = 0.1068 and df = 7, defeat vs defeat + Ile: p = 0.0082 and df = 6 and defeat vs defeat + Val: p < 0.0001 and df = 5. **(D)** Representative Western blot images depicting phosphorylated TRKB levels in hippocampus of control, defeat, defeat + Leu, defeat + Ile and defeat + Val animals. **(E)** Quantification of the phosphorylated TRKB western blots. Significance was measured versus defeat * p < 0.05. The n of hippocampi analyzed are 6,4,5,6, and 2 for control + saline, defeat + saline, defeat + Leu, defeat + Ile and defeat + Val groups respectively. Defeat vs control: p = 0.5853 and df = 9, defeat vs defeat + Leu: p = 0.0191 and df = 7, defeat vs defeat + Ile: p = 0.0022 and df = 8 and defeat vs defeat + Val: p = 0.0331 and df = 4.

the mice into susceptible or resilient by calculating the social interaction ratio. We found that all BCAA promote resilience to stress. The percentage of resilient mice within the defeat group increased from 33.3% (8/24) in the defeat group receiving saline to 76.5% (13/17) in the defeat group receiving Leu, 91.7% (11/12) in the defeat group receiving Ile and 84.6% (11/13) in the defeat group receiving Val (Fig. 1B).

3.2. BCAA enhance social interaction

In addition to promoting resilience to stress, BCAA also rescue social avoidance behavior characteristic of CSDS. Defeat mice receiving saline spent significantly less time interacting with the social stimulus as compared to the control mice (Fig. 1C). This social avoidance behavior was reversed by all three BCAA. The average time spent interacting with the social stimulus was significantly higher in the defeat mice receiving Leu, Ile and Val as compared to the defeat mice receiving saline (Fig. 1C). These results confirm that BCAA rescue the social behavior deficits induced by the CSDS paradigm.

3.3. BCAA partially decrease anxiety

We also assessed anxiety-like behaviors using the elevated plus maze (EPM). In the EPM, we measured the time spent exploring the closed arms of the maze to assess the anxiety levels of the different mice groups. A significant increase in the time spent exploring the closed arms of the maze is associated with anxious behavior. We found that mice subjected to CSDS (defeat + saline group) spent significantly more time in the closed arms of the maze as compared to the control mice (Fig. 1D). This effect was reversed only by Ile and Val, but not Leu administration. The defeat sessions and the different BCAA treatments did not affect locomotive behavior, as the average distance travelled by all mice groups in the open field was comparable (Fig. 1E). Our results demonstrate that BCAA promote resilience to stress and rescue social

