Research

Voluntary running depreciates the requirement of Ca^{2+}-stimulated cAMP signaling in synaptic potentiation and memory formation

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Mental health and cognitive functions are influenced by both genetic and environmental factors. Although having active lifestyle with physical exercise improves learning and memory, how it interacts with the specific key molecular regulators of synaptic plasticity is largely unknown. Here, we examined the effects of voluntary running on long-term potentiation (LTP) and memory formation in mice lacking type I adenylyl cyclase (ACI), a neurospecific synaptic enzyme that contributes to Ca^{2+}-stimulated cAMP production. Following I mo of voluntary running-wheel exercise, the impaired LTP and object recognition memory in ACI knockout (KO) mice were significantly attenuated. Running up-regulated exon II mRNA level of BDNF (brain-derived neurotrophic factor), though it failed to increase exon I and IV mRNAs in the hippocampus of ACI KO mice. Intrahippocampal infusion of recombinant BDNF was sufficient to rescue LTP and object recognition memory defects in ACI KO mice. Therefore, voluntary running and exogenous BDNF application overcome the defective Ca^{2+}-stimulated cAMP signaling. Our results also demonstrate that alteration in Ca^{2+}-stimulated cAMP can affect the molecular outcome of physical exercise.

[Supplemental material is available for this article.]

The impact of gene–environment interactions on human health has been reported for decades. Certain lifestyles such as physical exercise benefit cognitive function (Cotman et al. 2007; van Praag 2009) and ameliorate symptoms associated with aging and neurodegeneration. For instance, physical exercise has been reported to improve vocabulary learning in young and aged humans (Yaffe et al. 2001; Winter et al. 2007), increase hippocampus size and enhance memory (Erickson et al. 2011), and prevent cognitive decline in the elderly (Etnier et al. 2007; Deeny et al. 2008). Exercise also attenuates defective neuronal functions in animal models of spinocerebellar ataxia type 1 (Fryer et al. 2011), Parkinson's disease (Lau et al. 2011), and Alzheimer's disease (Adlard et al. 2005; Nichol et al. 2007). A significant knowledge gap in better understanding the beneficial effects and the underlying mechanism of physical exercise is whether it can exert its effects on defects in specific signaling pathways that regulate synaptic plasticity and memory formation.

The role of cAMP signaling in regulating synaptic plasticity and learning and memory has been strongly demonstrated in both invertebrates and vertebrates (Yin et al. 1994; Abel et al. 1997). As the Ca^{2+} influx through NMDA receptors and voltage-gated calcium channels is tightly coupled to neuronal activity, the Ca^{2+}-stimulated adenylyl cyclases (AC) are essential to sense neuronal stimulation and generate cAMP. As type 1 AC (AC1) is a major neurospecific Ca^{2+}-stimulated AC, it is hypothesized that the AC1-mediated cAMP production is required for activity-dependent neuronal signaling and neuroplasticity (Wang and Zhang 2012). Previous studies have demonstrated that AC1 is required for hippocampus-dependent spatial memory (Wu et al. 1995), remote fear memory (Shan et al. 2008), and synaptic long-term potentiation (LTP) in the cerebellum (Storm et al. 1998) and the anterior cingulated cortex (Liauw et al. 2005).

Although how cAMP signaling regulates synaptic plasticity and learning is not clear, previous studies implicated that the Ca^{2+}-stimulated adenylyl cyclases are required for the activity-dependent transcriptional up-regulation of plasticity-related and CREB (cAMP responsive element binding protein)-regulated genes including BDNF (Sindreu et al. 2007; Zheng et al. 2011, 2012). It is established that the up-regulation of BDNF is a major molecular outcome following physical exercise (Cotman et al. 2007), and the level of BDNF is directly related to the maintenance of LTP and long-term memory formation (Zheng et al. 2012). To better understand gene–environment interaction as well as the functional cross-talk between BDNF and cAMP signaling, we aim to determine whether lack of intact cAMP signaling in AC1 KO mice interferes the exercise-induced BDNF up-regulation, and

