

Skeletal Muscle PGC-1 α 1 Modulates Kynurenine Metabolism and Mediates Resilience to Stress-Induced Depression

Leandro Z. Agudelo,^{1,8} Teresa Femenía,^{1,2,8} Funda Orhan,³ Margareta Porsmyr-Palmertz,¹ Michel Goiny,³ Vicente Martinez-Redondo,¹ Jorge C. Correia,¹ Manizheh Izadi,¹ Maria Bhat,^{4,5} Ina Schuppe-Koistinen,^{4,5} Amanda T. Pettersson,¹ Duarte M.S. Ferreira,¹ Anna Krook,⁶ Romain Barres,⁶ Juleen R. Zierath,^{6,7} Sophie Erhardt,³ Maria Lindskog,^{2,*} and Jorge L. Ruas^{1,*}

¹Department of Physiology and Pharmacology, Molecular and Cellular Exercise Physiology, Karolinska Institutet, 17177 Stockholm, Sweden

²Department of Neuroscience, Karolinska Institutet, 17177 Stockholm, Sweden

³Department of Physiology and Pharmacology, Neuropsychimmunology, Karolinska Institutet, 17177 Stockholm, Sweden

⁴AstraZeneca R&D, Innovative Medicines, Personalized Healthcare and Biomarkers, Translational Science Center, Science for Life Laboratory, 17165 Solna, Sweden

⁵Department of Clinical Neuroscience, Karolinska Institutet, 17177 Stockholm, Sweden

⁶Department of Physiology and Pharmacology, Integrative Physiology, Karolinska Institutet, 17177 Stockholm, Sweden

⁷Department of Molecular Medicine and Surgery, Integrative Physiology, Karolinska Institutet, 17177 Stockholm, Sweden

⁸Co-first author

*Correspondence: mia.lindskog@ki.se (M.L.), jorge.ruas@ki.se (J.L.R.)

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SUMMARY

Depression is a debilitating condition with a profound impact on quality of life for millions of people worldwide. Physical exercise is used as a treatment strategy for many patients, but the mechanisms that underlie its beneficial effects remain unknown. Here, we describe a mechanism by which skeletal muscle PGC-1 α 1 induced by exercise training changes kynurenine metabolism and protects from stress-induced depression. Activation of the PGC-1 α 1-PPAR α / δ pathway increases skeletal muscle expression of kynurenine aminotransferases, thus enhancing the conversion of kynurenine into kynurenic acid, a metabolite unable to cross the blood-brain barrier. Reducing plasma kynurenine protects the brain from stress-induced changes associated with depression and renders skeletal muscle-specific PGC-1 α 1 transgenic mice resistant to depression induced by chronic mild stress or direct kynurenine administration. This study opens therapeutic avenues for the treatment of depression by targeting the PGC-1 α 1-PPAR axis in skeletal muscle, without the need to cross the blood-brain barrier.

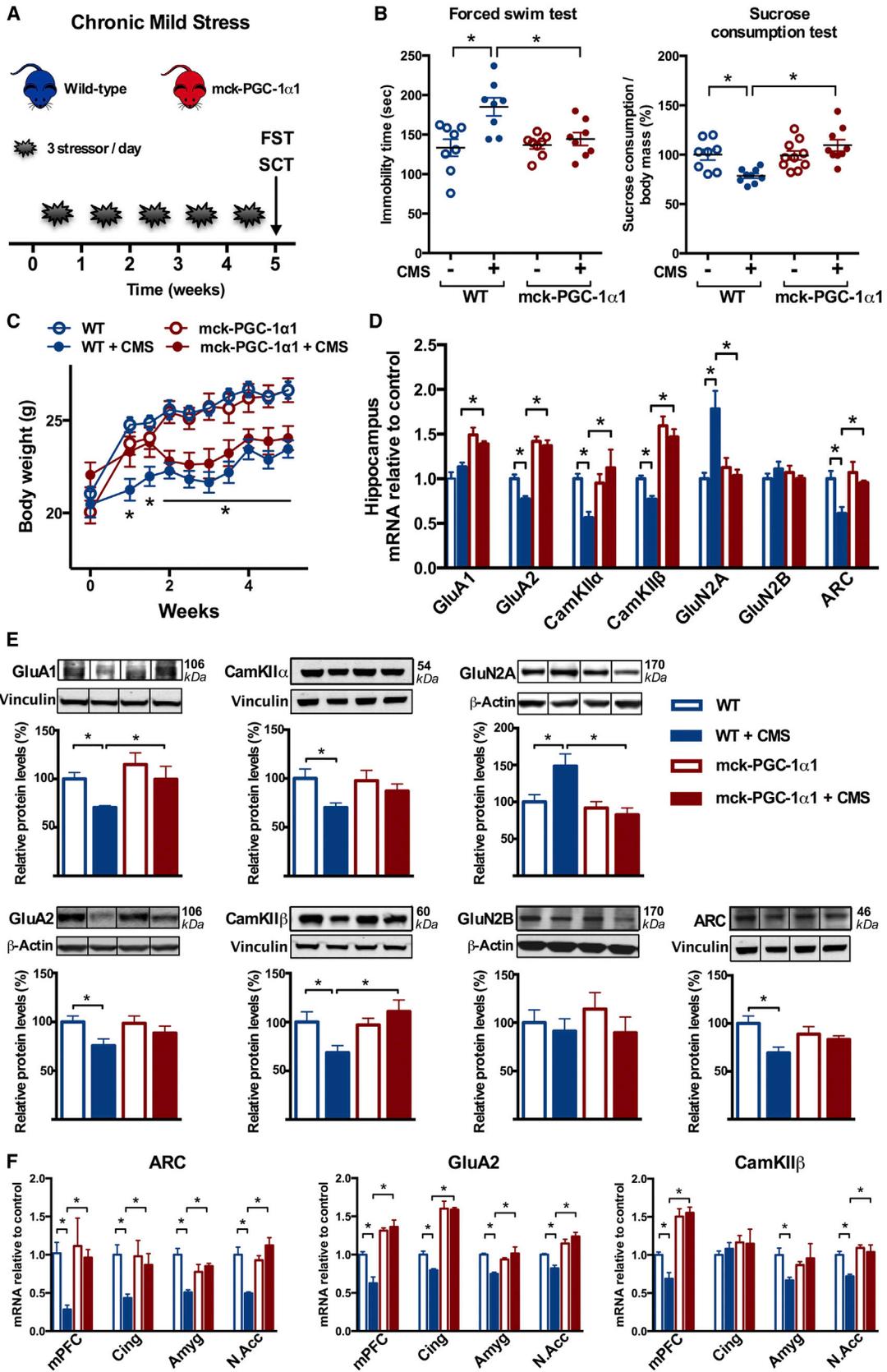
INTRODUCTION

The benefit of physical activity in depression is generally accepted (Lawlor and Hopker, 2001; Melanie et al., 2012), although the mechanisms that mediate these effects remain largely unknown. It is also unclear which components of the exercise program, such as skeletal muscle conditioning, cardio-

vascular effects, or even psychosocial influences, are therapeutic. PGC-1 α transcriptional coactivators are induced in skeletal muscle by exercise (Baar et al., 2002; Ruas et al., 2012; Short et al., 2003) and control many of the adaptations to physical activity. In particular, PGC-1 α 1 is activated in skeletal muscle by endurance-type activity and promotes mitochondrial biogenesis, fatty acid oxidation, angiogenesis, and resistance to muscle atrophy (Arany, 2008). Transgenic murine models with skeletal muscle-specific PGC-1 α 1 overexpression (mck-PGC-1 α 1 mice) show many of the adaptations to endurance-type training, without any exercise interventions (Lin et al., 2002). The mck-PGC-1 α 1 model has been extensively studied and has a lean, fatigue-resistant phenotype, with no differences at baseline in locomotor activity (Choi et al., 2008).

Depression is a heterogeneous disorder, and the exact neuronal mechanisms causing the disease are yet to be discovered. However, recent work suggests it is accompanied by an imbalance in glutamate transmission and/or decreased synaptic plasticity (Gómez-Galán et al., 2013; Pittenger, 2013; Sanacora et al., 2012).