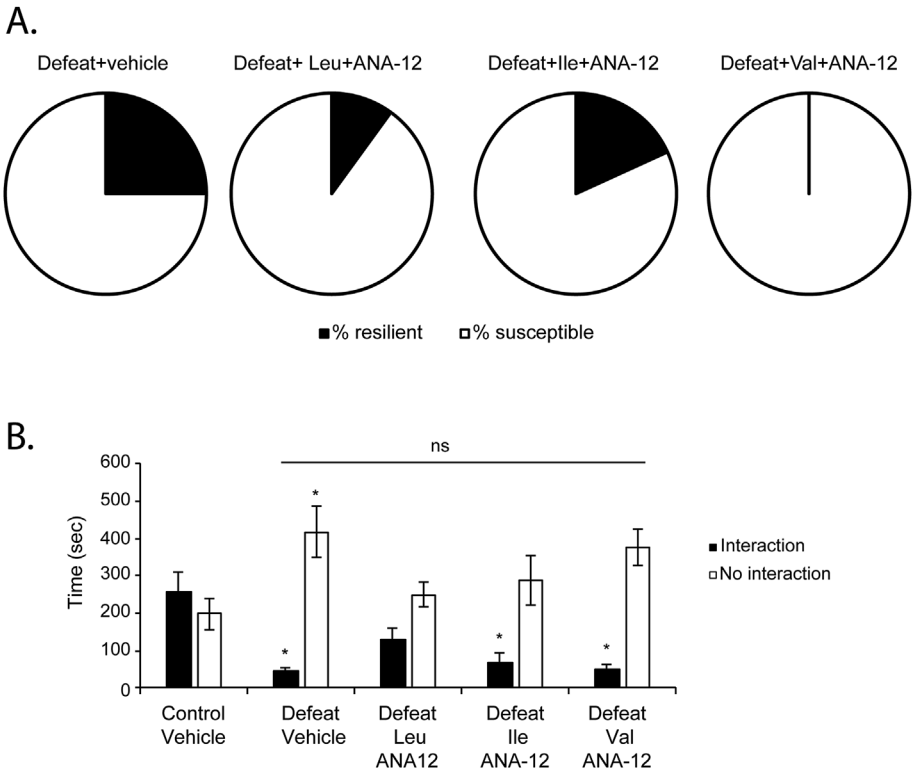

Fig. 3. BCAA mediate resilience to stress and rescue social avoidance behavior by activating BDNF/TRKB signaling. **(A)** The combined treatment of Leu, Ile and Val with the TRKB inhibitor ANA-12 failed to increase resilience to stress. In the group of mice receiving saline and subjected to CSDS, 25% are resilient to stress, whereas 75% are susceptible to stress. In the group of mice receiving Leu + ANA-12 and subjected to CSDS, 10% are resilient to stress, whereas 90% are susceptible to stress. In the group of mice receiving Ile + ANA-12 and subjected to CSDS, 18% are resilient to stress, whereas 82% are susceptible to stress. In the group of mice receiving Val + ANA-12 and subjected to CSDS, 0% are resilient to stress, whereas 100% are susceptible to stress. The n numbers for the control + vehicle, defeat + vehicle, defeat + Leu + ANA-12, defeat + Ile + ANA-12 and defeat + Val + ANA-32, 10, 11, 11. **(B)** Intraperitoneal injections of Leu, Ile and Val in combination with ANA-12 do not rescue the social avoidance phenotype associated with CSDS as shown by the lack of increase in the time spent in interaction zone of the social interaction test. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test. Significance was measured versus both control + vehicle or defeat + vehicle groups. *$p < 0.05$. The n numbers for the control + vehicle, defeat + vehicle, defeat + Leu + ANA-12, defeat + Ile + ANA-12 and defeat + Val + ANA-12 are 6, 8, 10, 11 and 11. Interaction: (defeat + vehicle vs control + vehicle: $p = 0.0268$, defeat + vehicle vs defeat + Leu: $p = 0.6559$, defeat + vehicle vs defeat + Ile: $p = 0.9930$ and defeat + vehicle vs defeat + Val: $p > 0.9999$) and No Interaction: (defeat + vehicle vs control + vehicle: $p = 0.0227$, defeat + vehicle vs defeat + Leu: $p = 0.0630$ defeat + vehicle vs defeat + Ile: $p = 0.2149$ and defeat + vehicle vs defeat + Val: $p = 0.9659$).

avoidance behavior. Their effects on anxiety were not as robust, but rather were divergent, suggesting as previously reported that the pathways regulating social avoidance behavior and anxiety maybe different (Karnib et al., 2019). In addition, our results are consistent with Ile and Val engaging pathways that protect against anxiety and Leu failing to activate these pathways. We next decided to understand how BCAA mediate resilience to stress.