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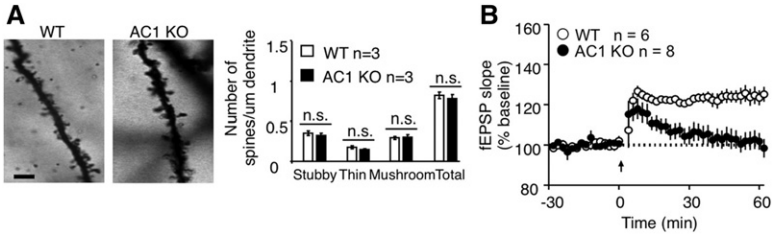

Figure 1. Mice lacking AC1 show defective LTP at the Schaffer collateral CA1 synapses in vivo. (*A*) Brain sections were obtained from WT and AC1 KO mice. Density of different types of dendritic spine (as indicated in the *right* panel) in the hippocampal CA1 region (*right* panel) was visualized and determined by Golgi staining. Scale bar: 8 μm. (n.s.) Not significant. (*B*) WT and AC1 KO mice were anesthetized, and subjected to electrophysiology recording. Following the establishment of baseline fEPSP at the Schaffer collateral-CA1 synapses in dorsal hippocampus, LTP was induced by a single HFS (100 Hz for 1-sec duration, as indicated by the arrow). Percentage of potentiation was determined by the ratio of post-HFS fEPSP to baseline fEPSP. Two-way repeated measures ANOVA reveals significant difference between WT and AC1 KO mice.

whether exercise and BDNF affect plasticity and memory in AC1 KO mice.

Here, we subjected mice lacking AC1 (i.e., AC1 KO mice) to voluntary wheel-running to determine how exercise interacts with the Ca^{2+}-stimulated cAMP signaling. First, we found that AC1 KO mice are defective in LTP at the CA1 synapses and object recognition memory. Next, we found that 1-mo voluntary running dampened these synaptic and behavioral impairments in AC1 KO mice. We further demonstrated that running elevated BDNF expression and application of exogenous BDNF mimicked the beneficial effects of exercise in AC1 KO mice. Interestingly, lack of AC1 activity altered the expression pattern of BNDF mRNA following running, indicating that genetic factors may affect the molecular outcomes of physical exercise. Our data identified a novel function of physical exercise to lessen the requirement of Ca^{2+}-stimulated cAMP signaling in synaptic potentiation and memory formation.

Results

AC1 is required for hippocampal LTP and object recognition memory

The expression of AC1 is restricted to the central nervous system (Xia et al. 1993). As AC1 activity accounts for the majority of the Ca^{2+}-stimulated AC activity in the hippocampus (Wu et al. 1995), it may play essential roles in regulating synaptic function. Here, we found that the dendritic spine morphology and density in the hippocampus were comparable between wild-type (WT) and AC1 KO mice (Fig. 1A). AC1 KO mice also showed normal basal neural transmission and paired-pulse facilitation in vivo (Supplemental Fig. S1). Previous studies demonstrated that the rising phase of LTP at the CA1 synapses is slightly impaired in hippocampal slices obtained from AC1 KO mice; the expression of LTP is relatively normal (Wu et al. 1995; Wong et al. 1999). Here, we found that, in anesthetized mice, genetic deletion of AC1 caused significant impairment in LTP induced by a single HFS (genotype: $F_{(1,12)} = 9.5$, $P < 0.01$; genotype × time: $F_{(34,408)} = 5.8$, $P < 0.01$) (Fig. 1B). A stronger induction paradigm with two trains of HFS resulted in comparable LTP in AC1 KO and WT mice (genotype: $F_{(1,10)} = 0.0$, $P = 1.0$; genotype × time: $F_{(34,340)} = 1.4$, $P = 0.09$) (Supplemental Fig. S2).