The modulation of glutamate transmission and plasticity by stress-induced neuroinflammatory pathways may constitute a link between depression and chronic stress (Foy et al., 1987; Liu et al., 2013; Yuen et al., 2012). In particular, the kynurenine pathway of tryptophan degradation is activated by stress as well as directly by inflammatory factors (Gibney et al., 2013; Liu et al., 2013). This pathway accounts for 90% of peripheral tryptophan metabolism and occurs mainly in the liver, kidney, and immune cells (Müller and Schwarz, 2007). Levels of kynurenine (KYN) and its metabolites 3-hydroxykynurenine (3HK) and kynurenic acid (KYNA) in patients are strongly correlated to depression (Claes et al., 2011; Müller and Schwarz, 2007). These compounds have a plethora of effects that could contribute to depression, including modulating neuronal cell death, glutamate



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transmission, and neuroinflammation (Myint and Kim, 2014; Schwarcz et al., 2012). Approximately 60% of brain KYN comes from the periphery because, as opposed to KYNA, it can readily cross the blood-brain barrier (Fukui et al., 1991; Gál and Sherman, 1980). In the brain, KYN is metabolized to KYNA by astrocytes and to 3HK in microglia and macrophages (Schwarcz et al., 2012).

Here, we show that modulation of skeletal muscle condition through PGC-1 α 1 expression mediates resilience to stress-induced depressive behavior. Together with PPAR α/δ , PGC-1 α 1 increases the expression of several kynurenine aminotransferases (KATs) in skeletal muscle. Importantly, this shifts peripheral metabolism of stress-induced and exogenous KYN into KYNA, thereby protecting against stress-induced neurobiological mechanisms of depression.

RESULTS

Skeletal Muscle-Specific PGC-1 α 1 Transgenic Mice Are Resilient to Developing Chronic Mild Stress-Induced Depression

To isolate the effects of skeletal muscle conditioning on depression, we subjected the mck-PGC-1 α 1 mice to a chronic mild stress (CMS) protocol (Figure 1A). This protocol involves multiple, daily, mild stressors at unpredictable time (Table S1 available online) validated to induce depressive behavior (Willner, 2005). As expected, after 5 weeks of CMS, wild-type (WT) mice displayed depressive behavior as shown by increased immobility time in forced swim tests (FST) (a measure of despair with good predictive value for antidepressant effects) and decreased sucrose consumption (a measure of anhedonia; Figure 1B), when compared to nonstressed control animals. Strikingly, these CMS effects were completely absent in the mck-PGC-1 α 1 mice (Figure 1B). Genotype had no effect on locomotor activity or baseline sucrose preference (Figures S1A–S1C). Mck-PGC-1 α 1 mice were not protected from all the effects of CMS and showed decreases in body weight gain similar to WT after the second week (Figure 1C).

To exclude any nonskeletal muscle transgene expression in mck-PGC-1 α 1 mice, we determined exogenous and endogenous PGC-1 α 1 levels in several tissues (Figure S1D). We detected PGC-1 α 1 transgenic expression in skeletal muscle, a small increase in the heart, but not in any of the other analyzed tissues (Figure S1E).

Imbalances in glutamate transmission and decreased synaptic plasticity have been suggested as possible mechanisms of depression (Duman and Aghajanian, 2012; Sanacora et al.,

2012). Accordingly, our analysis of synaptic proteins in WT mice after CMS revealed reduced hippocampal expression levels of proteins mediating synaptic plasticity: CamKII α and CamKII β and ARC, as well as the AMPA receptor subunits GluA1 and 2. Expression of the NMDA receptor subunit GluN2B remained unchanged whereas GluN2A protein levels increased after stress (Figures 1D–1F). In agreement with the behavioral test results, none of these stress-induced changes occurred in mck-PGC-1 α 1 mice (Figures 1D–1F). Similar gene expression profiles were observed in other brain regions (Figures 1F and S1F) known to be involved in depressive disorders (Femenía et al., 2012). Neurotrophic factors also play a role in depression (Anisman and Hayley, 2012; Duman and Monteggia, 2006). After exposure to CMS, only WT mice showed decreased hippocampal transcript levels for BDNF, GDNF, and VEGFA and B, whereas no changes were observed in mck-PGC-1 α 1 mice (Figure 2A). Nerve-growth factor (NGF) levels did not change in any experimental group (Figure 2A).

Reduced astrocytic regulation of synaptic function has been proposed to contribute to stress-induced depression (Gómez-Galán et al., 2013; Rajkowska and Miguel-Hidalgo, 2007). Consistent with earlier observations (Banasr et al., 2010), CMS reduced the amount of glial fibrillary acidic protein (GFAP) and excitatory amino acid transporter 1 (EAAT1) protein, but not EAAT2 protein abundance in WT mice (Figures 2B and 2C). Baseline GFAP transcript levels were higher in hippocampi of mck-PGC-1 α 1 mice than in controls, but remained unaffected by CMS (Figure 2B). Further supporting its effect on glutamatergic transmission, CMS decreased the mRNA levels of glutamine synthetase (GLNS) in the hippocampus of WT mice, whereas we observed the opposite effect in mck-PGC-1 α 1 mice after CMS (Figure 2D). When assessing gene expression of astrocytic proteins in other brain regions, a more complex picture emerged (Figure S2A), suggesting that astrocyte response is a fine-tuned result of many signaling pathways.

We next analyzed the expression of structural synaptic genes known to be decreased in patients with depression (Kang et al., 2012; Tochigi et al., 2008). After CMS, we observed a reduction in the hippocampus and mPFC expression levels of calmodulin-2, synapsin-3, and Rab3A, of WT mice (Rab4B and β -tubulin were also decreased in mPFC), whereas these levels were unchanged in mck-PGC-1 α 1 mice (Figures S2B and S2C). Collectively, these results indicate that PGC-1 α 1 expression in skeletal muscle protects synaptic transmission and plasticity from stress-induced alterations, rendering mck-PGC-1 α 1 animals resistant to developing depressive behavior.

Figure 1. Skeletal Muscle-Specific PGC-1 α 1 Transgenic Mice Are Resilient to Chronic Mild Stress-Induced Depressive Behavior

(A) A chronic mild stress (CMS) protocol was used to induce depressive behavior in wild-type (blue) and muscle-specific PGC-1 α 1 transgenic mice (mck-PGC-1 α 1, red).

(B) Immobility time in the forced swim test (left) and sucrose consumption normalized to body weight (right) shown for individual animals (n = 8–10).