3.4. BCAA increase hippocampal Bdnf expression and BDNF/TRKB signaling in mice exposed to CSDS

Because hippocampal *Bdnf* expression is necessary for the ability of antidepressants to promote resilience to CSDS and to rescue social avoidance behavior (reviewed in: Bjorkholm and Monteggia, 2016), we studied how BCAA affect *Bdnf* expression in the hippocampus. We measured *Bdnf exon 1 (BdnfI)* mRNA expression using RT Real Time PCR. The rodent *Bdnf* gene consists of eight non-coding exons and a single coding exon. Many transcripts are generated through alternative splicing of the different non-coding exons with the common coding exon (Pruunsild et al., 2011). We focused on *BdnfI* since it is both a neuronal activity-dependent (Tabuchi et al., 2002) and an exercise-dependent transcript (Sleiman et al., 2016; Tong et al., 2001). We found that defeat mice that received only Ile or Val, but not Leu had significantly increased hippocampal *BdnfI* mRNA when compared to defeat mice that received saline (Fig. 2A). We also assessed hippocampal BDNF protein levels using western blots. We found that defeat mice that received Ile and Val had significantly increased hippocampal BDNF protein levels as compared to defeat mice receiving saline (Fig. 2B and C). Even though we observed some increases in hippocampal BDNF protein levels in defeat mice receiving Leu, these increases were not statistically significant. To understand whether BCAA activate BDNF signaling, we analyzed the phosphorylation of the BDNF receptor TRKB using western blots. Interestingly, we found that all three BCAA significantly increased TRKB phosphorylation (Fig. 2D and E). Taken together, our results suggest that Leu, Ile and Val activate BDNF signaling in the hippocampi of defeat mice. In addition, our data suggest that both Ile and Val activate the BDNF/TRKB activity by inducing BDNF gene expression, whereas Leu activates the pathway at the level of the TRKB receptor potentially independent of significant effects on BDNF levels.

3.5. BCAA mediate resilience to stress and rescue social avoidance behavior by activating BDNF/TRKB signaling

In order to test whether BCAA promote resilience to stress by modulating hippocampal BDNF/TRKB signaling, we studied the effect of a combinatorial treatment of BCAA and a selective TRKB inhibitor, ANA-12, on social behavior in response to CSDS. ANA-12 is a potent and TRKB-selective ligand that prevents activation of this receptor by BDNF without altering TRKA and TRKC functions (Cazorla et al., 2011). ANA-12 crosses the blood-brain barrier and can be detected in the brain (Cazorla et al., 2011). In order to understand whether BCAA promote resilience to stress by modulating BDNF signaling, mice received daily injections of vehicle, Leu + ANA-12, Ile + ANA-12 or Val + ANA-12. After the injections, the mice were exposed to CSDS. We found that all of the combined treatments failed to promote resilience to stress (Fig. 3A). Taken together, these experiments suggest that BCAA mediate resilience to stress by activating BDNF/TRKB signaling. We also studied the social interaction behaviors of the mice receiving the combined treatment. The combined treatment of all BCAA with ANA-12 did not significantly rescue social avoidance phenotype (Fig. 3B). Indeed, no significant increase in the time spent interacting with the social stimulus was observed in mice exposed to CSDS and receiving either treatment as compared to defeat mice receiving vehicle (Fig. 3B). These results support the hypothesis that BCAA rescue social interaction deficits by restoring hippocampal BDNF signaling. We were next