We next examined hippocampus-dependent learning in AC1 KO mice. Consistent with the previous report (Wong et al. 1999), AC1 KO mice displayed normal passive avoidance memory when tested 1 and 31 d after training (Supplemental Fig. S3). We next examined object recognition memory, which requires neuronal activity in the hippocampus (Clark et al. 2000; Cohen et al. 2013) and does not involve aversive stimulus. During training, WT and AC1 KO mice showed equal preference to object A and object B (Fig. 2A). During testing, one familiar object (object B) was replaced by a novel object (object C). WT but not AC1 KO animals showed preference to the novel object C (Fig. 2B,C), indicating that AC1 activity is required for object recognition memory.

AC1 KO mice show normal behavioral responses to acute stress

As cognitive dysfunctions may be partially caused by abnormalities in anxiety and stress response, we performed several relevant behavioral examinations. In the light–dark test, AC1 KO mice showed normal transition between the lit and dark chambers, latency to enter the lit chamber, and time spent in the lit chamber (Supplemental Fig. S4). In a novel open field, WT and AC1 KO mice showed comparable locomotor activity. They also showed comparable activity in the center area of the open field arena and number of entries to the center (Supplemental Fig. S5). Thus, AC1 may not play a critical role in regulating anxiety.

The AC1 KO mice showed comparable immobility to that of WT animals in the tail suspension test (Supplemental Fig. S6). In the three-chamber social interaction test, AC1 KO mice showed less number of entry to the chamber with the stranger mouse (Supplemental Fig. S7a), but spent normal time in the "stranger mouse" chamber (Supplemental Fig. S7b) and displayed normal direct interaction with the stranger mouse (Supplemental Fig. S7c). These results indicate that AC1 KO mice are capable of coping with acute stresses and are emotionally stable.

Voluntary running attenuates the defective LTP and object recognition memory in AC1 KO mice

Previous reports have demonstrated that environmental factors and life styles may affect synaptic function and cognition. We examined whether voluntary wheel running affects LTP and memory formation in AC1 KO mice. Following 1 mo of voluntary running, AC1 KO mice showed significant LTP (Fig. 3A,B), though the level of synaptic potentiation was still less than the WT controls (one-way ANOVA, group: $F_{(2,18)} = 9.4$, $P < 0.01$) (Fig. 3B).

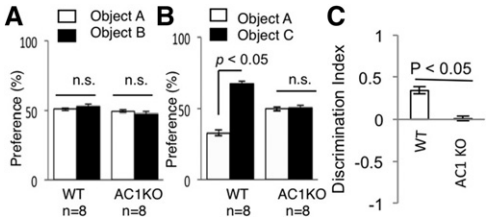

Figure 2. Mice lacking AC1 are defective in object recognition memory. During training, mouse was allowed to explore and interact with Object A and B for 10 min (*A*). During testing, the trained mouse was exposed to one old object (i.e., Object A) and one new object with different shape and color (Object C) (*B*,*C*) for 5 min. The number of sniffing/approaches for each object during training and testing was recorded. Object preference (*A*,*B*) and object discrimination index [(new object interaction − old object interaction)/total objects interaction] (*C*) are presented. (n.s.) Not significant.