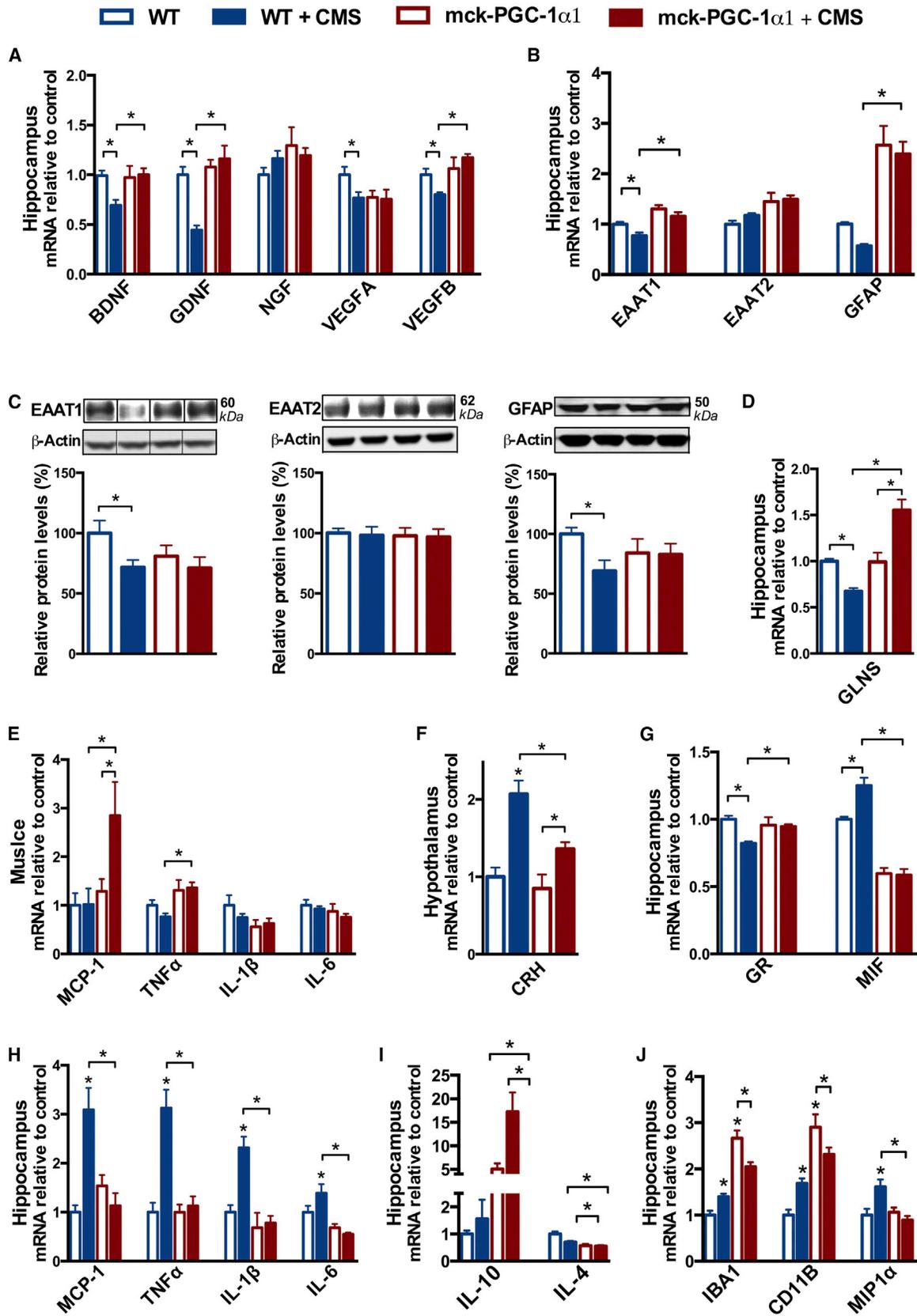
(C) Body weight throughout the CMS treatment (n = 8–10).

(D) Analysis of gene expression in hippocampus by quantitative real-time PCR (qRT-PCR) (n = 4–6).

(E) Representative immunoblots and quantification of hippocampal protein levels normalized to loading controls. Proteins were loaded randomly by an experimenter blinded to experimental conditions. Representative images come from the same gel and lines indicate they originate from different lanes (n = 5–8).

(F) Analysis of gene expression in medial prefrontal cortex (mPFC), cingulate cortex (Cing), amygdala (Amyg), and nucleus accumbens (N. Acc) by qRT-PCR (n = 4–6). Scale bars depict mean values expressed as percentage or fold change of wild-type nonstressed and error bars represent SEM, *p < 0.05.

See also Figure S1 and Tables S1, S2, and S3.



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Elevated Skeletal Muscle PGC-1 α 1 Expression Impacts Central Inflammatory Responses to Chronic Mild Stress

Proinflammatory processes and their effects on astrocyte function (McNally et al., 2008) are important promoting factors in the pathogenesis of stress-induced depressive disorders (Anisman and Hayley, 2012; Gibb et al., 2011). Despite the anti-inflammatory properties of PGC-1 α 1 in muscle (Eisele et al., 2013), chronic exposure of mck-PGC-1 α 1 mice to unpredictable stress increased skeletal muscle expression of the proinflammatory cytokines MCP-1 and TNF α (Figure 2E). This further indicates that mck-PGC-1 α 1 mice do respond to stress, despite their resilience to developing depressive behavior. Consistent with this, we observed that CMS increased corticotrophin-releasing hormone (CRH) expression (Deussing and Wurst, 2005; Lloyd and Nemeroff, 2011) in hypothalamus of mck-PGC-1 α 1 transgenic mice, although to a lesser extent than in WT mice (Figure 2F). WT mice (but not mck-PGC-1 α 1) responded to CMS with decreased hippocampus glucocorticoid receptor expression (Froger et al., 2004), increased macrophage migration inhibitory factor levels (Figure 2G), and increased levels of stress-induced inflammatory markers (Figures 2H and S2C). Expression of the anti-inflammatory cytokine IL-10 was increased upon CMS in hippocampus of mck-PGC-1 α 1 mice, whereas IL-4 expression was reduced in all groups compared to WT controls (Figures 2I and S2D). IL-10 and IL-4 levels were unchanged in skeletal muscle (Figure S2E). Lastly, expression of macrophage/microglia activity markers ionized calcium-binding adaptor molecule (IBA1), cyclin-dependent kinase 11b (CD11b), and macrophage inflammatory protein 1 α (MIP1 α) was increased in hippocampus of WT mice after CMS but reduced (or unchanged) in mck-PGC-1 α 1 mice under the same conditions (Figure 2J). These results show that although mck-PGC-1 α 1 mice are sensitive to stress, they are protected from brain neuroinflammation.

PGC-1 α 1 Induces Expression of Kynurenine Aminotransferases in Skeletal Muscle

To identify pathways that mediate the effects of skeletal muscle PGC-1 α 1 on the central nervous system we analyzed gene expression array data from *in vivo* and *in vitro* muscle PGC-1 α 1 overexpression (Ruas et al., 2012). This bioinformatic analysis uncovered a putative role for PGC-1 α 1 in the control of the kynurenine pathway of tryptophan degradation in skeletal muscle (kynurenine pathway; Figures 3A, S3A, and S3B). Conversion of tryptophan to KYN is emerging as a main mediator

of stress-induced depression (Dantzer et al., 2008; Müller and Schwarz, 2007). By examining the skeletal muscle expression levels of rate limiting enzymes in the kynurenine pathway, we found that exposure to CMS increased the expression of indoleamine 2, 3-dioxygenase 1 and 2 (IDO1 and IDO2), tryptophan 2, 3-dioxygenase 1 and 2 (TDO1 and TDO2), and kynurenine 3-monooxygenase (KMO) only in WT mice (Figures 3B and 3C). Liver IDO1 expression was increased by CMS in WT mice, to levels higher than those observed in skeletal muscle (Figure S3C). IDO and TDO levels were unchanged in mck-PGC-1 α 1 skeletal muscle, which instead showed high expression of kynurenine aminotransferases (KAT) 1, 3, and 4, further elevated by CMS (Figures 3D and 3E). These enzymes catalyze the conversion of KYN to KYNA (Han et al., 2010), an end metabolite of this pathway that contrary to KYN cannot cross the blood-brain barrier (BBB) (Fukui et al., 1991). KAT 2 could not be detected in murine skeletal muscle (Figure 3D) and no changes in liver KAT levels were observed (Figure S3C). Although PGC-1 α 1 levels are slightly elevated in the heart of mck-PGC-1 α 1 mice, this did not affect cardiac KAT expression (Figure S3D).

Skeletal Muscle PGC-1 α 1 Enhances Peripheral Metabolism of Kynurenine to Kynurenic Acid during Chronic Mild Stress

Our results suggest that the mck-PGC-1 α 1 mice are better equipped to metabolize circulating KYN produced in response to CMS. Indeed, exposure to CMS elevated plasma KYN levels in WT, but not in mck-PGC-1 α 1 mice (Figure 3F). Conversely, only mck-PGC-1 α 1 mice exposed to CMS showed significantly higher KYNA plasma levels (Figure 3F). Given that KYN crosses the BBB and is rapidly converted to 3HK or KYNA (Fukui et al., 1991; Schwarcz et al., 2012), we measured the levels of these metabolites in the brain. 3HK levels were robustly increased in brains of WT mice after CMS, while unaltered in mck-PGC-1 α 1 mice (Figure 3G). We observed no differences in brain KYNA levels in any group (Figure 3G). Highlighting the importance of shifting peripheral KYN to KYNA balance, we found a significant correlation between plasma KYN and brain 3HK levels but not with KYNA (Figure 3H). CMS responses increased the expression of enzymes of the kynurenine pathway in brains of WT mice, whereas mck-PGC-1 α 1 mice were exempt from these changes (Figure S3E). An increased breakdown of kynurenine could lead to tryptophan depletion and result in decreased serotonin levels, a proposed mechanism of depression. However, we did not observe any reduction in tryptophan, serotonin, or its

Figure 2. Skeletal Muscle PGC-1 α 1 Expression Impacts Central Inflammatory Responses to CMS

