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How muscle talks to brain: apelin protein mediates exercise-induced antidepressant effects

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Physical exercise alleviates depressive symptoms and enhances hippocampal plasticity, but the mediators of muscle-brain crosstalk underlying these effects are not fully understood. We evaluated apelin as a novel mediator of the antidepressant effects of physical exercise, specifically testing the hypothesis that exercise-induced increases in skeletal muscle-derived apelin enhance hippocampal plasticity via apelin and its receptor APJ signaling. Voluntary running for 4 weeks alleviated depression-like behaviors and increased serum and hippocampal apelin levels, with skeletal muscles (tibialis anterior and gastrocnemius) as primary apelin sources. Muscle-specific apelin knockout abolished the antidepressant and pro-neurogenic effects of running, whereas muscle-targeted apelin overexpression mimicked the benefits of running in wild-type mice. Mechanistically, myokine apelin enhanced NMDA receptor-mediated neurotransmission via receptors APJ on hippocampal glutamatergic neurons. Specific knockdown of APJ diminished the pro-neurogenic and antidepressant effects of running. Furthermore, apelin/APJ signaling activated casein kinase 2, which phosphorylated the GluN2B subunit at serine 1480, thereby enhancing NMDA receptor function and activating downstream calpain-2 signaling. Our findings reveal a muscle-brain axis where exercise-induced myokine apelin coordinates hippocampal neuroplasticity and antidepressant responses, offering new therapeutic avenues for depression.

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INTRODUCTION

Major depressive disorder (MDD), characterized by persistent low mood, anhedonia, and cognitive impairments, is a profound global health challenge affecting millions worldwide [1, 2]. While physical exercise is recognized as a promising non-pharmacological intervention to alleviate depressive symptoms [3], primarily through enhancement of hippocampal plasticity, including adult neurogenesis and synaptic remodeling [4–7], and its role in improving muscle function is also well established [8, 9]. However, the precise molecular mediators underlying the muscle-brain crosstalk that contributes to the antidepressant effects of physical exercise have not been identified.

Recent advances have identified exercise-induced myokines as key players in muscle-brain communication, with emerging evidence suggesting their involvement in alleviating depressive symptoms [10, 11]. Among these, apelin, a multifunctional myokine upregulated by exercise, has received attention for its dual role in regulating both muscle health and brain function [12–15]. Produced from a 77-amino-acid precursor, apelin is

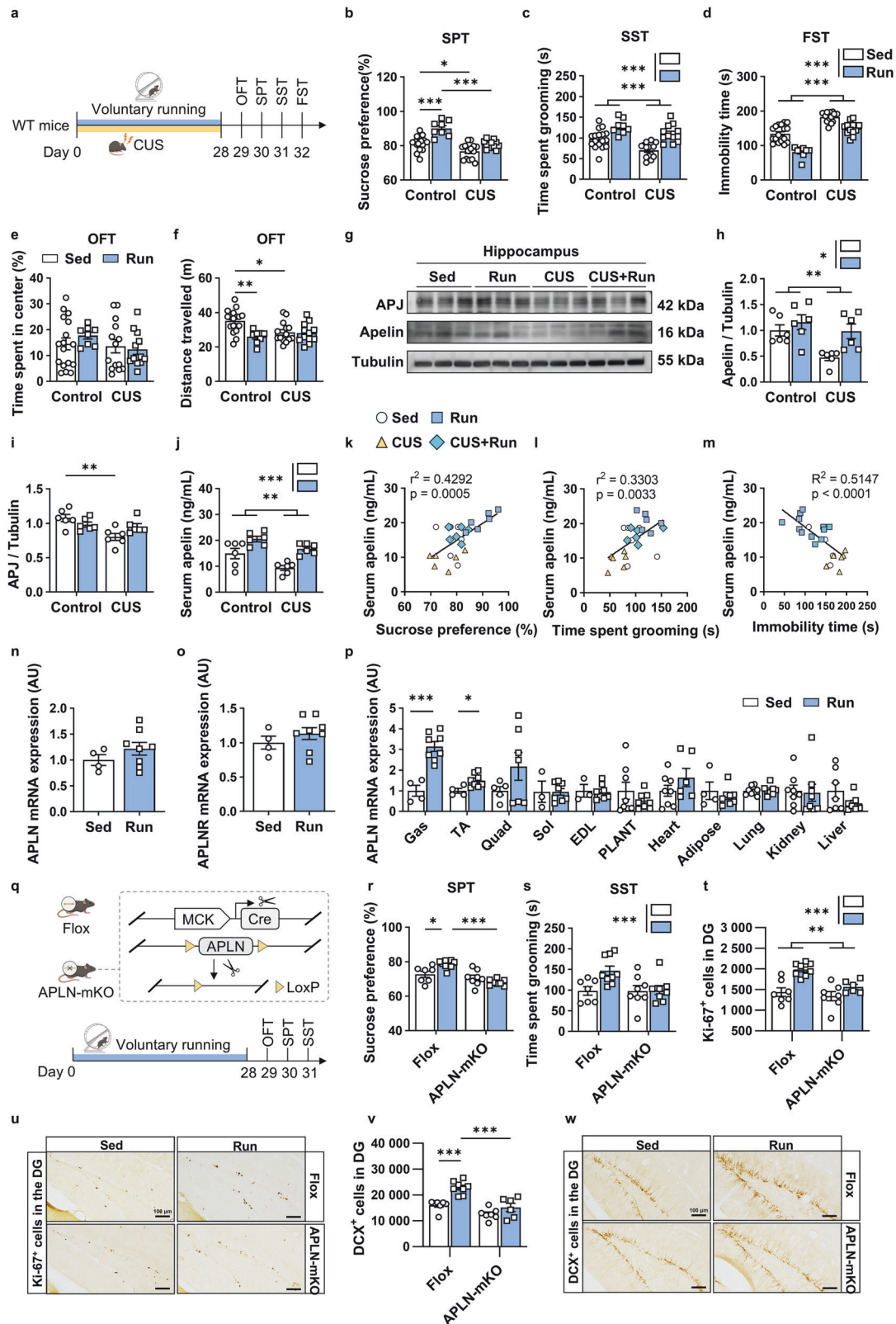
cleaved into bioactive isoforms such as apelin-13, -17, and -36, which activate the APJ receptor to modulate cardiovascular homeostasis, energy balance, and neuroendocrine signaling [16–18]. Notably, apelin levels reduced in sarcopenic individuals, and supplementation has been shown to improve muscle function [13, 19], while physical exercise improves sarcopenic muscle degeneration via increasing apelin levels in aged mice and humans [12]. Beyond its peripheral role, apelin exhibits neuroprotective and antidepressant properties by promoting hippocampal neurogenesis [20, 21], suppressing apoptosis [22–24], and mitigating neuroinflammation [25–27]. Furthermore, administration of apelin has been found to reverse depressive behaviors and enhance synaptic function [20, 28–30]. Although previous studies have implicated apelin's function in neuroprotection, the detailed molecular mechanisms by which it may exert these effects are still unclear. Furthermore, its role as a myokine that relays exercise signals to the brain has yet to be explored.

We address this critical knowledge gap by evaluating apelin as a novel mediator of the antidepressant effects of physical exercise.

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Specifically, we hypothesize that physical exercise-induced increases in skeletal muscle-derived apelin enhance hippocampal plasticity via APJ signaling pathway, thereby alleviating depressive behaviors. Our study provides the first direct test that apelin serves as a pivotal exercise-responsive myokine, bridging

peripheral muscle adaptations to central plasticity mechanisms in depression, offering a potential therapeutic target for the treatment of depression, particularly in aging individuals with muscle loss.