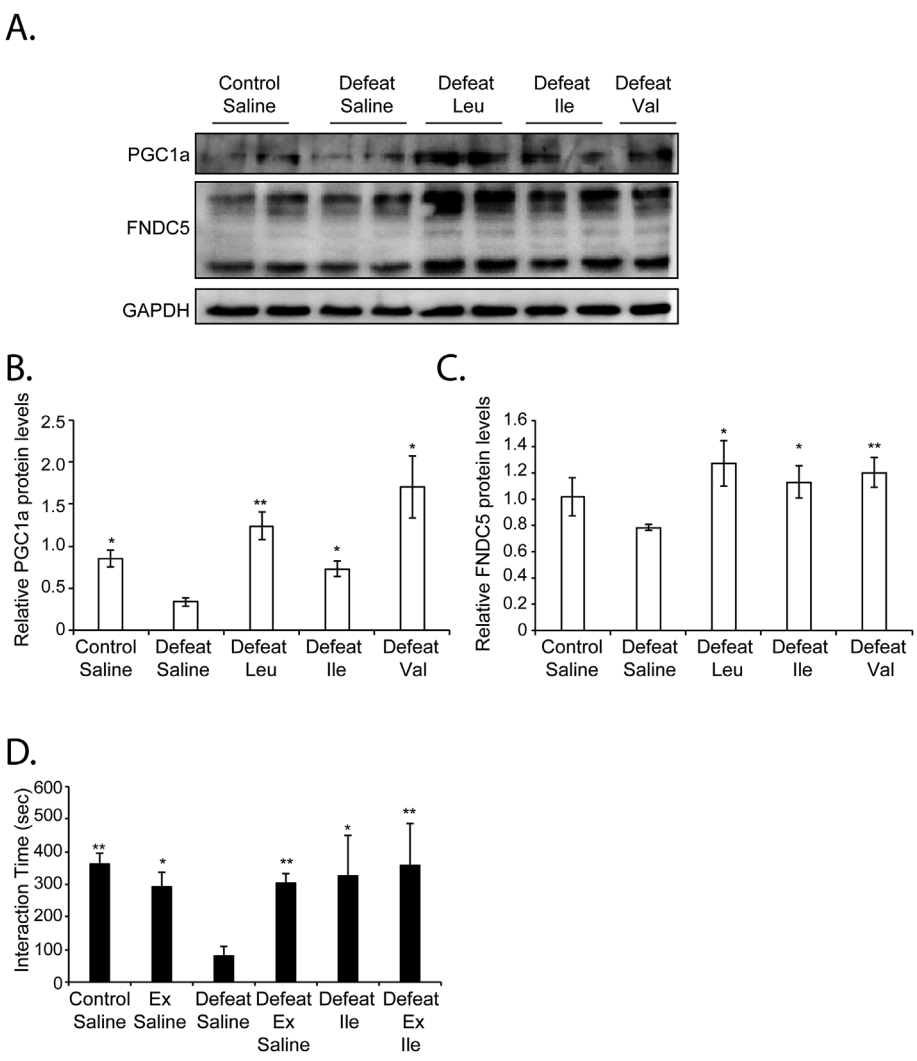

Fig. 4. BCAA activate the PGC1A/FNDC5 pathway known to activate BDNF signaling in the hippocampus (**A**) BCAA restore control hippocampal levels of PGC1a and FNDC5 in animals subjected to CSDS. Representative Western blot image depicting hippocampal PGC1a and FNDC5 in control, defeat, defeat + Leu, defeat + Ile and defeat + Val mice. **(B)** Quantification of the PGC1A western blots. Statistical significance was measured by the unpaired *t*-test. Significance was measured versus defeat * $p < 0.05$. The n of hippocampi analyzed are 3 for control + saline, defeat + saline, defeat + Leu, defeat + Ile and defeat + Val groups respectively. Defeat + saline vs control = saline: $p = 0.0102$ and $df = 4$, defeat + saline vs defeat + Leu: $p = 0.0063$ and $df = 4$, defeat + saline vs defeat + Ile: $p = 0.0175$ and $df = 4$ and defeat + saline vs defeat + Val: $p = 0.0213$ and $df = 4$ (**C**) Quantification of the FNDC5 western blots. Statistical significance was measured by the unpaired *t*-test. Significance was measured versus defeat * $p < 0.05$. The n of hippocampi analyzed are 4,4,3,3, and 3 for control + saline, defeat + saline, defeat + Leu, defeat + Ile and defeat + Val groups respectively. Defeat vs control: $p = 0.1572$ and $df = 6$, defeat vs defeat + Leu: $p = 0.0226$ and $df = 5$, defeat vs defeat + Ile: $p = 0.0226$ and $df = 5$ and defeat vs defeat + Val: $p = 0.0090$ and $df = 5$. **(D)** VWR and intraperitoneal injections of Ile alone or combined reverse the chronic social defeat phenotype as shown by the increase in the time spent in interaction zone of the social interaction test. Statistical significance was measured by 1way Anova followed by Dunnett's multiple comparison test. Significance was measured versus the defeat groups. *$p < 0.05$ and **$p < 0.01$. The n numbers for the control + saline, exercise + saline, defeat + saline, defeat + Exercise (Ex), defeat + Ile and defeat + Ex + Ile are 7, 6, 7, 8, 3 and 4 respectively. Defeat + saline vs control + saline: $p = 0.0013$, defeat + saline vs Ex + saline: $p = 0.0244$, defeat + saline vs defeat + Ex + saline: $p = 0.0090$, defeat + saline vs defeat + Ile: $p = 0.0375$ and defeat + saline vs defeat + Ex + Ile: $p = 0.0071$.

interested in understanding the molecular pathways activated by the BCAA to induce BDNF signaling.