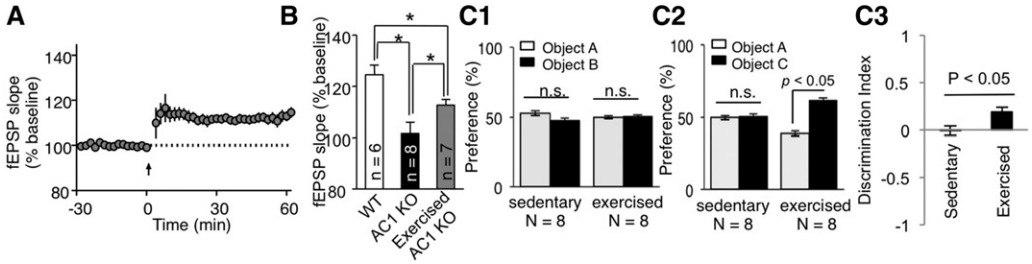

Figure 3. Voluntary running attenuates the impaired synaptic potentiation and object recognition memory in AC1 KO mice. AC1 KO mice were subjected to a locked (the sedentary group) or unlocked running wheel (the exercised group) for 1 mo, during which the animal was individually housed and have full-time free access to the running wheel. (*A*) Following the 1-mo voluntary running, mouse was anesthetized and fEPSP at the Schaffer collateral-CA1 synapses in dorsal hippocampus was determined. LTP was induced by a single HFS (100 Hz for 1-sec duration, as indicated by the arrow in *A*). Percentage of potentiation was determined by the ratio of post-HFS fEPSP to baseline fEPSP. (*B*) The average of synaptic potentiation during the last 10 min of recording was compared among WT, sedentary AC1 KO, and exercised AC1 KO mice. (*C*) To examine object recognition memory, the sedentary and exercised AC1 KO mice were exposed to two objects (objects A and B) for 10 min during training, and showed comparable preference to the two objects (*C*1). During the 5-min testing, object B was replaced by the novel object C (*C*2,*C*3). Object preference (*C*1,*C*2) (% interaction with object A and B during training or % interaction with object *A* and *C* during testing) and object discrimination index [(novel object interaction − old object interaction)/total objects interaction] (*C*3) are presented. The exercised but not the sedentary AC1 KO mice showed preference to the novel object (i.e., object C) during testing (*C*2,*C*3). (n.s.) Not significant. (*) $P < 0.05$ between the indicated groups.

Next, we examined whether voluntary running can overcome the deficits in cAMP signaling and rescue memory formation. Comparing to the sedentary AC1 KO mice, there was significant object recognition memory in the exercised AC1 KO mice (Fig. 3C).

Voluntary exercise enhances BDNF expression in ACI KO mice

It has been demonstrated that voluntary exercise increases BDNF expression in rodents (Neeper et al. 1995; Adlard et al. 2004). As BDNF facilitates synaptic potentiation and memory formation (Figurov et al. 1996; Gorski et al. 2003; Waterhouse and Xu 2009), we surmise that the rescue of LTP and memory deficits in exercised AC1 KO mice may correlate with increased BDNF expression following running. We found that 1-mo voluntary running increased total BDNF mRNA in the hippocampus of both WT and AC1 KO mice (Fig. 4A1,B1). Interestingly, the pattern of the increased BDNF mRNA isoforms in AC1 KO hippocampus was different from that in WT samples. WT mice showed increase in exon I-, II-, and IV-containing BDNF mRNAs (Fig. 4A2), whereas only exon II mRNA was significantly increased in AC1 KO mice (Fig. 4B2).

To further investigate whether AC1 activity is required for the up-regulation of specific isoforms of BDNF mRNA, we subjected mice to short-term running, which has been shown to only induce exon I expression in the hippocampus (Adlard et al. 2004). In contrast to WT mice (Fig. 4C), one-day running did not increase exon I-containing BDNF mRNA in AC1 KO animals (Fig. 4D). These data demonstrate that the running-induced transcription of exon I and exon IV BNDF mRNA requires AC1 activity.

To exclude the possibility that lack of specific exon up-regulation in exercised AC1 KO is due to activity difference, we compared running behavior in WT and AC1 KO mice. First, AC1 KO and WT mice showed comparable running time, running distance, and maximal speed (Supplemental Fig. S8), indicating that lack of AC1 expression does not affect motivation and physical strength. The general locomotor activity in the home cage was also not different between WT and AC1 KO animals. The activity pattern was similar between WT and AC1 KO animals. Quantitative analysis revealed no significant difference in total daily activity or in activities during the daytime and the nighttime (Supplemental Fig. S9).

Exogenous BDNF rescues impaired LTP and recognition memory in ACI KO mice

The correlation between BDNF up-regulation and correction effects on LTP and memory deficits suggests a possibility that elevation of BDNF level may override the impaired cAMP signaling and correct the defective LTP and object recognition memory in AC1 KO mice. To test this possibility, we infused recombinant BDNF protein into the dorsal hippocampus of the AC1 KO mice.