Fig. 1 Muscle-specific apelin knockout diminishes the beneficial effects of voluntary running on antidepressant-like behaviors and adult neurogenesis. **a** Experimental timeline of 4-week chronic unpredictable stress (CUS) and voluntary running (Run). CUS increased depression-like behavior which could be restored by running, as shown by **b** increasing sucrose preference in sucrose preference test (SPT): Interaction $F(1, 47) = 3.866$, $P > 0.05$; CUS $F(1, 47) = 35.94$, $P < 0.001$; Run $F(1, 47) = 30.35$, $P < 0.001$. **c** CUS reduced self-grooming time in sucrose splash test (SST), but running showed opposite effects. Interaction $F(1, 47) = 0.717$, $P > 0.05$; CUS $F(1, 47) = 14.51$, $P < 0.001$; Run $F(1, 47) = 34.84$, $P < 0.001$. **d** CUS promoted immobility time in forced swimming test (FST) but running showed opposite effect. Interaction $F(1, 47) = 3.123$, $P > 0.05$; CUS $F(1, 47) = 107.0$, $P < 0.001$; Run $F(1, 47) = 58.87$, $P < 0.001$. **e** Neither CUS nor running affected time spent in the center in the open field test (OFT). Interaction $F(1, 47) = 0.836$, $P = 0.365$; CUS $F(1, 47) = 1.636$, $P > 0.05$; Run $F(1, 47) = 0.171$, $P > 0.05$. **f** Running and CUS decreased distance travelled in the OFT: Interaction $F(1, 47) = 6.384$, $P < 0.05$; CUS $F(1, 47) = 1.790$, $P > 0.05$; Run $F(1, 47) = 8.028$, $P < 0.001$. **g-i** Western blotting analysis. Running attenuated CUS-decreased hippocampal protein levels of apelin: Interaction $F(1, 20) = 2.157$, $P > 0.05$; CUS $F(1, 20) = 8.607$, $P < 0.01$; Run $F(1, 20) = 7.844$, $P < 0.05$, but did not restore decrease in APJ: Interaction $F(1, 20) = 7.127$, $P = 0.010$; CUS $F(1, 20) = 10.72$, $P < 0.01$; Run $F(1, 20) = 0.347$, $P > 0.05$. **j** Running increased and CUS suppressed serum apelin levels. Interaction $F(1, 20) = 0.478$, $P > 0.05$; CUS $F(1, 20) = 13.26$, $P < 0.01$; Run $F(1, 20) = 25.46$, $P < 0.001$. **k** A positive correlation between sucrose preference in SPT and serum apelin levels (Linear regression analysis $R^2 = 0.4292$, $P < 0.001$). **l** A positive correlation between grooming time in SST and serum apelin levels (Linear regression analysis $R^2 = 0.3303$, $P < 0.001$). **m** A negative correlation between immobility time in FST and serum apelin levels (Linear regression analysis $R^2 = 0.5147$, $P < 0.0001$). **n-o** Voluntary running did not alter the mRNA expression levels of apelin (APLN) ($P > 0.05$) and APLNR (apelin receptor) in hippocampi ($P > 0.05$). **p** Running specifically promoted apelin mRNA expression in gastrocnemius (Gas, $P < 0.001$) and tibialis anterior (TA, $P < 0.05$) muscles, but did not affect quadriceps (Quadri.), soleus (Sol), extensor digitorum longus (EDL), plantaris (Plant.), and other organs (heart, adipose tissue, lung, kidney, liver; $P > 0.05$). APLN and APLNR mRNA expression normalized to HPRT as the housekeeping gene; data are presented as relative fold-changes compared to the Sed group **n-p**. **q** Experimental timeline with a 4-week voluntary running in APLN-mKO mice (muscle-specific apelin knockout mice) and Flox mice (control). Sed: sedentary mice; Run: voluntary running. Muscle apelin knockout blocked antidepressant effects of running. Running produced antidepressant effects in wildtype mice with increase in: **r** sucrose preference in SPT: Interaction $F(1, 28) = 11.44$, $P < 0.01$; APLN-mKO $F(1, 28) = 26.14$, $P < 0.001$; Run $F(1, 28) = 1.686$, $P > 0.05$. But apelin knockout has no effects on **s** self-grooming time in SST: Interaction $F(1, 28) = 4.057$, $P > 0.05$; APLN-mKO $F(1, 28) = 4.146$, $P > 0.05$; Run $F(1, 28) = 4.891$, $P < 0.05$. **t-w** Both muscle apelin deficiency and running modulated hippocampal Ki-67⁺ cells: Interaction $F(1, 24) = 3.341$, $P > 0.05$; APLN-mKO $F(1, 24) = 8.904$, $P < 0.01$; Run $F(1, 24) = 19.76$, $P < 0.001$; and muscle apelin knockout blocked running-induced increase **v** doublecortin (DCX⁺) cells in the hippocampal dentate gyrus: Interaction $F(1, 24) = 4.304$, $P < 0.05$; APLN-mKO $F(1, 24) = 29.11$, $P < 0.001$; Run $F(1, 24) = 21.02$, $P < 0.001$ (scale bars, 100 μ m in 100 $\times$). Data are presented as mean $\pm$ SEM. Statistical significance was determined by two-way ANOVA with Tukey's HSD post hoc test for panels **b-f**, **h-j**, **r-v**, unpaired t-test for panels **n-o**, and linear regression analysis for panel **p**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. CUS: chronic unpredictable stress; APLN: apelin gene; OFT: Open field test; SPT: sucrose preference test, SST: sucrose splash test, FST: forced swim test, Gas: gastrocnemius, TA: tibialis anterior, Sol: soleus, EDL: extensor digitorum longus, DG: dentate gyrus, APLN-mKO: muscle-specific apelin knockout.

METHODS AND MATERIALS

A detailed description of the experimental procedures, including animal models, adeno-associated virus (AAV) injections, behavioral assays, histological and biochemical analyses, and electrophysiological recordings, is provided in the Supplementary Information.

RESULTS

Voluntary running alleviates stress-induced depression via muscle-secreted apelin

We first demonstrated antidepressant effects of physical running in the mouse model of depression induced by chronic unpredictable stress (CUS; Fig. 1a). CUS significantly increased depression-like behavior, as evidenced by decreased sucrose preference in the sucrose preference test (SPT; Fig. 1b), reduced grooming time in the splash test (SST; Fig. 1c), and increased immobility in the forced swim test (FST; Fig. 1d). These behaviors were improved by running (Fig. 1b–d). Neither CUS nor running altered anxiety-like behavior as indicated by the lack of significant differences in the time spent in the center of the open field test (OFT) among the groups (Fig. 1e). Both CUS and running reduced total distance traveled in the OFT (Figs. 1f; S1a, b), possibly due to exercise-induced fatigue observed in behavioral tests conducted 24 h after running.

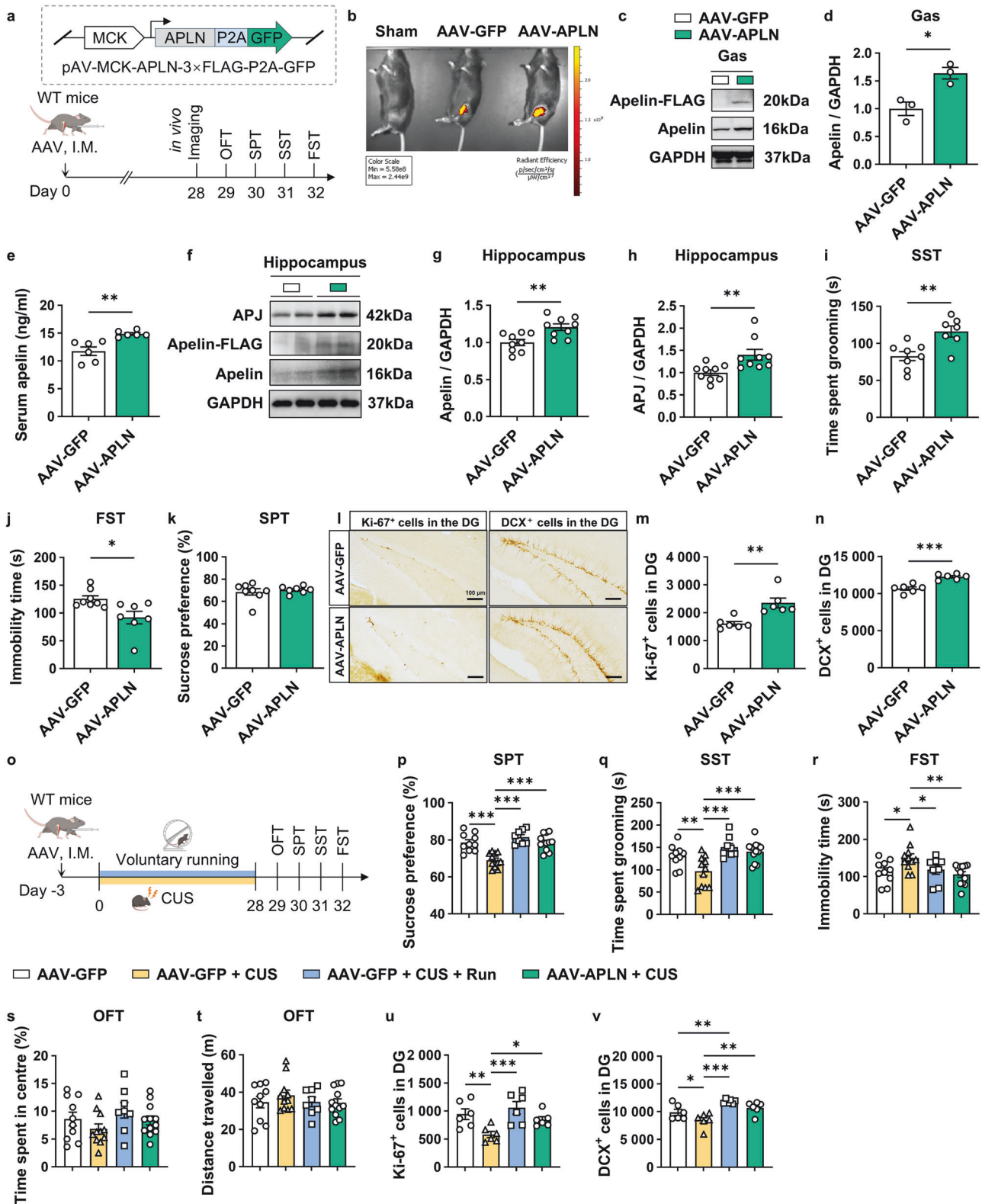
Our previous work revealed that voluntary running promotes neuroplasticity in the hippocampus [5, 31–35]. Our results showed that running promoted hippocampal apelin levels (Fig. 1g–h), although it did not affect APJ levels (Fig. 1i). Additionally, running increased serum apelin levels, whereas CUS exposure markedly reduced serum apelin concentrations. (Fig. 1j). Significant correlations were found between serum apelin levels and behavioral measures across various tests, indicating apelin's role in modulating depression-like behaviors. Specifically, higher serum apelin levels were positively correlated with sucrose preference in the SPT (Fig. 1k) and grooming time in the splash test (SST; Fig. 1l), while they were negatively correlated with immobility time in the

FST (Fig. 1m). These findings suggest that apelin may play a crucial role in alleviating depressive behaviors.