(A and B) qRT-PCR gene expression analysis of (A) neurotrophic factors and (B) astrocytic proteins from hippocampus (n = 4–6).
 (C) Representative immunoblots and quantification of hippocampal protein levels normalized to loading controls. Proteins were loaded randomly by an experimenter blinded to experimental conditions. Representative images come from the same gel and lines indicate they originate from different lanes (n = 4–8).
 (D) Glutamine synthetase (GLNS) mRNA levels in hippocampus (n = 4–6) determined by qRT-PCR.
 (E) qRT-PCR analysis of gene expression for inflammatory cytokines in skeletal muscle (n = 4–6).
 (F) Corticotrophin-releasing hormone (CRH) gene expression levels in hypothalamus (n = 4–6).
 (G) Gene expression levels of glucocorticoid receptor (GR) and macrophage migration inhibitory factor (MIF) in hippocampus (n = 4–6).
 (H and I) qRT-PCR analysis of gene expression of (H) inflammatory and (I) anti-inflammatory cytokines from hippocampus (n = 4–6).
 (J) Analysis of gene expression by qRT-PCR of macrophage/microglial markers from hippocampus (n = 4–6). Scale bars represent mean values and error bars represent SEM. ns, nonsignificant. *p < 0.05.
 See also Figure S2 and Tables S2 and S3.

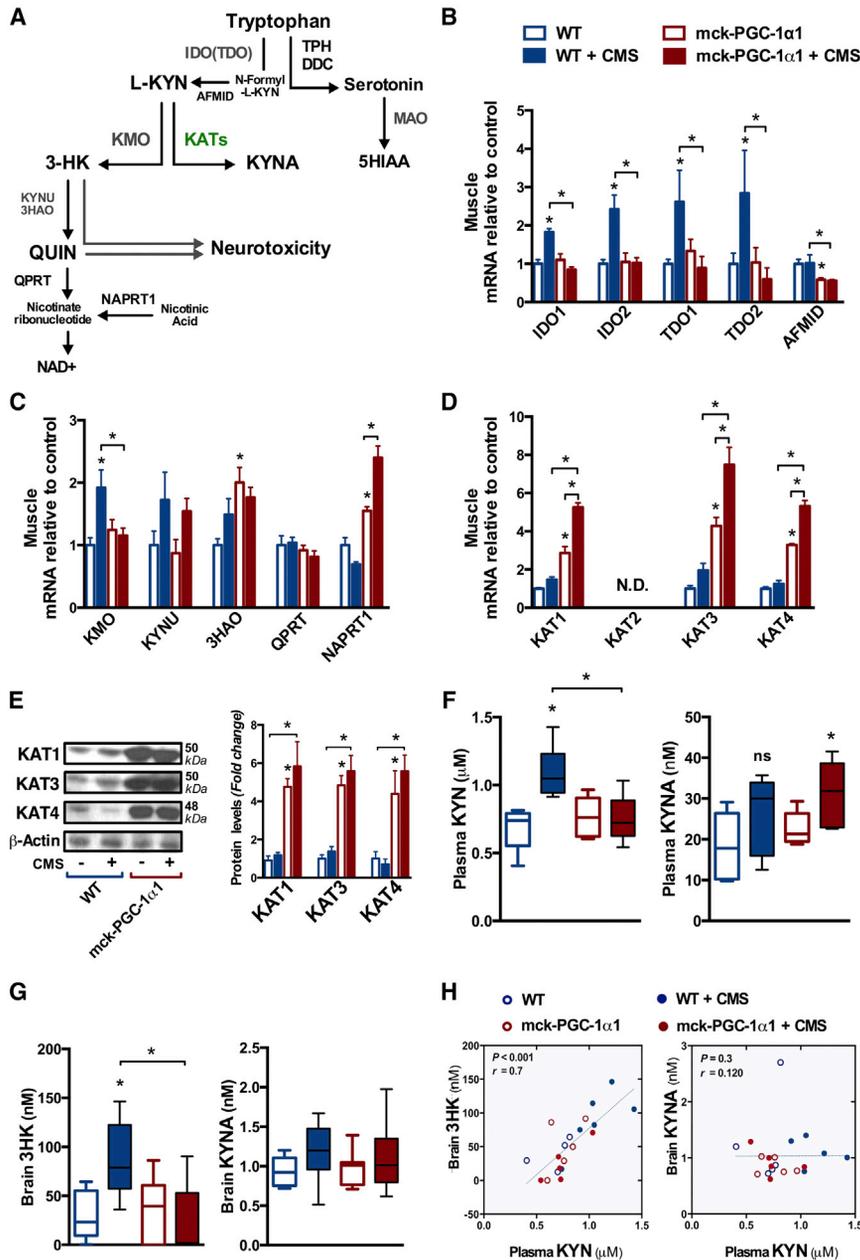


Figure 3. Mck-PGC-1α1 Transgenic Mice Have Enhanced Peripheral Kynurenine Catabolism after Stress

(A) Representation of the kynurenine pathway. (B–D) Analysis of gene expression by qRT-PCR using primers specific to the indicated genes ($n = 4–6$). (E) KAT1, KAT3, and KAT4 protein levels in the gastrocnemius muscle ($n = 4$). Representative immunoblots (left) and average protein levels relative to controls (right) from gastrocnemius ($n = 4$). (F) Box plots of plasma concentrations of kynurenine (KYN) and kynurenic acid (KYNA) ($n = 5–7$). (G) Box plots of 3-hydroxykynurenine (3HK) and KYNA concentration in brain tissue ($n = 6–10$). (H) Correlation between plasma KYN levels and brain 3HK and KYNA levels with each circle representing an individual animal ($n = 20$). Scale bars represent mean values and error bars represent SEM. N.D., not detectable. * $p < 0.05$. See also Figure S3 and Tables S2 and S3.

metabolite 5-hydroxyindoleacetic acid (5HIAA) levels under any conditions (Figure S3F).

Peripheral Kynurenine Administration Induces Depressive Behavior in Wild-Type, but Not mck-PGC-1α1, Mice

Our data suggest that stress-induced increases in plasma KYN levels have central effects associated with depression. Indeed, KYN plasma levels directly correlated with hippocampal gene expression of proinflammatory markers MCP-1, TNFα, and IL-1β (Figure S4A). We also found that plasma KYN levels inversely correlate with the synaptic changes shown in Figure 1 (Figure 4A). If mck-PGC-1α1 mice are protected from these pathological ef-

fects by converting KYN to KYNA in skeletal muscle, this mechanism should also protect from exogenous KYN administration. We determined that a single intraperitoneal 2 mg/kg L-KYN dose results in circulating levels similar to those observed in WT animals exposed to CMS (i.e., 1 μM, see Figure 4F). L-KYN injection resulted in a striking reduction in sucrose consumption in WT mice but not in mck-PGC-1α1 mice (Figure 4B). Moreover, KYN injection in WT mice mimicked the CMS-induced brain pattern of gene expression including proinflammatory (Figure 4C), kynurenine pathway (Figure 4D), and synaptic genes (Figure 4E). This transcriptional signature and the concomitant elevation of plasma KYN levels (Figure 4F) were not observed in mck-PGC-1α1 animals (Figures 4C and 4F). Consistent with our hypothesis, circulating KYNA levels were increased in mck-PGC-1α1 mice after KYN administration (Figure 4F).