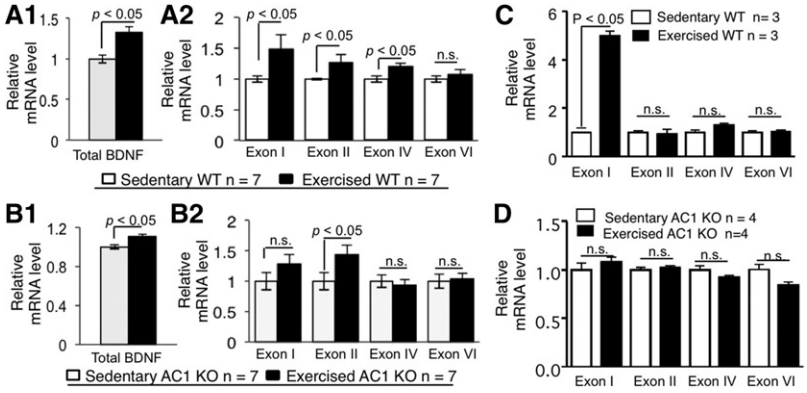

Figure 4. Voluntary running up-regulates specific isoform of BDNF mRNA in the hippocampus of AC1 KO mice. The hippocampus was collected from mice following voluntary wheel running for 1 mo (*A*,*B*) or 1 d (*C*,*D*), during which the sedentary and exercised mouse were individually housed and had full-time free access to locked and unlocked running wheel, respectively. The level of total BDNF mRNA (*A*1,*B*1) and different isoforms of BDNF mRNA (*A*2,*B*2,*C*,*D*) were determined by quantitative RT-PCR, and compared between the sedentary and the exercised groups as indicated. The level of different BDNF mRNA isoforms was normalized to the level of GAPDH mRNA, which is comparable among all four experimental groups. The level of different BDNF mRNA isoforms in the sedentary WT group was defined as 1, and used to determine the relative changes in the other three groups including exercised WT, sedentary AC1 KO, and exercised AC1 KO mice. (n.s.) Not significant.

While vehicle infusion did not significantly alter LTP in WT and AC1 KO mice (Fig. 5A,B), infusion of BDNF did not cause measurable changes in WT mice but elevated LTP in AC1 KO mice to the WT level (Fig. 5A,B). We next examined whether exogenous BDNF can correct memory deficits in AC1 KO mice (Fig. 5C1). Pretraining intrahippocampus infusion of BDNF did not affect animals' interaction with the objects during training (Fig. 5C2). Although BDNF did not enhance novel object preference in WT mice during testing, the BDNF-injected but not the vehicle-injected AC1 KO mice showed preference for the novel object (Fig. 5C3). The novel object discrimination index in the BDNF-injected AC1 KO mice was comparable to that of vehicle- and BDNF-injected WT mice (Fig. 5C4). These results demonstrate that BDNF is sufficient to override the requirement of Ca^{2+}-stimulated cAMP signaling in synaptic plasticity and memory formation.