Running did not alter hippocampal mRNA levels of apelin and APJ (Fig. 1n–o), suggesting that changes in hippocampal apelin levels are not due to transcriptional regulation in the hippocampus. To identify the major peripheral sources of apelin in response to running, we conducted quantitative PCR analysis. Our results indicated that the gastrocnemius (Gas) and tibialis anterior (TA) muscles of the lower hindlimb are the primary peripheral sources, as running specifically increased apelin (Fig. 1p) and APJ mRNA levels in these muscles (Fig. S1j), consistent with previous reports [12, 36]. In contrast, no significant differences in apelin mRNA levels were observed in other organs (Fig. 1p). Furthermore, while chronic unpredictable stress (CUS) reduced apelin mRNA levels in the gastrocnemius muscle without affecting the TA, running increased apelin expression in both muscles (Fig. S1c–d). These findings confirm that skeletal muscle is a key source of exercise-induced apelin release.

Muscle-secreted apelin is required for running to elicit antidepressant effects

We investigated whether skeletal muscle-derived apelin mediates the antidepressant effects of running by utilizing muscle-specific apelin knockout mice (APLN-mKO; APLN^{fl/y} $\times$ MCK-Cre) and their floxed littermates as control (Flox; APLN^{fl/y} without MCK-Cre expression) (Fig. 1q). At 10 weeks of age, APLN-mKO mice exhibited no APLN mRNA expression in muscles and demonstrated reduced grip strength and impaired performance on the rotarod test, although their muscle mass remained unchanged (Fig. S2b–i). Despite having comparable running activity (Fig. S2j–k), APLN-mKO mice showed significantly lower serum apelin levels (Fig. S2n). The absence of muscle apelin eliminated the antidepressant effects of voluntary running, which was evidenced by the lack of increase in sucrose preference in SPT and grooming time in SST in APLN-mKO mice following running (Fig. 1r–s). Neither running nor muscle apelin knockout affected



the time spent in the center or locomotor activity in the OFT (Fig. S2l–m). These findings indicate that muscle-derived apelin is essential for voluntary running to exert its antidepressant effects.

The increase in hippocampal neurogenesis is significantly associated with the antidepressant effects of physical exercise [37–40]. We quantified changes in proliferating cells (Ki-67⁺ cells)

and immature neurons (DCX⁺ cells; Fig. 1t–w). Voluntary running led to a significant increase in the number of Ki-67⁺ cells and DCX⁺ cells in both dorsal and ventral dentate gyrus (DG) (Figs. 1t–w; S2o–r). However, this increase was not observed in APLN-mKO mice, indicating that the absence of muscle-derived apelin impairs the neurogenic response to exercise. These findings suggest that

Fig. 2 Muscle-secreted apelin is involved in the antidepressant effect of voluntary running. **a** Treatment timeline for overexpression of apelin by intramuscular injections of adeno-associated viruses (AAV) in wildtype mice. AAV-GFP (AAV-MCK-Control-P2A-GFP): control; AAV-APLN (AAV-MCK-APLN-3xFLAG-P2A-GFP): muscle specific apelin overexpression. **b** Confirmation of intramuscular injection of AAV showing reporter gene expression of GFP in TA by the IVIS Spectrum system (PerkinElmer). Signal intensity is presented as a heat map and quantified as total radiant efficiency (p/s/cm²/sr)/(μW/cm²) using Living Image software. **c–d** Western blotting analysis confirming overexpression of apelin in muscle ($P < 0.05$). **e** Overexpression of apelin in muscles increased serum apelin levels ($P < 0.01$). **f–h** Western blotting analysis. Overexpression of muscle apelin increased protein expression of apelin ($P < 0.01$) and APJ in the hippocampi ($P < 0.01$). **i** Muscle apelin overexpression increased self-grooming time in SST ($P < 0.01$), and **j** decreased immobility time in FST ($P < 0.05$), **k** but did not alter sucrose preference in SPT ($P > 0.05$). **l–n** Immunohistochemical staining showed that muscle apelin overexpression increased Ki-67⁺ cells in DG ($P < 0.001$) and DCX⁺ cells in DG ($P < 0.001$) (scale bars, 100 μm in 100x). **o** Treatment timeline for wildtype mice receiving intramuscular injection AAV. AAV-GFP + CUS: stressed non-runner control; AAV-GFP + CUS + Run: stressed runners; AAV-APLN + CUS: stressed non-runner with muscle apelin overexpression. **p–s** Overexpression of apelin mimicked antidepressant effects of running in (p) Increasing sucrose preference in SPT: $F(3,37) = 16.76$, $P < 0.001$; **q** Increased self-grooming time in SST: $F(3,37) = 8.033$, $P < 0.001$; and (r) Decreasing immobility time in FST: $F(3,37) = 4.116$, $P < 0.05$; **s** No significant changes in time in center in OFT: $F(3,37) = 1.549$, $P > 0.05$; or in **t** distance travelled in OFT: $F(3,37) = 0.5614$, $P > 0.05$. **u–v** Overexpression of muscle apelin mimicked running-induced increases in hippocampal neurogenesis. Comparable increase between runners and muscle apelin overexpression groups in (u) Ki-67⁺ cells in DG: $F(3,20) = 6.046$, $P < 0.01$; **v** DCX⁺ cells in DG: $F(3,20) = 9.446$, $P < 0.001$. Data are presented as mean ± SEM. Statistical significance was determined by one-way ANOVA with Tukey's HSD post hoc test for **p–v** or unpaired t-test for **c–n**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. MCK muscle creatine kinase, P2A porcine teschovirus-1 2A, GFP green fluorescent protein, APLN apelin gene, OFT open field test, SPT sucrose preference test, SST sucrose splash test, FST forced swim test, DCX doublecortin, DG dentate gyrus.

apelin plays a critical role in mediating the effects of running on adult hippocampal neurogenesis, which may underlie its antidepressant benefits.

Muscle apelin overexpression mimics antidepressant effects of running

To test whether muscle-secreted apelin can mimic beneficial effects of physical exercise, we overexpressed apelin by intramuscular injections of adeno-associated viruses (AAV-MCK-APLN) into the gastrocnemius and tibialis anterior muscles (Fig. 2a–b). This intervention significantly increased apelin levels in the Gas and TA muscles (Figs. 2c–d; S3a–c) and serum (Fig. 2e), along with elevated hippocampal apelin and its receptor APJ levels (Figs. 2f–h; S3a), echoing the finding that apelin crosses the blood-brain barrier [41, 42].

Muscle apelin overexpression reduced depression-like behavior, as evidenced by increased grooming time in SST (Fig. 2i) and reduced immobility in FST (Fig. 2j). However, it did not affect sucrose preference in SPT (Fig. 2k) or exploration time in the center of OFT (Fig. S3d–e). Immunostaining revealed that muscle apelin overexpression significantly increased the number of Ki-67⁺ and DCX⁺ cells in the DG (Figs. 2l–n & S3f–i), indicating that the increase in muscle apelin elicits antidepressant effects in conjunction with enhanced adult hippocampal neurogenesis. These findings suggest that muscle-derived apelin plays a pivotal role in mediating the neurogenic and behavioral benefits associated with exercise.

We compared the antidepressant effects of muscle apelin overexpression to those of voluntary running in stressed mice (Fig. 2o). Both interventions demonstrated comparable efficacy in reversing the depression-like behaviors induced by chronic stress, as evidenced by improvements in sucrose preference in SPT (Fig. 2p), increased grooming time in SST (Fig. 2q), and reduced immobility time in FST (Fig. 2r). Neither intervention affected anxiety-like behaviors (Fig. 2s–t). Furthermore, immunostaining results revealed that muscle apelin overexpression restored hippocampal neurogenesis in stressed mice to a level similar to that achieved by running (Figs. 2u–v; S3j–m). These findings suggest that muscle apelin overexpression effectively mimics the antidepressant-like behavior and neurogenic effects of voluntary running.

Muscle apelin enhances hippocampal glutamatergic transmission and NMDA receptor function