Skeletal Muscle-Specific PGC-1α Deletion Sensitizes to KYN-Induced Depressive Behavior

To determine if PGC-1α is not only sufficient, but also required for the conversion of KYN to KYNA in skeletal muscle, we next analyzed mice with skeletal muscle-specific genetic deletion of PGC-1α (hereafter MKO-PGC-1α) (Chinsomboon et al., 2009). Loss of PGC-1α expression in skeletal muscle resulted in decreased KAT expression levels (Figures 5A and S5A). When evaluated in sucrose consumption tests, MKO-PGC-1α mice displayed anhedonic behavior even under control conditions

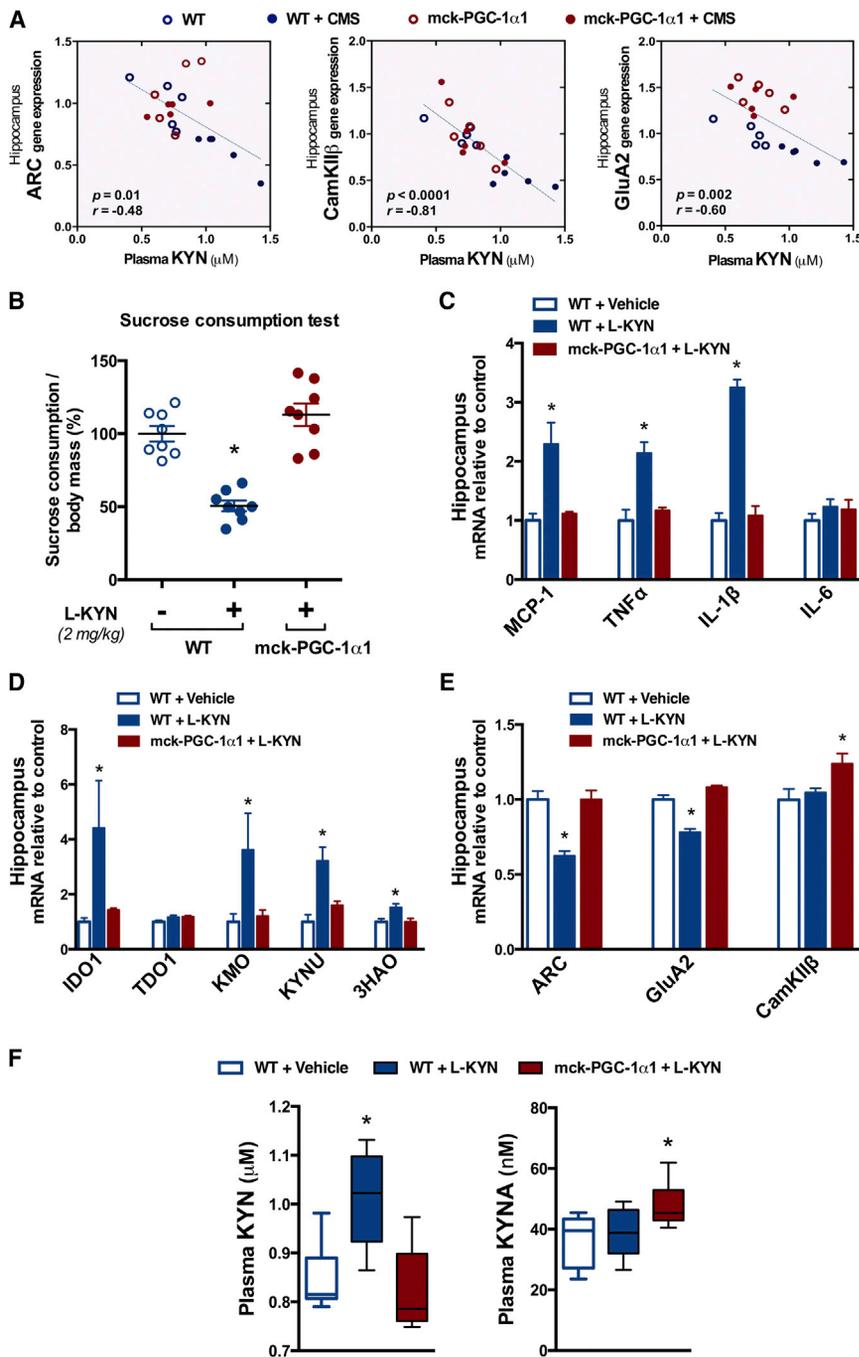


Figure 4. Skeletal Muscle PGC-1 α 1 Protects from Developing Depressive Behavior Induced by Direct Kynurenine Administration

(A) Correlation between plasma KYN-levels and hippocampal gene expression of indicated genes (same mice as in Figures 1, 2, and 3) with each circle representing an individual mice ($n = 20$).

(B) Amount of sucrose consumed normalized to body weight after KYN treatment versus vehicle-treated wild-type, unless otherwise indicated ($n = 8$).

(C–E) mRNA levels of indicated genes in hippocampus from the same animals as in (B).

(F) Plasma concentrations of KYN and KYNA shown as box plots ($n = 5–7$). Scale bars represent mean values and error bars represent SEM. * $p < 0.05$.

See also Figure S4 and Table S3.

KYN levels even more than in treated WT mice, accompanied by lower KYNA levels (Figure 5G). We did not observe changes in tryptophan or serotonin levels in the MKO-PGC-1 α 1 mice (Figures S5C and S5D). These findings indicate that acute peripheral KYN administration can drive changes in the brain similar to those induced by CMS, which can be controlled by modulating PGC-1 α levels in skeletal muscle.

A PGC-1 α 1-PPAR α/δ Partnership Regulates KAT Expression in Myotubes

To determine if PGC-1 α 1 effects are cell-autonomous we used primary myotube cultures in gain- and loss-of-function experiments. Myotubes transduced with recombinant adenovirus to overexpress PGC-1 α 1 (Figure S6A) showed higher KAT1, KAT3, and KAT4 mRNA levels without significant effects on other members of the pathway (Figures 6A and S6B). In agreement with the results obtained in vivo (Figure 5A), MKO-PGC-1 α 1 myotubes had decreased KAT1, KAT3, and KAT4 gene expression,

which could be rescued by exogenous PGC-1 α 1 expression (Figure 6B).

PGC-1 α 1 coactivators interact with specific DNA-binding transcription factors to exert their biological functions (Lin et al., 2005). Analysis of genomic regions surrounding the KAT1, KAT3, and KAT4 genes revealed an overrepresentation of direct repeat 1 (DR1) sequences (Figure S6C). These elements are recognized by PPAR α and δ , well-known PGC-1 α 1 partners that also increase in skeletal muscle with exercise (Arany, 2008; Schmitt et al., 2003; Wang et al., 2004). In accordance,

compared to WT mice (Figures 5B and S5B). This behavior was considerably worsened after exogenous KYN administration and even more pronounced than in the corresponding WT mice (Figure 5C). As before, (Figures 4C–4E), KYN administration induced hippocampal expression of proinflammatory and kynurenine pathway genes in WT mice, but even more clearly in mice lacking muscle PGC-1 α 1 (Figures 5D and 5E). The expression of synaptic proteins, with the exception of ARC, was equally reduced in WT and MKO-PGC-1 α 1 mice after KYN treatment (Figure 5F). Finally, KYN administration to MKO-PGC-1 α 1 mice increased circulating