3.6. BCAA activate the exercise-regulated PGC1a/FNDC5 pathway known to induce Bdnf expression

Like BCAA, exercise is a potent inducer of BDNF signaling in the hippocampus (El Hayek et al., 2019; Lourenco et al., 2019; Oliff et al., 1998; Sleiman and Chao, 2015; Sleiman et al., 2016; Wrann et al., 2013). Indeed, the exercise-activated pathway that leads to hippocampal BDNF induction has been deciphered (El Hayek et al., 2019; Wrann et al., 2013). Exercise increases the levels of the transcriptional coactivator PGC1a, which in turn activates the expression of *Fndc5*. FNDC5, is a protein that is processed and secreted, and that induces BDNF/TRKB activation through an unknown mechanism (El Hayek et al., 2019; Lourenco et al., 2019; Wrann et al., 2013). Since both exercise and diet are environmental factors that lead to BDNF signaling in the hippocampus, we tested whether BCAA also activate the PGC1a/FNDC5 pathway. Indeed, we found that the administration of BCAA increases the protein levels of PGC1a (Fig. 4A and B) and FNDC5 (Fig. 4A and C) in the hippocampus. Considering the already well-established causal relationship between the PGC1a/FNDC5 pathway and hippocampal BDNF signaling, our data is consistent with BCAA activating this pathway to promote resilience to stress and rescue social avoidance behavior. Interestingly, we found that even though both voluntary wheel running (VWR) and Ile can rescue social avoidance behavior as measured by the increase in the time spent interacting with a social stimulus, we do not observe synergistic nor additive effects when we combine VWR with Ile treatment (Fig. 4D). These results are consistent with the hypothesis that both exercise and BCAA mediate resilience to stress by engaging the BDNF/TRKB signaling pathway. Indeed, previous work has already established that exercise reverses chronic stress-induced memory deficits via BDNF induction (Kim and Leem, 2016).

3.7. High protein diet (HPD) promotes resilience to stress and rescues social deficits

We next decided to test whether like BCAA, a high protein diet (HPD) can also promote resilience to stress and rescue social avoidance behavior. Several studies have associated carbohydrate-enriched and high fat diets with social behavior, yet little is known about the effect of HPDs. For example, carbohydrate-enriched diets increase anxiety and depression after exposure to stress (Santos et al., 2018). High-fat diets, on the other hand, are initially anxiolytic, but prolonged use promotes anxiety and depression (Del Rio et al., 2016; Hassan et al., 2018; Xu et al., 2018). To test whether a HPD mediates resilience to CSDS, mice were either fed a standard diet (STD) or a HPD for two weeks then were