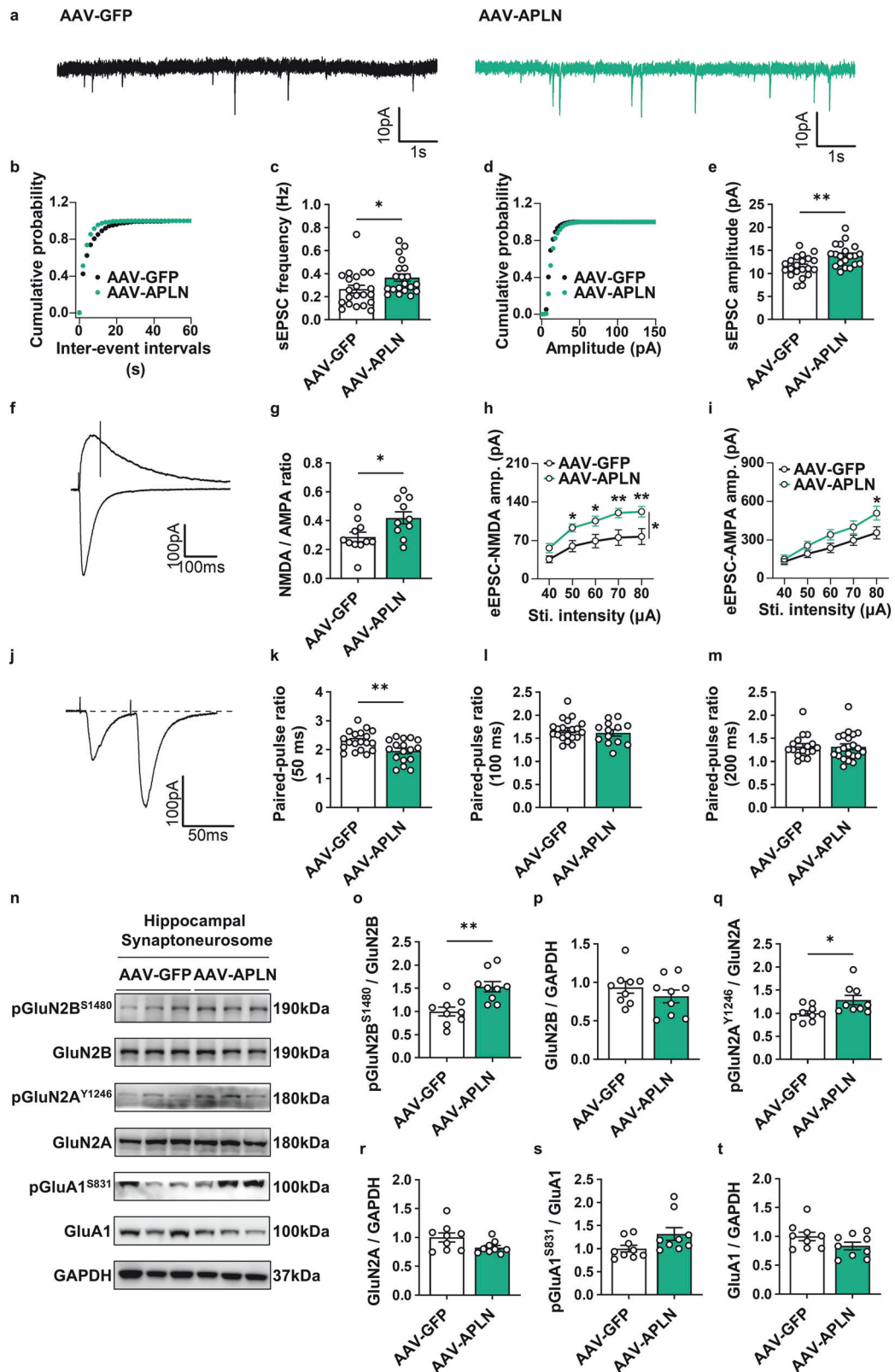
In addition to promoting hippocampal neurogenesis in DG, exercise also enhances synaptic neurotransmission in the cornu ammonis 1 (CA1) region [43, 44], both of which are involved in the

antidepressant effects [38, 45, 46]. However, whether the myokine apelin exerts effects similar to those of physical exercise remains unknown. Using whole-cell patch-clamp recordings in hippocampal CA1 pyramidal neurons, we found that muscle apelin overexpression resulted in higher frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs; Fig. 3a–e), indicating enhanced excitatory synaptic transmission. Moreover, muscle apelin overexpression increased the ratio of N-methyl-D-aspartate (NMDA) to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) currents (Fig. 3g), as well as both NMDA receptor (NMDAR)-mediated and AMPAR-mediated EPSCs (Fig. 3h–i). Interestingly, the paired-pulse ratio at a 50 ms stimulation interval was altered (Fig. 3k), while it remained unchanged at 100 and 200 ms intervals (Fig. 3l–m). These findings indicate that muscle-derived apelin enhances hippocampal glutamatergic transmission and NMDA receptor function, which may contribute to the synaptic plasticity associated with physical exercise.

We further investigated whether muscle apelin overexpression affects the expression of glutamatergic receptors in the hippocampus. GluN2A and GluN2B are key subunits of the NMDA receptor [47, 48]. Western blot analysis (Fig. 3n) revealed that muscle apelin overexpression increased phosphorylation of the GluN2B subunit at serine 1480 (S1480; Fig. 3o) and tyrosine 1472 (Y1472; Fig. S3o), as well as phosphorylation of the GluN2A subunit at tyrosine 1246 (Y1246; Fig. 3q), without altering the total expression levels of GluN2A and GluN2B (Fig. 3p & r). However, muscle apelin overexpression did not affect the phosphorylation of the AMPA receptor subunit GluA1 at serine 831 (S831; Fig. 3s–t), nor did it alter the expression of postsynaptic density-95 (PSD-95) or the presynaptic marker synaptophysin in hippocampal synaptoneurosome extractions (Fig. S3p–q). These findings suggest that muscle apelin overexpression enhances excitatory neurotransmission and NMDAR-mediated synaptic plasticity, contributing to enhanced synaptic plasticity and thus its potential antidepressant effects.

Apelin receptor in hippocampal glutamatergic neurons is essential for the antidepressant effects of running

The apelin receptor, APJ, is expressed in neurons, astrocytes, and oligodendrocytes but not in microglia or monocytes [49, 50]. We next sought to determine the presence of APJ in the hippocampal neurons and the role of apelin/APJ involvement in glutamatergic transmission. Co-labeling in the hippocampal dentate gyrus (DG) showed that APJ is predominantly expressed in glutamatergic neurons, as indicated by its co-localization with the neuronal marker Camk2a, and to a lesser extent with the GABAergic marker



GAD67 (Figs. 4a; S4a). In addition, immunostaining confirmed that APJ is present in the DG, CA1, and CA3 regions of the hippocampus (Fig. S4b). To determine whether APJ expression in the hippocampal glutamatergic neurons are necessary for the antidepressant effects of voluntary running, we performed intra-

ventral hippocampal injections of Cre-dependent AAV-shAPLN to knock down APJ expression specifically in the ventral hippocampal CA1 region (Fig. 4b–d). Following recovery from the surgical procedure, mice were subjected to four weeks of voluntary running. All mice exhibited similar levels of running activity

Fig. 3 Muscle apelin overexpression enhances excitatory neuronal transmission and NMDA receptor expression in the hippocampus. **a** Representative traces of sEPSCs in CA1 pyramidal neurons from AAV-GFP (Control) and AAV-APLN mice. **b** Cumulative probability plots for inter-event intervals (s); **d** Amplitudes (pA) of sEPSCs. Muscle apelin overexpression increased: **c** sEPSC frequency ($P < 0.05$). **e** sEPSC amplitude ($P < 0.01$). **f** Maximal AMPA and NMDA currents in CA1 of AAV-GFP and AAV-APLN mice. Overexpression of muscle apelin increased: **g** NMDA/AMPA ratio ($P < 0.05$) and **i** NMDA current amplitude ($P < 0.05$) without altering **h** AMPA current amplitude ($P > 0.05$). **j** Muscle apelin overexpression decreased pair-pulse ratio at **k** 50 ms inter-pulse interval ($P < 0.01$), with no significant effects at **l** 100 ms or **m** 200 ms interval ($P > 0.05$). **n** Western blotting analysis using hippocampal synaptoneurosome proteins. Apelin overexpression increased expression levels of **o** phospho-GluN2B (S1480, $P < 0.01$) and **q** phospho-GluN2A (Y1246, $P < 0.05$) but did not affect **p** total GluN2B or **r** total GluN2A ($P > 0.05$). There was no significant effect on **s** phospho-GluA1 (S831) and **t** total GluA1 ($P > 0.05$). Data are shown as mean $\pm$ S.E.M. Statistical analysis is performed using an unpaired t-test, with $*P < 0.05$ and $**P < 0.01$. CA1: Cornu ammonis 1; GFP green fluorescent protein, APLN apelin gene, sEPSCs spontaneous excitatory postsynaptic currents, NMDA N-methyl-D-aspartate, AMPA α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

(Fig. S4c–d), indicating that APJ knockdown did not affect their overall activity levels.

However, APJ knockdown significantly attenuated the antidepressant effects of voluntary running, as evidenced by the lack of improvement in sucrose preference in SPT, grooming time in SST, and immobility time in FST (Fig. 4e–g). There were no significant effects on anxiety-like behavior in OFT (Fig. S4e–f). These findings suggest that the expression of APJ in hippocampal glutamatergic neurons is crucial for mediating the antidepressant effects of voluntary running.

We further explored whether apelin signaling through APJ is essential for running-induced increases in adult hippocampal neurogenesis. As anticipated, voluntary running significantly increased the number of Ki-67⁺ proliferating cells and DCX⁺ immature neurons, effects that were absent in mice with APJ knockdown (Figs. 4h–j; S4g–j). Additionally, running enhanced the number of BrdU⁺ survival cells and increased the proportion of BrdU⁺DCX⁺ co-labeled cells, indicating improved cell survival and neuronal differentiation. However, these effects were absent in hippocampal APJ-knockdown mice (Figs. 4k–l; S4k–n). These findings collectively demonstrate that APJ is crucial for the running-induced enhancement of adult neurogenesis, including cell proliferation, survival, and neuronal differentiation of adult-born neurons. This underscores the importance of APJ-mediated apelin signaling in enabling the neurogenic effects of physical exercise.

To further investigate the mechanistic role of apelin signaling in hippocampal synaptic plasticity, we examined glutamate receptor expression in synaptoneurosome extracted from the ventral hippocampus (Fig. 5a). Western blot analysis revealed that hippocampal APJ knockdown attenuated the running-induced increase in phosphorylation of the GluN2B subunit at serine 1480 (S1480) and tyrosine 1472 (Y1472; Figs. 5b–c; S4o–p), as well as phosphorylation of the GluN2A subunit at tyrosine 1246 (Y1246) and the GluA1 subunit at serine 831 (S831; Fig. 5d–g). However, there were no significant differences in the expression levels of postsynaptic density-95 (PSD-95) and the presynaptic marker synaptophysin among the groups (Fig. 5h–i). These findings suggest that apelin signaling is essential for the running-induced enhancement of hippocampal NMDAR function, highlighting its role in enabling synaptic plasticity associated with the antidepressant effects of exercise.