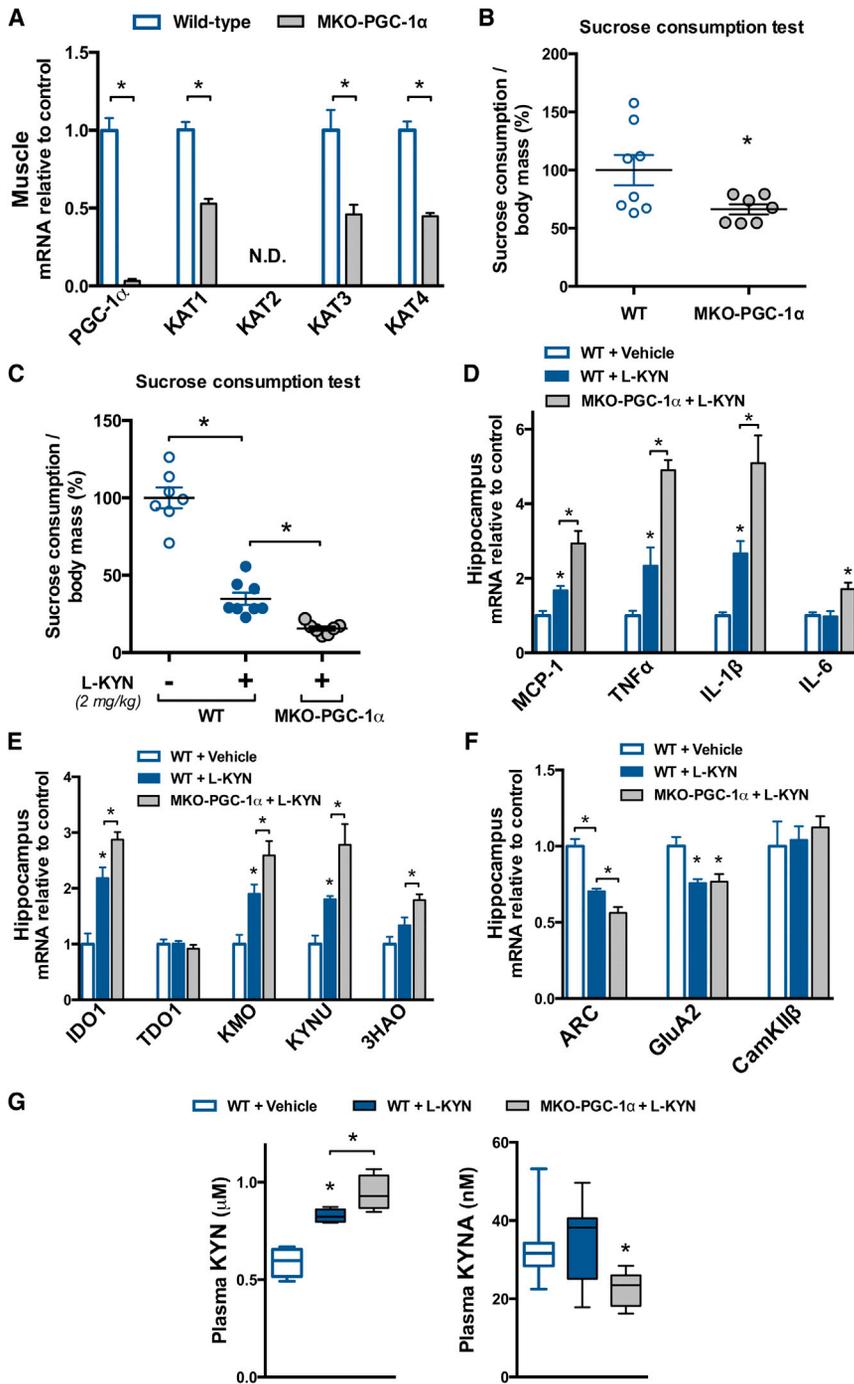


Figure 5. Skeletal Muscle-Specific PGC-1 α Deletion Sensitizes to KYN-Induced Depressive Behavior

(A) mRNA levels in gastrocnemius muscle from WT and muscle-specific PGC-1 α knockout mice (MKO-PGC-1 α ; n = 4–6). (B) Sucrose consumption expressed as amount consumed normalized to body weight before KYN administration (SCT; n = 7–8). (C) Sucrose consumption expressed as amount consumed normalized to body weight 2 hr after KYN administration in the same animals as in (B) (n = 7–8). (D–F) Analysis of gene expression in hippocampus by qRT-PCR using primers specific to the indicated genes (n > 5). (G) Plasma KYN and KYNA concentration shown as box plots (n = 5–7). Scale bars represent mean values and error bars represent SEM. N.D., not detectable. *p < 0.05. See also Figure S5 and Table S3.

δ activation resulted in the highest increase in KAT gene expression (Figure 6C). Conversely, reducing PPAR α levels in myotubes resulted in decreased KAT levels, whereas silencing PPAR δ expression affected only KAT1 (Figure 6D). This was verified also in the presence of overexpressed PGC-1 α 1 (Figure 6D). By using in vivo chromatin immunoprecipitation experiments we could confirm that, in mck-PGC-1 α 1 skeletal muscle, PPAR α , PPAR δ , and PGC-1 α 1 can be found associated with discrete regulatory regions upstream of the transcription start site of the KAT1, KAT3, and KAT4 genes (Figure 6E). These results suggest the PGC-1 α 1-PPAR α / δ partnership is required for full effects on KAT gene expression in skeletal muscle in a cell-autonomous manner.

Exercise Training Increases Murine and Human KAT Expression in Skeletal Muscle

To verify if the PGC-1 α 1-KAT-KYN mechanism is part of the physiological response to exercise training, we analyzed WT mice after 8 weeks of free

mck-PGC-1 α 1 animals had increased skeletal muscle PPAR α and δ expression, which was further enhanced by CMS (Figure S6D). Treatment of WT myotubes with a selective PPAR α agonist increased KAT1, KAT3, and KAT4 expression, while a selective PPAR δ agonist affected only KAT1 levels (Figure S6E). PPAR ligand-mediated increase in KAT1 and KAT3 expression was strictly dependent on PGC-1 α 1, whereas KAT4 was still responsive to PPAR α activation even in MKO-PGC-1 α myotubes (Figure S6E). Combining PGC-1 α 1 expression with PPAR α or

wheel running. This exercise intervention resulted in a gene expression profile that significantly overlapped with ectopic PGC-1 α 1 expression in skeletal muscle (Figure 7A). Notably, endurance exercise training increased skeletal muscle expression of KAT1, KAT3, and KAT4, decreased TDO1 and KMO expression (Figures 7A and S7A), and increased plasma KYNA levels (Figure 7B). Plasma KYNA levels directly correlated with murine skeletal muscle gene expression of KAT1, KAT3, and KAT4 (Figure S7B). We next determined the expression of

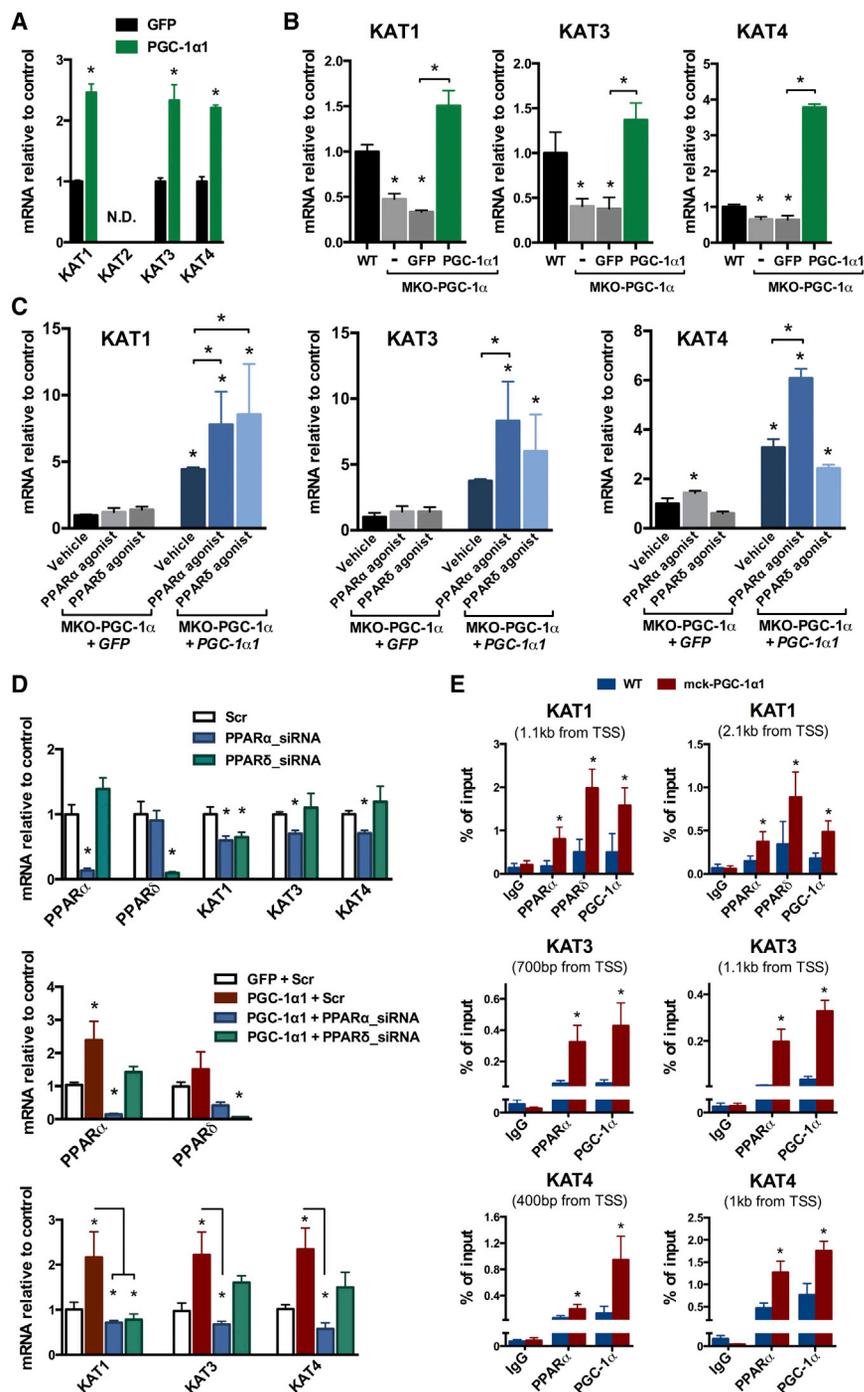


Figure 6. A PGC-1α1-PPARα/δ Partnership Regulates Myotube KATs Expression

(A and B) KAT mRNA levels in primary myotubes from WT or MKO-PGC-1α mice transduced with adenovirus expressing GFP alone or together with PGC-1α1 (n = 3).