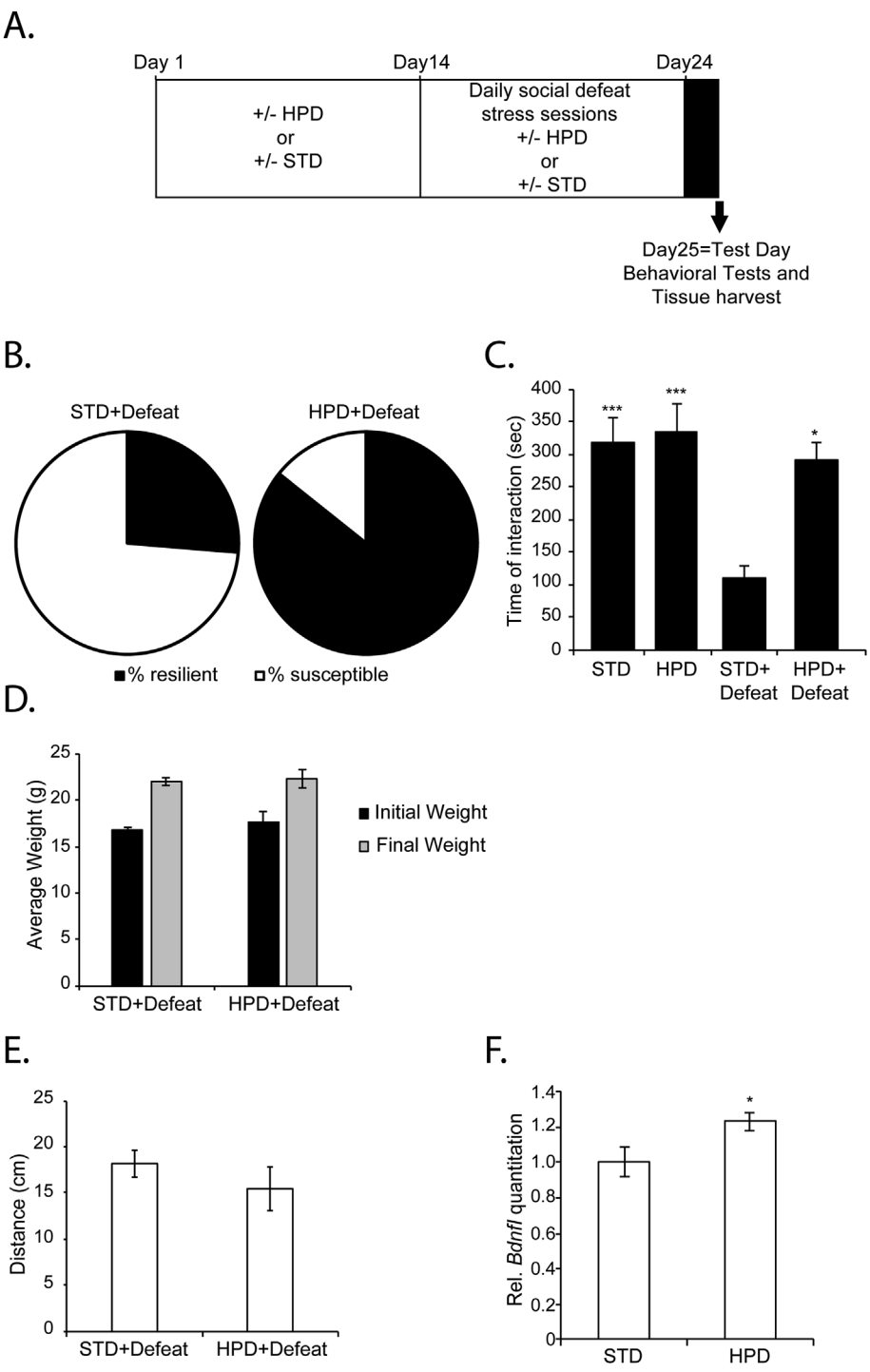

Fig. 5. HPD promotes resilience to CSDS, rescues social avoidance behaviors and induces hippocampal *Bdnf* expression. **(A)** Animals were fed either STD or HPD for two weeks before being subjected to CSDS. Animals continued to receive the different diets during the CSDS. On day 24, behavioral tests and brain tissue collection were conducted. **(B)** HPD increase resilience to stress. In the group of mice (n = 19) receiving a STD and subjected to CSDS, 26% are resilient to stress. In the group of mice (n = 7) receiving a HPD and subjected to CSDS, 85.7% are resilient to stress. **(C)** HPD reverses the social avoidance phenotype as shown by the increase in the time spent in interaction zone of the social interaction test. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test. Interaction: $F(1,43) = 4.886$, $p = 0.0324$, Row Factor $F(1,43) = 6.864$, $p = 0.0121$ and column factor $F(1,43) = 10.84$, $p = 0.02$. STD + defeat vs HPD + Defeat: $p = 0.0153$, STD + defeat vs STD: $p = 0.001$ and STD + defeat vs HPD: $p = 0.0002$. **(D)** No significant changes in the weight were observed between the mice receiving a STD or HPD at the end of the experiment. **(E)** There was no significant difference in the distance travelled in the open field between the different mice groups. **(F)** Mice receiving a HPD for 4 weeks had significantly increased *Bdnfl* expression levels as compared to mice receiving a STD. Statistical significance was measured by unpaired *t*-test groups. *$p < 0.05$. The n number for mice receiving STD and HPD is 10 and 12 respectively.