We next examined whether hippocampal APJ is required for muscle-derived apelin to modulate synaptic function. To selectively knock down APJ in glutamatergic neurons in the ventral hippocampus, WT mice received bilateral injections of AAV-DIO-shAPLN together with AAV-Camk2a-Cre into the ventral hippocampus. In parallel, apelin was overexpressed in skeletal muscle via intramuscular injection of AAV-MCK-APLN (Fig. S5a). Control mice received corresponding control AAVs.

Whole-cell patch-clamp recordings from hippocampal CA1 pyramidal neurons revealed that APJ knockdown significantly attenuated the apelin-induced increase in the NMDA/AMPA ratio by selectively reducing NMDA receptor-mediated EPSCs, without

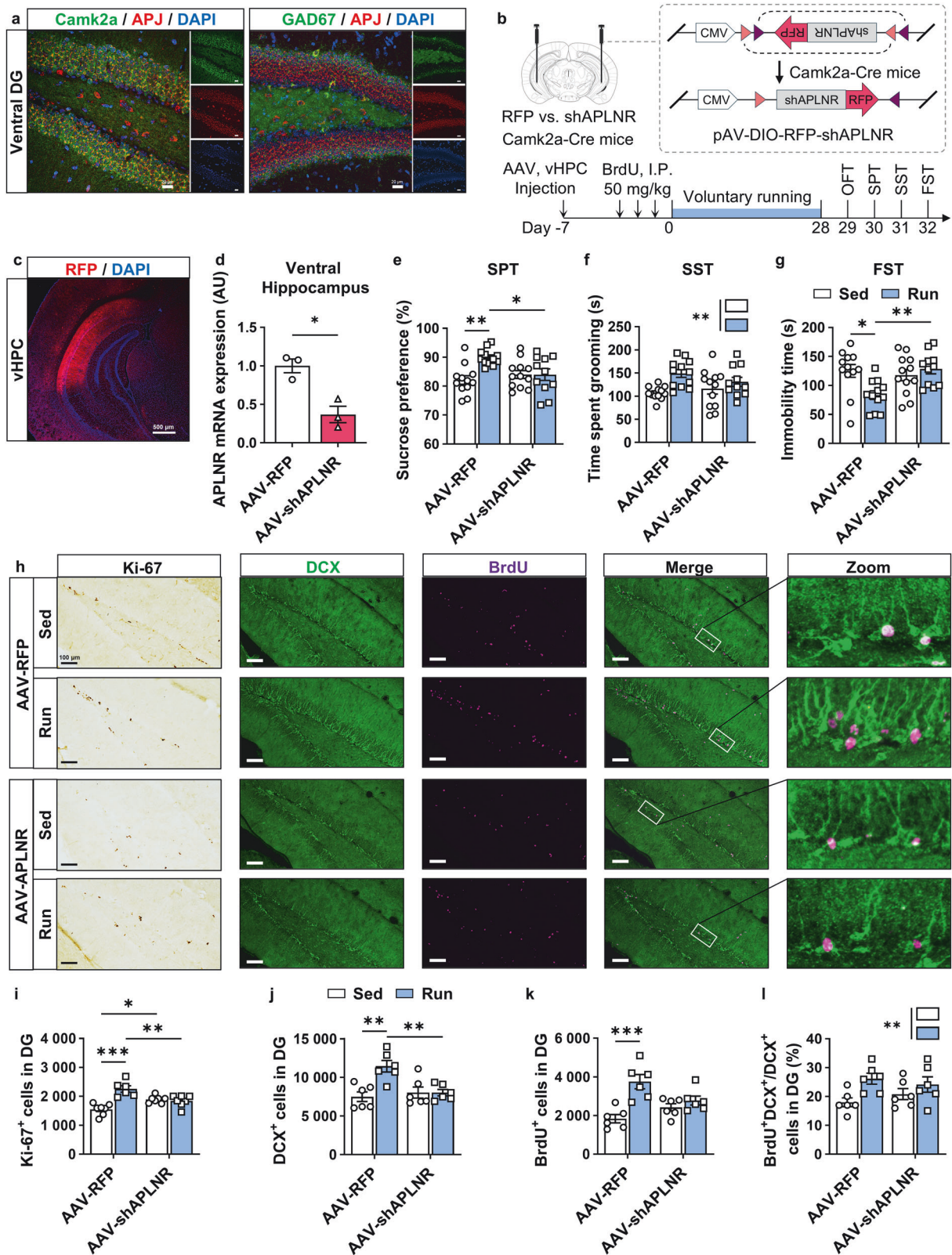
affecting AMPA receptor-mediated currents (Fig. S5b–d). Notably, muscle apelin decreased the paired-pulse ratio (PPR) at a 50-ms inter-pulse interval, and this presynaptic effect was unchanged by APJ knockdown (Fig. S5e). In contrast, neither muscle apelin nor APJ knockdown altered PPRs at 100- or 200-ms inter-pulse intervals (Fig. S5f–g), indicating that the presynaptic effect of apelin is restricted to short inter-stimulus intervals and that APJ knockdown does not affect presynaptic release probability. Moreover, muscle apelin overexpression significantly increased frequency and amplitude of sEPSCs which were attenuated by APJ knockdown (Fig. S5h–l). Together, these findings confirm that muscle-derived apelin enhances hippocampal glutamatergic transmission primarily through APJ-dependent modulation of NMDA receptor function.

Apelin/APJ signaling enhances NMDAR function via modulating casein kinase 2 activity

The mechanism by which apelin modulates NMDAR functions is not fully understood. An in vitro study has suggested that the apelin/APJ pathway regulates NMDAR and calpain activity through casein kinase 2 (CK2) [51]. CK2 interacts with APJ receptor [52] and regulates the phosphorylation of GluN2B-S1480 subunit [53, 54]. Our western blot analysis revealed that muscle apelin overexpression increased the levels of the catalytic subunit CK2 α and CK2 activity, but did not affect the levels of the regulatory CK2 β dimer (Figs. 6b–d; S6b–c). Consistently, voluntary running increased synaptic CK2 α levels and CK2 activity, the effect that was absent in APJ knockdown (Fig. 6e–f). There were no effects on CK2 β levels (Fig. 6g). In non-synaptic (cytosolic) fractions (Fig. S6a), muscle apelin overexpression increased both CK2 α and CK2 β levels (Fig. S6d–f). Furthermore, voluntary running increased cytosolic CK2 α levels, an effect that was prevented by APJ knockdown (Fig. S6g–i). Overall, these results suggest that apelin/APJ signaling interacts with CK2 α to enhance NMDAR function.

To further investigate the role of CK2 in apelin's enhancement of NMDAR function, we inhibited CK2 using 4,5,6,7-tetrabromobenzotriazole (TBB), which significantly reduced synaptic CK2 activity (Fig. S6j–k), while maintaining stable synaptic transmission [55]. Western blot analysis (Fig. 6m) showed that TBB blocked the effects of muscle apelin on increasing phosphorylation of GluN2B at the S1480 site (Fig. 6n), but did not affect phosphorylation at the Y1472 site and total GluN2B levels, or phosphorylation of GluN2A at the Y1246 site (Figs. 6o; S6l–o). Calcium-activated neutral proteases, such as calpain-1 and calpain-2, are activated by NMDAR-induced Ca²⁺ influx and are involved in promoting spine enlargement and AMPAR/NMDAR trafficking [56, 57]. Our findings demonstrated that muscle apelin overexpression significantly increased synaptic calpain-2 expression levels, but not calpain-1 (Figs. 6p; S3r–s). However, TBB decreased muscle apelin-mediated calpain-2 expression (Fig. 6p), confirming apelin's action on regulating CK2 activity to regulate calpain-2 expression.

In behavioral tests, inhibiting CK2 activity by TBB attenuated the antidepressant effects of muscle apelin overexpression, as shown in SPT, SST, and FST, but not anxiolytic behavior in OFT (Figs. 6j–l;



S6p-q). Taken together, these data suggest that CK2 is a critical mediator of apelin's action in modulating NMDAR function, and consequently, regulate downstream calpain-2 signaling in the hippocampus to enhance synaptic plasticity and adult neurogenesis.

To determine whether CK2 activity is required for the antidepressant effects of voluntary running, 6-week-old wild-type male mice were subjected to 4 weeks of wheel running, followed by intraperitoneal administration of the CK2 inhibitor TBB