(C) KAT mRNA levels in MKO-PGC-1α myotubes treated with vehicle, or selective PPARα or PPARδ agonists upon GFP or PGC-1α1 expression (n = 3).

(D) mRNA levels in myotubes from WT mice treated with siRNA for PPARα or PPARδ (n = 3), transduced as in (A).

(E) In vivo chromatin immunoprecipitation of PPARα-, PPARδ-, and PGC-1α1-associated DNA regions in skeletal muscle of WT and mck-PGC-1α1 animals (n = 4–6), shows occupancy of KAT gene regulatory region containing PPRE motifs. Scale bars represent mean values and error bars represent SD. N.D., not detectable. *p < 0.05. Transcription start site (TSS). See also Figure S6 and Table S3.

of KAT1–KAT4 in skeletal muscle (Figure 7D), after the exercise training program (Figure 7E).

DISCUSSION

Many of the difficulties in treating depression stem from the considerable heterogeneity of the disease and a lack of defined etiology. Mechanisms suggested to contribute to depression include decrease in neurogenesis (Kheirbek et al., 2012), changes in levels of serotonin and other monoamines (Hirschfeld, 2000), neuroinflammation and astrocyte activation (Rajkowska and Miguel-Hidalgo, 2007), brain plasticity (Castrén, 2013), and glutamate imbalance (Sanacora et al., 2012). All of these aspects are affected by physical activity (Brené et al., 2007; Eyre and Baune, 2012), which has emerged as an important therapeutic alternative in affective disorders. In this study, we identify a mechanism by which skeletal muscle PGC-1α1 induced by exercise training changes tryptophan-kyurenine metabolism and protects from stress-induced depression. This mechanism is mediated by the actions of PGC-1α1 and the PPARα/δ transcription factors that together induce the expression of KAT enzymes, thus shifting peripheral KYN to KYNA.

Peripheral tryptophan conversion to KYN under proinflammatory and stress conditions is linked to neuroinflammation and considered to contribute to the pathogenesis of depression (Schwarcz et al., 2012). In this study, we observe a clear relationship between plasma KYN levels and expression of both inflammatory and synaptic proteins. Because KYNA, in contrast

KATs in human skeletal muscle before and after a 3-week training program (Czepluch et al., 2011). Percutaneous biopsies of the *vastus lateralis* skeletal muscle were obtained for each individual at baseline and 48 hr after the last training session. In agreement with previous reports (Mahoney and Tarnopolsky, 2005; Ruas et al., 2012; Schmitt et al., 2003), 3 weeks of physical exercise increased the expression of PGC-1α, PPARα, and PPARδ in human skeletal muscle (Figure 7C). Notably, the same individuals showed an increase in the expression levels

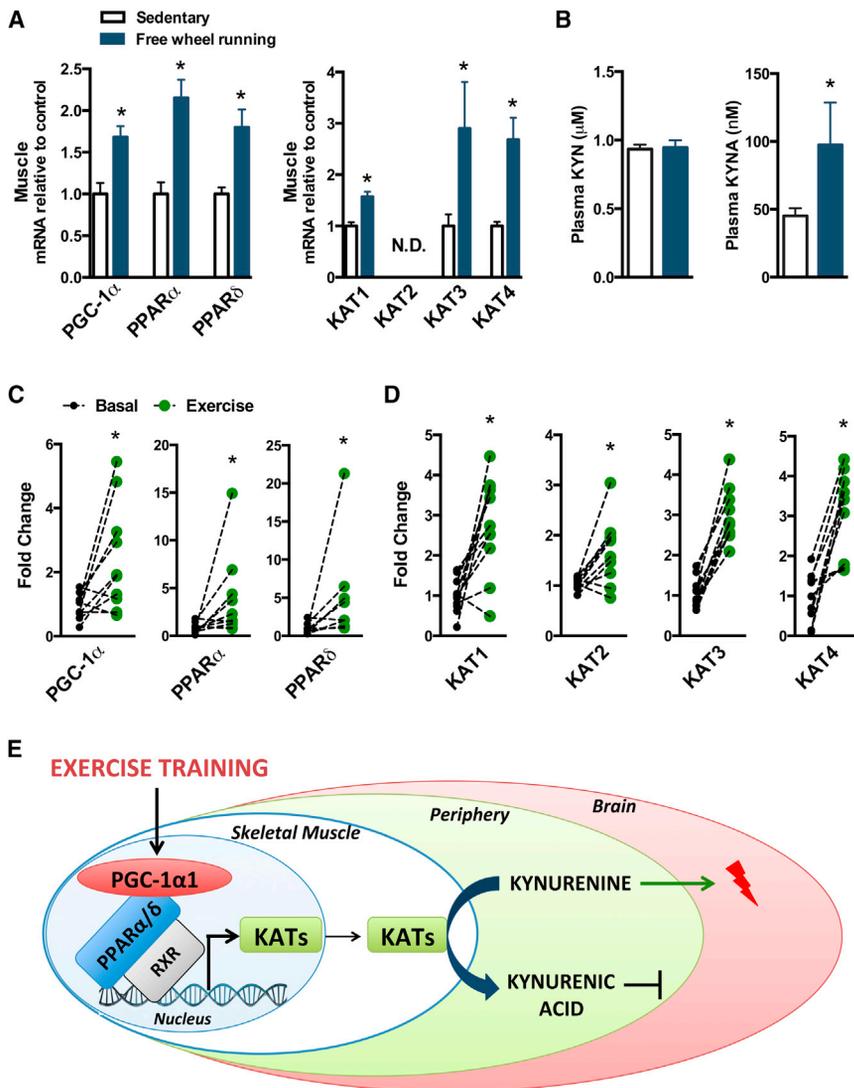


Figure 7. Exercise Training Increases Murine and Human KAT Expression in Skeletal Muscle

(A) Analysis of gene expression by qRT-PCR in gastrocnemius from sedentary and exercise-trained WT mice (n = 4–6).

(B) Plasma KYN and KYNA concentration (n = 6–8).

(C and D) Individual exercise training-induced changes in gene expression determined by qRT-PCR of *vastus lateralis* skeletal muscle from healthy volunteers (n = 8–10).

(E) Synoptic figure highlighting the molecular mechanism by which skeletal muscle PGC-1 α shifts peripheral metabolism of KYN into KYNA. Scale bars represent mean values and error bars represent SEM. N.D., not detectable. *p < 0.05. See also Figure S7 and Table S3.

pairs endurance performance in forced exercise tests (Handschin et al., 2007). This could be a confounding factor for behavioral analysis relying on motor activity such as forced swim tests, thus we used only the sucrose consumption test. Even so, we cannot exclude that the effect on sucrose consumption elicited by the KYN injections is due to sickness behavior rather than anhedonia per se (Dantzer et al., 2008). Nevertheless, this does not affect the conclusion that PGC-1 α -mediated regulation of skeletal muscle KAT levels and the consequent breaking down of KYN prevents a central response.

Diabetic and/or obese mice and humans have reduced PGC-1 α levels in skeletal muscle, which has been suggested to lead or contribute to metabolic

to KYN, does not pass the blood-brain barrier (Fukui et al., 1991), increased peripheral conversion of KYN to KYNA observed in the mck-PGC-1 α mice should have a protective effect on the brain. Indeed, while the CMS treatment increases microglia and neuroinflammatory markers in WT mice, these changes do not occur in mck-PGC-1 α mice. In addition, synaptic proteins, as well as proteins mediating synaptic plasticity, are reduced by CMS in WT but not in mck-PGC-1 α mice. However, this is not due to an overall protection from stress or stress-induced inflammation, as stressed mck-PGC-1 α mice display weight loss, increased skeletal muscle inflammatory markers, and higher CRH levels than unstressed transgenic mice.