subjected to CSDS (Fig. 5A). 26% of the mice that received the STD and that were subjected to CSDS were resilient to stress, whereas 85.7% of the mice that received the HPD and that were subjected to CSDS were resilient to stress (Fig. 5B). Indeed, the mice that received the HPD and that were subjected to CSDS spent significantly more time interacting with a social stimulus as compared to mice that were subjected to CSDS and that received the STD (Fig. 5C). The effects of the HPD on resilience to stress and social behavior were independent of mice weight (Fig. 5D) or changes in locomotor behavior (Fig. 5E). Interestingly, mice that received the HPD had significantly higher hippocampal *Bdnfl* mRNA expression as compared to mice that received the STD (Fig. 5F). Taken together, our data are consistent with HPD, BCAA and exercise protecting against depression by inducing hippocampal BDNF/TrkB signaling (Fig. 6).

4. Discussion

Changes in the levels of BCAA have been detected in depressed patients. However, our knowledge of their specific roles in the development of depression is limited. Leu acts as an antidepressant in an inflammation-induced depression model (Walker et al., 2018). It produces its antidepressant effects by preventing the transport of the inflammation-induced neurotoxic metabolite kynurenine to the brain (Walker et al., 2018). In this study, we showed that all BCAA promote resilience to CSDS and prevent social avoidance behavior. These observations suggest that the decreased BCAA levels in depressed patients

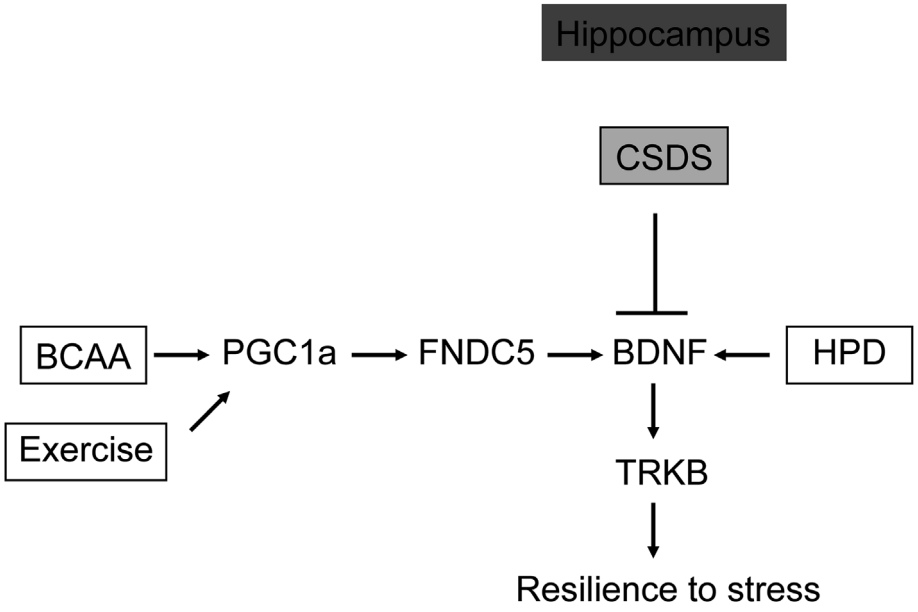

Fig. 6. BCAA mediate resilience to stress by inducing the hippocampal BNDF/TRKB signaling through the PGC1a/FNDC5 pathway.

are not circumstantial, but rather relevant to the prevention of the disease. Interestingly, the anxiolytic effects of BCAA were less robust and variable. Both Ile and Val prevented anxious behavior, whereas Leu was not anxiolytic suggesting that BCAA activate different pathways to regulate anxiety. Our results are consistent with previous studies suggesting that BCAA have important functions in the brain (Sperringer et al., 2017). Interestingly BCAA supplementation improves cognitive function in mice subjected to traumatic brain injury (Cole et al., 2010; Paterno et al., 2018) and extends life span (D'Antona et al., 2010; Mansfeld et al., 2015). One critical aspect in considering BCAA supplementation to enhance cognitive function and rescue depression is dosing. Elevated levels of BCAA are associated with a high risk of metabolic disorder such as insulin resistance as well as maple syrup urine disorder (Al-Haddad et al., 2016; Lynch and Adams, 2014).