Fig. 4 Knockdown of APJ in hippocampal glutamatergic neurons diminished anti-depressant effects of running. **a** APJ expressions primarily co-labelled with Camk2a⁺ neurons (glutamatergic neurons), rather than GAD67⁺ neurons (GABAergic neurons) in hippocampal dentate gyrus of wild-type C57BL/6J mice (scale bars, 20 μ m at 400 $\times$). **b** Treatment timeline of Camk2a-Cre mice with APJ knockdown using intra-ventral hippocampal injections with AAV. AAV-RFP: AAV-CMV-DIO-RFP-shControl; AAV-shAPLNR: AAV-CMV-DIO-RFP-shAPLNR; Sed: Sedentary; Run: Runner. **c** Confirmation of AAV injection site with RFP expression (scale bars, 500 μ m at 40 $\times$), and **d** knockdown of APJ mRNA expression in ventral hippocampus ($P < 0.05$). APLNR mRNA expression normalized to GAPDH as the housekeeping gene. (**e–g**) Ventral hippocampal APJ knockdown in glutamatergic neurons reduced the antidepressant effect of running, as shown by absence of running-induced increase in **e** sucrose preference in SPT: Interaction $F(1,43) = 7.453$, $P < 0.01$; shAPLNR $F(1,43) = 2.014$, $P > 0.05$; Run $F(1,43) = 7.256$, $P < 0.01$. **f** Running increased grooming time in SST, but APJ knockdown did not show significant effect. Interaction $F(1,43) = 2.327$, $P > 0.05$; shAPLNR $F(1,43) = 0.275$, $P > 0.05$; Run $F(1,43) = 10.17$, $P < 0.01$. **g** and decrease in immobility time in FST: Interaction $F(1,43) = 7.873$, $P < 0.01$; shAPLNR $F(1,43) = 4.100$, $P < 0.05$; Run $F(1,43) = 2.724$, $P > 0.05$. **h** Representative images of immunohistochemical staining Ki-67⁺ (proliferating cells), DCX⁺ (immature neurons) and DCX⁺/BrdU⁺ co-labelling (neuronal differentiation) in the hippocampal dentate gyrus (scale bars, 100 μ m in 100 $\times$). Knockdown of APJ diminished running-induced increases in hippocampal neurogenesis including **i** Ki-67⁺ cells: Interaction $F(1,23) = 23.93$, $P < 0.001$; shAPLNR $F(1,23) = 0.247$, $P > 0.05$; Run $F(1,23) = 17.79$, $P < 0.001$. **j** DCX⁺ cells in DG: Interaction $F(1,20) = 9.807$, $P < 0.01$; shAPLNR $F(1,20) = 5.144$, $P < 0.05$; Run $F(1,20) = 9.650$, $P < 0.01$. **k** BrdU⁺ cells: Interaction $F(1,20) = 9.093$, $P < 0.01$; shAPLNR $F(1,20) = 0.589$, $P > 0.05$; Run $F(1,20) = 19.33$, $P < 0.001$. **l** BrdU⁺DCX⁺/DCX⁺ ratio in DG: Interaction $F(1,20) = 1.406$, $P > 0.05$; shAPLNR $F(1,20) = 0.042$, $P > 0.05$; Run $F(1,20) = 7.959$, $P < 0.05$. Data are presented as mean $\pm$ S.E.M. Statistical significance was assessed by two-way ANOVA with Tukey's post hoc test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. APJ apelin receptor, MCV cytomegalovirus, DIO double-floxed inverse orientation, RFP red fluorescent protein, APLNR apelin receptor gene, OFT open field test, SPT sucrose preference test, SST sucrose splash test, FST forced swim test, DCX doublecortin, DG dentate gyrus.

or vehicle for 4 consecutive days prior to behavioral testing (Fig. S7a).

CK2 inhibition significantly attenuated the running-induced increase in sucrose preference (Fig. S7b), indicating a role for CK2 in mediating running-induced anhedonia relief. In contrast, TBB treatment did not significantly alter grooming behavior in the SPT (Fig. S7c), immobility time in the FST (Fig. S7d), anxiety-like behavior in the OPF (Fig. S7e), or locomotor activity (Fig. S7f). These results indicate that pharmacological inhibition of CK2 diminished the antidepressant effects of running.

DISCUSSION

This study uncovers a novel mechanism by which physical exercise alleviates depression, highlighting the pivotal role of muscle-derived apelin. We demonstrate for the first time that exercise-induced apelin, a myokine, enhances NMDA receptor function in hippocampal glutamatergic neurons through its receptor APJ, which interacts with CK2. This interaction activates downstream calpain signaling, leading to improved hippocampal structural and synaptic plasticity, and resulting in antidepressant effects. Importantly, our findings not only reveal a previously unrecognized pathway mediating muscle-brain communication, but also identify myokine apelin as a promising therapeutic target for depression, particularly for aging populations with muscle loss.

Voluntary running elicits antidepressant effects that correlated with serum apelin levels. Our findings are supported by earlier research demonstrating elevated apelin levels in muscle and serum by physical exercise [12, 14, 58]. A key finding of our study is that muscle-derived apelin can cross the blood-brain barrier (BBB), as evidenced by increased hippocampal apelin levels and the detection of AAV-mediated, Flag-tagged apelin in the hippocampus following muscle-specific apelin overexpression (Figs. 2c–g; S3a–c). This finding aligns with prior evidence that circulating apelin can reach hippocampal regions, possibly through transport or diffusion [41, 42].

Notably, muscle-specific apelin knockout (APLN-mKO) reduced the antidepressant effects of running but did not affect baseline depression-like behavior in the mice. Muscle apelin is crucial for exercise benefits, since muscle apelin knockdown blocked exercise-induced enhancement in muscle strength [12]. However, our finding contrasts with Bullich et al.'s study, which found spontaneous antidepressant-like behavior in systemic apelin knockout mice [59]. These conflicting results highlight the need to distinguish between the central and peripheral roles of apelin. Systemic knockout may trigger compensatory mechanisms, potentially masking the loss of apelin. In contrast, muscle-

specific knockout allows us to isolate the endocrine role of muscle-derived apelin and demonstrates its essential contribution to the antidepressant effects of exercise.

Previous studies have implicated apelin in neuroprotection and antidepressant effects [25, 60], but its role as a myokine that relays exercise signals to the brain remained unknown. Our results showed that APJ knockdown in ventral hippocampal glutamatergic neurons blocked the antidepressant effects of running (Fig. 4e–g). Conversely, elevating apelin levels in muscle mimicked antidepressant-like effects of exercise. This is consistent with findings by previous studies that 7-day or acute administration of apelin-13 reverses depressive behaviors [24, 25, 60–63]. However, the antidepressant role of apelin has been controversial. Lv et al. showed that acute administration of apelin-13 induced the opioid receptor-mediated despair [64], while our study found that APJ knockdown did not affect baseline immobility. This finding aligns with observations from systemic APJ knockout mice, which do not exhibit spontaneous depressive phenotypes [59]. A further consideration is the pharmacokinetic profile of apelin. As apelin peptides are characterized by a short half-life [65], the acute administration studies could not compare the antidepressant effect of long-term exercise-induced endogenous apelin. Notably, the antidepressant effect of apelin in the context of exercise had not been previously evaluated, and our findings provide the first direct evidence for its essential role in mediating exercise-induced mood benefits.

The hippocampus is a key brain region for the antidepressant effects of apelin [61, 66, 67], however, previous studies have not addressed the underlying mechanisms on hippocampal plasticity. Our findings demonstrate that apelin/APJ signaling is required for running to promote both adult neurogenesis and synaptic plasticity in the hippocampal DG. Previous studies have shown that apelin-13 stimulates hippocampal progenitor proliferation [68] and preserves synaptic long-term potentiation in Parkinson's disease [28]. Our research expands our understanding of neuroprotective effects of apelin on promoting both structural and functional plasticity [25, 69]. Beyond its antidepressant effects, the apelin/APJ signaling identified here likely plays a broader role in hippocampal-dependent cognition. Hippocampal synaptic plasticity and adult neurogenesis are fundamental substrates for both mood regulation and the formation of learning and memory [37, 70]. Consistent with this, apelin-13 has been shown to rescue cognitive impairments in various neurodegenerative models, including Alzheimer's and Parkinson's diseases, by enhancing synaptic plasticity and increasing BDNF expression [20, 71, 72]. Although some reports suggest complex effects on short-term memory [73, 74], the enhancement of hippocampal plasticity