The importance of the enhanced peripheral KYN breakdown is illustrated by the fact that mck-PGC-1 α mice are protected from developing depressive behavior, even upon direct KYN administration. Conversely, MKO-PGC-1 α mice display signs of depressive behavior at baseline, which worsens with KYN administration. The genetic loss of skeletal muscle PGC-1 α im-

disease. In line with this, the link between insulin resistance, inflammation, and the kynurenine pathway is an emerging theme in recent literature (Oxenkrug, 2013). This is particularly evident in the context of aging (also associated with reduced muscle PGC-1 α expression) (Johnson et al., 2013). Our results imply that dysregulation in the PGC-1 α /kynurenine pathway could contribute to the increased risk for depression in type 2 diabetes patients (Roy and Lloyd, 2012; Stuart and Baune, 2012).

In general, the stress-induced molecular signature we observe in the brains of WT mice was not seen in mck-PGC-1 α mice. The fact that the levels of astrocytic proteins EAAT1 and GFAP are affected by CMS supports the role of astrocytes in mediating stress-induced depression (Bajramović et al., 2000; Banasr et al., 2010; Bechtholt-Gompf et al., 2010; Czéh et al., 2006). Of note, some of the molecular pathways analyzed are already changed in brains of mck-PGC-1 α mice at baseline, even without the CMS challenge. This suggests that other, not yet identified, signaling pathway(s) from skeletal muscle to brain might be active in mck-PGC-1 α mice.

Skeletal muscle can act as an endocrine organ, secreting diverse myokines in a context-dependent manner (Brandt and Pedersen, 2010). One such myokine under PGC-1 α 1 control is cleaved from FNDC5 and secreted as Irisin, shown to impact adipose tissue function and systemic energy expenditure (Boström et al., 2012). It has been recently reported that peripheral FNDC5 delivery by viral vectors has a neuroprotective effect mediated by increased neuronal BDNF expression (Wrann et al., 2013). As previously reported, we observed that CMS induces a reduction in BDNF expression in WT mice (Gibney et al., 2013; Smith et al., 1995). However, BDNF levels remained unchanged in mck-PGC-1 α 1 mice, indicating that the FNDC5/BDNF pathway is unlikely to be a major component in the protection against stress-induced depression.

The use of PGC-1 α genetic models allowed us to isolate skeletal muscle conditioning from other exercise components. The relevance of the proposed mechanism is supported by the fact that aerobic exercise training interventions in mice and humans induced skeletal muscle PGC-1 α , PPAR α/δ , and KAT expression. It will be interesting to expand this study design to a larger cohort of human volunteers, to include also patients with depression. The exercise training protocol used in this study was sufficient to increase plasma KYNA levels in rodents. In agreement with this, human plasma KYNA levels have been reported to increase after extensive endurance exercise (Lewis et al., 2010).

Depression is one of the world's leading causes of disease burden and time lived with disability (Whiteford et al., 2013) and current antidepressant treatments are insufficient (McClintock et al., 2011). By inducing exercise-mimetic changes specifically in skeletal muscle we have identified a mechanism that reduces the detrimental effects of stress and enhances the resilience to depression. Our work suggests that there is great therapeutic potential in targeting the PGC-1 α 1-PPAR α/δ -KAT-Kynurenine pathway in depressive disorders (Schwarcz et al., 2012; Wu et al., 2014) thus harnessing one of the many beneficial effects of exercise training (Liu et al., 2013).

EXPERIMENTAL PROCEDURES

Please see the [Extended Experimental Procedures](#) for additional details.

Animal Experimentation

Mck-PGC-1 α and MKO-PGC-1 α animals (all on C57BL/6J background), a kind gift from Dr. Bruce Spiegelman (Harvard Medical School, Boston, MA), have been previously described (Chinsomboon et al., 2009; Lin et al., 2002). All experiments and protocols were approved by the regional animal ethics committee of Northern Stockholm.

Chronic Mild Stress

Male mice (8- to 10-week-old at the start of experiments) were subjected to stressors several times a day, applied at different time points to avoid habituation (Table S1). Behavioral testing was carried out 24 hr or more after the last stressor to evaluate depressive-like behavior.

Exercise Training

C57BL/6J male mice (9 weeks) were single housed with or without access to a running wheel for 8 weeks. Only mice that had run more than 4 km/night were selected for subsequent experiments.

Vastus lateralis skeletal muscle biopsies were obtained from healthy volunteers as previously described (Barrès et al., 2012). Muscle biopsies were obtained before training and 48 hr after the last exercise training session (Cze-

pluch et al., 2011). All participants provided written informed consent and all protocols were approved by the Karolinska Institutet Ethics Committee (Czepluch et al., 2011).

Behavior

Forced Swim Test

Each mouse was placed in a cylinder containing 15 cm water for 15 min. After 24 hr, the animals were placed again in the cylinder and the duration of immobility (including movements to keep afloat, but not active swimming) was scored for a 5 min period.

Sucrose Consumption Test

Mice were individually housed, deprived of food for 6 hr, and water for 12 hr before the test, and then given access to 1% sucrose solution during 1 hr. The amount consumed was normalized to body weight. For L-Kynurenine treatments, 2 mg/kg of L-KYN (Sigma-Aldrich) or saline were injected intraperitoneally. Sucrose consumption was assessed 2 hr after injection.

Cell Culture

Primary myoblast cultures and adenovirus expressing PGC-1 α 1 or GFP (green fluorescent protein) have been previously described (Ruas et al., 2012). Fully differentiated myotubes were treated overnight with 1 μ M CP775146 (PPAR α agonist), or GW0742 (PPAR δ agonist) (Tocris bioscience). Myoblasts were transfected with siRNA for PPAR α and PPAR δ and then differentiated.

Western Blot

Brain tissue samples were sonicated in RIPA-buffer with protease inhibitors. Muscle tissue was lysed in Isol-RNA lysis reagent (5 Prime). All primary antibodies used are listed in Table S2.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) experiments were performed as previously described (Ruas et al., 2012) with some modifications.

Gene Expression Analysis

Total RNA was isolated using Isol-RNA Lysis Reagent (5 PRIME). Quantitative real-time PCR was performed and analysis of gene expression was performed using the $\Delta\Delta$ Ct method. Primer sequences are listed in Table S3.

High-Performance Liquid Chromatography

Briefly, brain-tissue homogenate or plasma was analyzed for KYN, KYNA, and 3-HK levels using an isocratic reversed-phase high-performance liquid chromatography (HPLC) system.

Liquid Chromatography and Mass Spectrometry

Brain-tissue homogenate and plasma were prepared by ultracentrifugation and solid extraction and used for tryptophan and serotonin analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Statistical Analysis

Data are expressed as average \pm SD for in vitro and \pm SEM for in vivo experiments. Statistical analyses were performed using GraphPad Prism 6. Unpaired Student's t test was used when two groups were compared, and one-way ANOVA followed by a protected Fisher's least significance difference (LSD) test for post hoc comparisons was used to compare multiple groups. Statistical significance was defined as $p < 0.05$. Correlations were calculated by Spearman rank correlation.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, seven figures, and three tables and can be found with this article at <http://dx.doi.org/10.1016/j.cell.2014.07.051>.

AUTHOR CONTRIBUTIONS

M.L. and J.L.R. conceptualized and supervised the study. L.Z.A. and T.F. contributed to the study design. L.Z.A., T.F., and M.L. performed and analyzed

behavior tests. L.Z.A. performed and analyzed tissue culture experiments, gene expression, and chromatin immunoprecipitations, with contributions from M.P.-P., V.M.-R., J.C.C., A.T.P., and D.M.S.F. T.F. performed and analyzed immunoblots. F.O., M.G., and S.E. performed and analyzed tryptophan and serotonin metabolite measurements. M.B. and I.S.-K. performed and analyzed tryptophan and serotonin measurements. A.K., R.B., and J.R.Z. performed the human exercise study and contributed muscle samples. A.K., J.R.Z., and S.E. edited the manuscript. L.Z.A., T.F., M.L., and J.L.R. wrote the manuscript.

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