We also discovered that BCAA promote resilience to stress by activating hippocampal BDNF/TRKB signaling. BDNF signaling regulates neuronal survival, synaptic plasticity, neurotransmitter release, long-term potentiation and memory formation. Disruption of BDNF signaling is associated with the pathophysiology of many psychiatric disorders such as depression and anxiety (Mitre et al., 2017). Specifically, decreases in BDNF levels and TRKB signaling in the hippocampus have been associated with depression and increased susceptibility to CSDS (Bjorkholm and Monteggia, 2016). Indeed, antidepressants can mediate their effects by restoring BDNF signaling in the hippocampus (Bjorkholm and Monteggia, 2016; Monteggia et al., 2004, 2007; Tsankova et al., 2006). Even though all three BCAA induced TRKB signaling, our data suggest that Ile and Val specifically affect *BdnfI* gene expression.

In addition to antidepressants, voluntary exercise as well as lactate, a metabolite produced during exercise, promote resilience to CSDS and anxiety disorders (Karnib et al., 2019; Mattar et al., 2017; Mul, 2018; Mul et al., 2018; Rizk et al., 2018) and increase BDNF signaling. Multiple reports have deciphered the exercise-regulated molecular pathways responsible for hippocampal BDNF activation and identified the PGC1a/FNDC5 as a major BDNF inducer (El Hayek et al., 2019; Sleiman et al., 2016; Wrann et al., 2013). PGC1a induces the expression of the transmembrane protein FNDC5 which is cleaved and secreted as irisin. Irisin is thought to activate BDNF signaling and this exercise-induced pathway is critical to rescue cognitive impairment associated with Alzheimer's disease mouse models (Lourenco et al., 2019; Wrann et al., 2013). In this work, we discovered that like exercise, BCAA can engage this hippocampal pathway known to induce BDNF activity to promote resilience to CSDS. Indeed, we found that combining exercise with Ile did not induce synergistic effects on resilience to stress suggesting that both paradigms mediate their effects by engaging similar pathways. These data are consistent with previous reports suggesting that exercise reverses chronic-stress induced cognitive functions by inducing hippocampal BDNF signaling (Kim and Leem, 2016).

Finally, we were also able to expand our results to show that HPD rich in BCAA also promote resilience to stress and rescue social avoidance. Associations between life-style choices including diets and psychiatric disorders have been established (Doumit et al., 2016, 2018; Mattar et al., 2019; Quirk et al., 2013; Sanchez-Ruiz et al., 2019; Zeeni et al., 2018). Previous work has implicated prolonged use of both carbohydrate-enriched diets and high fat diets in promoting depression and anxiety, but no information was available about how HPD affect social behavior (Del Rio et al., 2016; Eudave et al., 2018; Hassan et al., 2018; Santos et al., 2018; Xu et al., 2018). Our work suggests that short-term use of HPD and BCAA can be useful. In future, further investigation may be required to distinguish between effects of short-term use of BCAA and long-term use, a phenomenon that is prevalent with athletes, on resilience to stress and depression. Even though our results are consistent with BCAA mediating the positive effects of the HPD, we have evidence that suggests that these positive effects are not exclusively mediated by BCAA. Indeed, preliminary results suggest that the amino acid methionine (Met) promotes resilience to CSDS and rescues social avoidance behavior (SFS, unpublished results). This is of great interest considering that Met is the precursor of S-Adenosyl Methionine (SAM), which is a cofactor for DNA and histone methyltransferases. Alternatively, how Tryptophan (Trp) affects CSDS may also be relevant since Trp is a precursor of serotonin and since recent studies have revealed that histones can be serotonylated (Farrelly et al., 2019). Ultimately, the effects of Met and Trp on CSDS should be addressed because these amino acids affect epigenetic modifications which can lead to changes in brain gene expression patterns, a hallmark of depression (Nestler et al., 2016).

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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