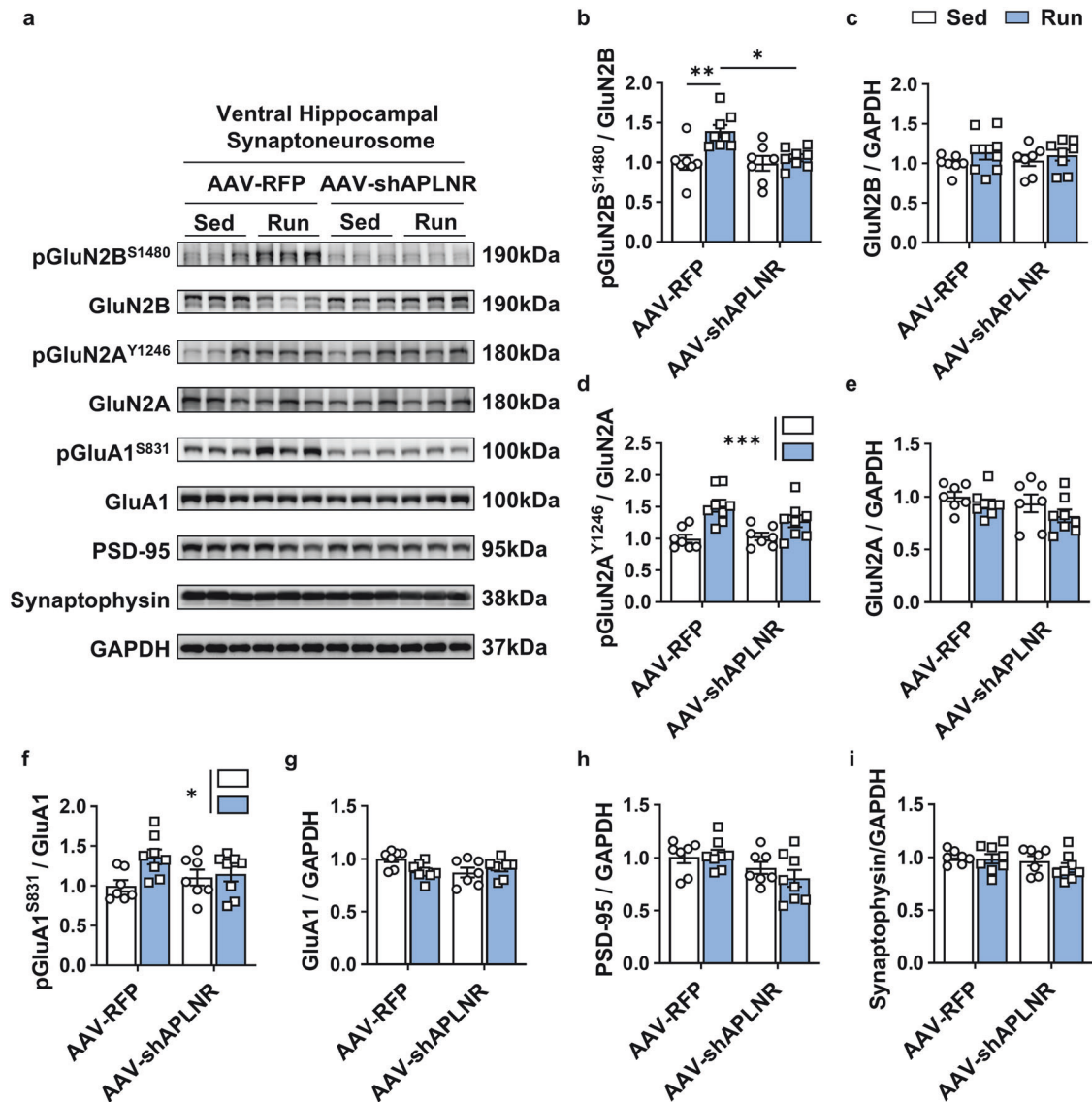
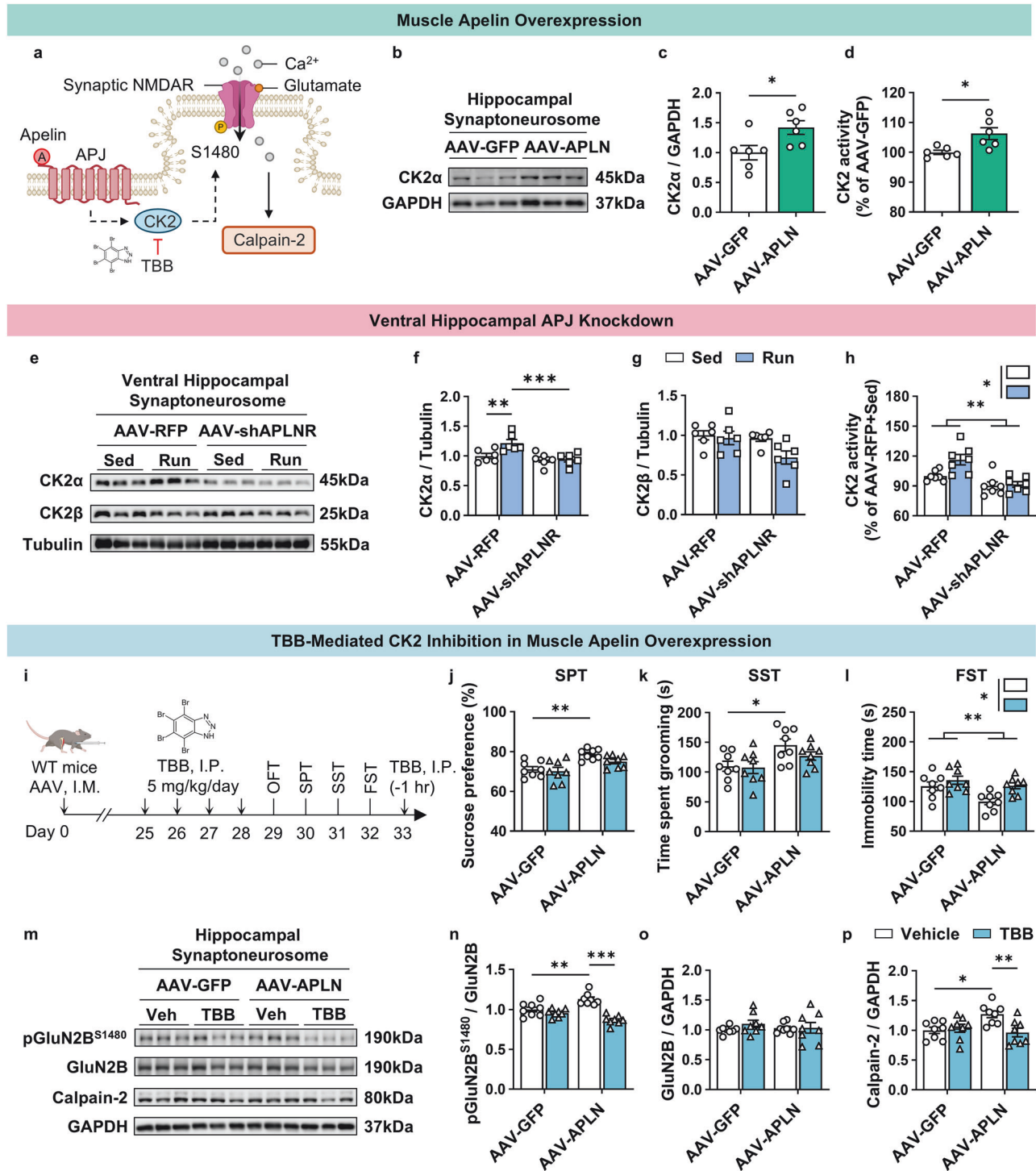


Fig. 5 Knockdown of APJ in hippocampal glutamatergic neurons diminishes the beneficial effects of running on enhancing synaptic NMDA receptor expression. **a** Western blotting analysis using hippocampal synaptoneurosomes. AAV-RFP: Camk2a-Cre + AAV-DIO-RFP; AAV-shAPLNR: Camk2a-Cre + AAV-DIO-shAPLNR; Sed: Sedentary; Run: Runner. **b** Knockdown of APJ attenuated running-induced increases in phosphorylation of GluN2B S1480: Interaction $F(1,26) = 4.535$, $P < 0.05$; shAPLNR $F(1,26) = 5.129$, $P < 0.05$; Run $F(1,26) = 9.211$, $P < 0.01$. Running increased phosphorylation of **d** GluN2A, but APJ knockdown had no significant effects: Interaction $F(1,26) = 2.694$, $P > 0.05$; shAPLNR $F(1,26) = 1.555$, $P > 0.05$; Run $F(1,26) = 20.38$, $P < 0.001$. **f** GluA1 S831: Interaction $F(1,26) = 3.157$, $P > 0.05$; shAPLNR $F(1,26) = 0.399$, $P > 0.05$; Run $F(1,26) = 5.100$, $P < 0.05$. Neither APJ knockdown nor running altered total protein levels of: **c** GluN2B: Interaction $F(1,26) = 0.264$, $P > 0.05$; shAPLNR $F(1,26) = 6.513 \times 10^{-7}$, $P > 0.05$; Run $F(1,26) = 2.037$, $P > 0.05$. **e** GluN2A: Interaction $F(1,26) = 0.177$, $P > 0.05$; shAPLNR $F(1,26) = 2.189$, $P > 0.05$; Run $F(1,26) = 2.435$, $P > 0.05$. **g** GluA1: Interaction $F(1,26) = 5.522$, $P > 0.05$; shAPLNR $F(1,26) = 2.156$, $P > 0.05$; Run $F(1,26) = 1.245$, $P > 0.05$. **h** PSD-95: Interaction $F(1,26) = 0.873$, $P > 0.05$; shAPLNR $F(1,26) = 6.574$, $P < 0.05$; Run $F(1,26) = 0.395$, $P > 0.05$. **i** Synaptophysin: Interaction $F(1,26) = 0.364$, $P > 0.05$; shAPLNR $F(1,26) = 2.032$, $P > 0.05$; Run $F(1,26) = 0.838$, $P > 0.05$. Data are presented as mean $\pm$ SEM. Statistical significance was assessed by two-way ANOVA with Tukey's HSD post hoc test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Sed sedentary group, Run running group, RFP red fluorescent protein, APLNR apelin receptor gene, PSD-95 postsynaptic density protein 95.

observed in our study suggests that exercise-induced apelin may serve a dual role in both alleviating behavioral despair and improving cognitive flexibility. Future research utilizing a broader battery of cognitive assays, such as the Y-maze or novel object recognition, to evaluate hippocampal-dependent cognitive function will be necessary to strengthen the behavioral evidence and improve the translational relevance of the findings to humans.

Mechanistically, our findings highlight the novel role of muscle-secreted apelin in modulating NMDAR in the hippocampus. Apelin enhances NMDAR function with its promoting effect on phosphorylation of the GluN2B subunit at S1480 and Y1472, as well as

phosphorylation of the GluN2A subunit at Y1246, without altering the phosphorylation of AMPA receptors. Functionally, phosphorylation at GluN2B-S1480 regulates receptor localization and is implicated in the maturation of synaptic transmission [53, 75, 76]. Apelin-36 is known to promote neuroprotection by phosphorylating GluN2B at S1480, thereby reducing calpain activation and Ca^{2+} accumulation [51]. Meanwhile, phosphorylation at GluN2B-Y1472 prevents clathrin-mediated endocytosis and increase surface retention of GluN2B-containing NMDARs, which enhances synaptic strength and long-term potentiation [77–80]. Similarly, GluN2A-Y1246 phosphorylation has been reported to reduce receptor



internalization and promote surface expression, supporting stable synaptic function [80–83]. By enhancing phosphorylation at these critical sites, apelin could potentiate synaptic NMDA currents. Thus, apelin enhances synaptic functions by its action on enhancing the NMDAR function.

An important mechanistic question arising from these findings is how enhanced NMDA receptor signaling and adult neurogenesis are functionally related in mediating the antidepressant effects of apelin. Although our data demonstrate that muscle-derived apelin promotes both hippocampal neurogenesis and NMDA receptor-dependent synaptic plasticity, these processes

are unlikely to represent independent parallel mechanisms. Extensive prior evidence indicates that adult hippocampal neurogenesis can be regulated by NMDA receptor [84]. In particular, dentate gyrus-specific deletion of the obligatory NMDA receptor subunit NR1 (GluN1) impairs adult neurogenesis [85], while NMDA receptor-dependent long-term potentiation enhances the survival of newborn neurons during a critical postmitotic window [86]. These effects are further shaped by the developmentally regulated switch from GluN2B- to GluN2A-containing NMDA receptors, which differentially support early dendritic growth and synaptic integration versus mature synaptic

Fig. 6 Apelin/APJ interacts with Casein Kinase 2 to phosphorylate GluN2B-pS1480 of NMDA receptor. **a** Mechanistic hypothesis: Apelin binding to APJ interacts with casein kinase 2 (CK2), leading to phosphorylates GluN2B at S1480, to potentiates NMDAR function, consequently upregulate calpain-2 to enhance hippocampal plasticity. Figure created with BioRender.com. **b** Western blotting analysis showing hippocampal synaptoneurosome CK2 levels in AAV-GFP (Control) mice and AAV-APLN mice. **c** Muscle apelin overexpression increases CK2 α in hippocampal synaptoneurosome ($P < 0.05$). **d** Muscle apelin overexpression promotes CK2 activity in hippocampal synaptoneurosome ($P < 0.05$). **e** Western blotting analysis shows CK subunits in ventral hippocampal synaptoneurosome. AAV-RFP: Camk2a-Cre + AAV-DIO-RFP; AAV-shAPLN: Camk2a-Cre + AAV-DIO-shAPLN; Sed: sedentary; Run: runner. Knockdown of APJ attenuates running-induced increases in CK2 α , CK2 β expression: **f** CK2 α : Interaction $F(1, 20) = 4.769, P < 0.05$; shAPLN $F(1, 20) = 11.61, P < 0.01$; Run $F(1, 20) = 5.119, P < 0.05$. **g** CK2 β : Interaction $F(1, 20) = 2.288, P > 0.05$; shAPLN $F(1, 20) = 4.311, P > 0.05$; Run $F(1, 20) = 4.037, P > 0.05$. **h** Running promoted hippocampal CK2 activity, while APJ knockdown showed opposite effects. Interaction $F(1, 24) = 4.128, P > 0.05$; shAPLN $F(1, 24) = 21.73, P < 0.01$; Run $F(1, 24) = 5.794, P < 0.05$. **i** Wild-type mice received intramuscular injections of AAVs overexpressing apelin (AAV-APLN) or GFP control (AAV-GFP), followed by intraperitoneal administration of the CK2 inhibitor TBB or vehicle (DMSO) daily for 4 consecutive days before behavioral tests and a single injection 1 h before hippocampal collection. AAV-GFP + Vehicle: Control + DMSO; AAV-GFP + TBB: Control + TBB; AAV-APLN + Vehicle: Muscle apelin overexpression + DMSO; AAV-APLN + TBB: Muscle apelin overexpression + TBB. CK2 inhibition reduced the antidepressant effect of apelin, as shown by TBB blocked apelin's effects on increasing: **j** sucrose preference in SPT: Interaction $F(1,28) = 0.960, P > 0.05$; AAV $F(1,28) = 20.24, P < 0.001$; TBB $F(1,28) = 2.755, P > 0.05$. **k** grooming time in SST: Interaction $F(1,28) = 0.783, P > 0.05$; AAV $F(1,28) = 9.325, P < 0.01$; TBB $F(1,28) = 1.307, P > 0.05$. **l** Running reduced immobility time in FST, while CK2 inhibition by TBB promoted immobility: Interaction $F(1, 28) = 1.697, P > 0.05$; AAV $F(1, 28) = 7.881, P < 0.01$; TBB $F(1, 28) = 8.767, P < 0.01$. **m-p** Western blotting analysis using hippocampal synaptoneurosome proteins. CK2 inhibition by TBB treatment blocked effects of apelin on enhancing p-GluN2B and calpain-2. **n** GluN2B S1480 phosphorylation: Interaction $F(1,28) = 20.32, P < 0.001$; AAV $F(1,28) = 0.739, P > 0.05$; TBB $F(1,28) = 41.07, P < 0.001$. **o** Total GluN2B: Interaction $F(1,28) = 0.677, P > 0.05$; AAV $F(1,28) = 0.091, P > 0.05$; TBB $F(1,28) = 0.884, P > 0.05$. **p** Calpain-2: Interaction $F(1,28) = 7.499, P < 0.05$; AAV $F(1,28) = 2.443, P > 0.05$; TBB $F(1,28) = 4.647, P < 0.05$. Data are presented as mean $\pm$ SEM. Statistical significance was assessed by two-way ANOVA with Tukey's HSD post hoc test for **f-p** and unpaired t-test for **c-d**, as appropriate. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. CK2 casein kinase 2, TBB 4,5,6,7-tetrabromobenzotriazole, CK2 inhibitor, GFP green fluorescent protein, APLN apelin gene, RFP red fluorescent protein, APLNR apelin receptor gene, SPT sucrose preference test, SST sucrose splash test, FST forced swim test. WT wild-type mice.

transmission and long-term potentiation [87]. The enhancement of NMDA receptor function observed in our study likely acts upstream to facilitate the neurogenic effects of apelin. The present study does not directly dissociate the relative contributions of neurogenesis versus glutamatergic synaptic modulation to the observed antidepressant-like behaviors. While selectively blocking either adult neurogenesis or NMDA receptor-mediated transmission would provide further mechanistic resolution.

Importantly, CK2 plays a crucial role in the communication between the APJ and the NMDAR subunit GluN2B. CK2 specifically targets the GluN2B-pS1480 residue, but does not phosphorylate the adjacent Y1472 site [53]. In contrast, GluN2B-pY1472 is a canonical site for Src-family kinases, whose phosphorylation enhances PSD-95 binding and stabilizes NMDA receptors [76]. Similarly, the analogous tyrosine residue on GluN2A (at Y1246) is phosphorylated by non-CK2 kinases, such as Src/Pyk2-family tyrosine kinases [88]. Our experiments showed that CK2 inhibition specifically blocked muscle apelin-mediated GluN2B S1480 phosphorylation, without affecting Y1472 or GluN2A Y1246 phosphorylation. This finding supports Cook et al.'s report on apelin-36-driven GluN2B S1480 phosphorylation in neuroprotection [51]. The specificity of CK2-mediated signaling allows for the selective remodeling of synaptic NMDAR by activating GluN2B subunits, without altering the broader tyrosine-phosphorylation code that governs receptor anchoring [76].

Apelin-13 enhances CK2 α expression and activity by interacting with the Gai and Gq sites of the APJ receptor, which alleviate ER stress-mediated neuronal apoptosis [52]. Additionally, the coupling of CK2 α to calpain-2 upregulation further extends apelin's role by linking NMDAR to calpain-dependent synaptic remodeling [89–92]. Phosphorylation at the S1480 site stabilizes NMDAR at synapses, increasing Ca²⁺ influx and activating calpain-2 (Figs. 6p; S3r), this activation promotes LTP consolidation through mTOR-dependent protein synthesis [92]. Unlike calpain-1, which is involved in the acute induction of LTP [90], calpain-2 serves as a novel effector of apelin's sustained synaptic effects, highlighting its unique role in maintaining synaptic plasticity over time.

We observed no changes in synaptic markers despite enhanced neurogenesis and NMDAR. This finding is consistent with Luo et al.'s study in healthy mice, where apelin-13 increased synaptic activity without affecting synaptophysin levels [93]. This suggests that apelin's effects are mediated through post-translational modifications, such as phosphorylation, rather than through structural synaptic changes. Our results identify apelin as the myokine that promotes both hippocampal neurogenesis and

NMDAR-dependent synaptic plasticity through the APJ/CK2 signaling pathway following exercise (Fig. S8).

While the present study establishes a mechanistic link between exercise-induced apelin and hippocampal plasticity using male mice, it remains unclear whether these findings generalize to females. Given that progesterone modulates synaptic plasticity [94] and estrogen generally up-regulating while progesterone down-regulating apelin/APJ expression signaling, this study was limited to male mice [95, 96]. Females have a higher prevalence of depression [97] and lower muscle mass compared to males [98], raising the question of whether females produce comparable levels of apelin in response to exercise. Sex-specific differences in muscle mass, basal apelin levels [99, 100], and hormonal regulation of synaptic plasticity may alter the kinetics or magnitude of the muscle apelin production in females in response to physical exercise. Future studies should include female mice to examine whether difference in muscle mass affect apelin production in response to physical exercise. Furthermore, although our study utilized an AAV construct encoding full-length preproapelin, which can be processed into multiple isoforms, we did not specifically determine which apelin isoform(s) are predominantly induced by exercise or responsible for the observed effects; future studies employing isoform-specific detection methods will be necessary to clarify the precise contributions of individual apelin isoforms to hippocampal plasticity.

This study significantly advances our understanding of the muscle-brain axis by establishing apelin as a key myokine that coordinates hippocampal neurogenesis and NMDAR-dependent plasticity through the APJ/CK2 pathway. It offers novel insights into the molecular mechanisms of exercise-induced antidepressant effects. Moreover, the findings underscore the role of muscle-derived apelin in modulating hippocampal plasticity, highlighting the potential impact of sarcopenia-related apelin disruption in depression. This discovery has significant implications for understanding how decreased apelin levels associated with sarcopenia may increase the risk of depression in the elderly and paves the way for therapeutic strategies targeting muscle health for depression treatment.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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