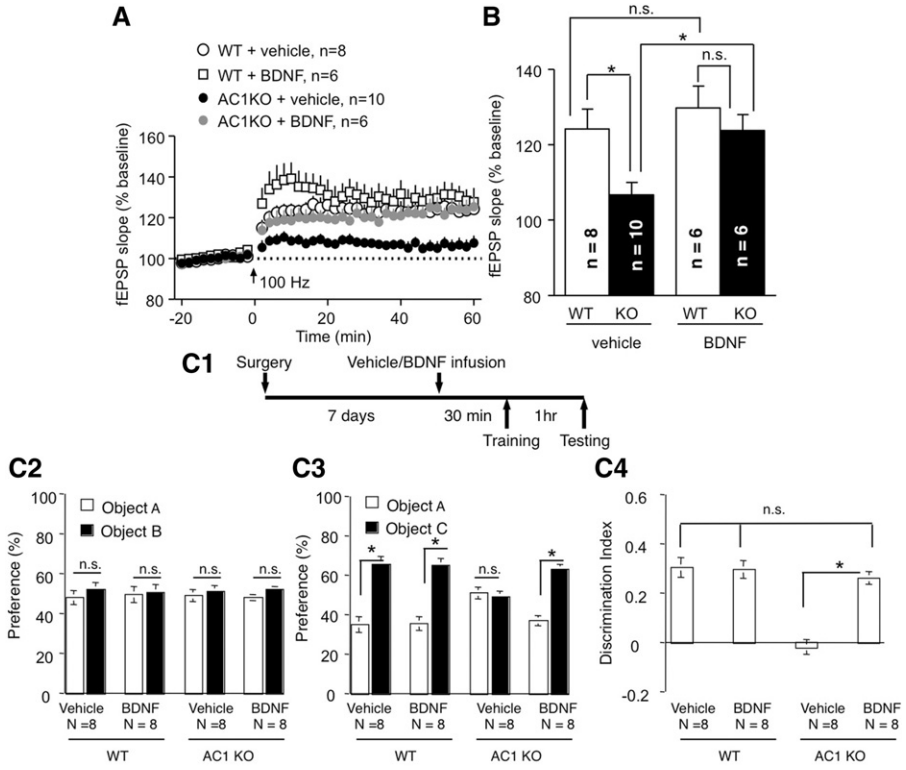

Figure 5. Intrahippocampal infusion of recombinant BDNF rescues the defective LTP and object recognition memory in AC1 KO mice. (*A*) Following anesthetization, BDNF or vehicle was delivered to the dorsal CA1 region of WT and AC1 KO mice. After baseline fEPSP was established at the Schaffer collateral-CA1 synapses, LTP induced by a single HFS train (100 Hz, 1 sec, as indicated by the arrow) was examined and compared. (*B*) The average of synaptic potentiation during the last 10 min of recording is shown. (*C*1) The protocol used to examine the effect of BDNF or vehicle infusion to the dorsal hippocampus of WT and AC1 KO mice on object recognition memory. (*C*2) Thirty minutes following infusion of vehicle or BDNF to the dorsal hippocampus, AC1 KO mice were exposed to two objects (objects A and B) during the 10-min object recognition memory training. The number of sniffing/approaches for object A and B was recorded. The vehicle- and BDNF-treated AC1 KO mice showed comparable preference for object A and B (i.e., percentage of total object interaction). (*C*3,*C*4) During the 5-min testing, AC1 KO mice infused with vehicle or BDNF were exposed to one old object (i.e., object A) and a novel object (i.e., object C). The number of sniffing/approaches for object A and C was recorded. Infusion of BDNF but not vehicle resulted in robust preference to the novel object C during testing. Object preference (*C*3) and object discrimination index [(novel object interaction − old object interaction)/total objects interaction] (*C*4) are presented. Two-way ANOVA followed by pairwise comparison was used for statistics determination. (n.s.) Not significant. (*) $P < 0.05$.

Discussion

Lines of evidence support the beneficial effects of physical exercise on higher order brain functions such as mental health and cognition. However, as a critical step to better understand gene–environment interaction, how exercise can overcome the specific defects in intracellular signaling has not been investigated. Individuals with different genotypes may also respond differently to exercise and show different outcomes. One novel aspect of this study is that voluntary running attenuates the defective LTP and hippocampus-dependent memory in mice lacking a key enzyme that links neuronal activity and Ca^{2+} to cAMP signaling. We chose voluntary exercise, as it is a voluntary decision and occurs naturally without exogenous intervention. Although forced exercise can be considered as a consequence of stressful intervention, some reports show that forced exercise also increases BDNF and has some beneficial effects on neuronal function (Lin et al. 2015). It would be interesting to test how forced exercise affects LTP and memory in AC1 KO mice in future studies. We further demonstrate that running induces BDNF elevation, and application of exogenous BDNF is sufficient to rescue synaptic and memory defects in AC1 KO mice. It is important to emphasize that exercise may alter numerous molecular changes other than BDNF. As complete depletion or excessive inhibition of BDNF impairs LTP and memory, future study should aim to determine the necessary role of exercise-induced BDNF up-regulation by precise dampening of BDNF to the pre-exercise level. Our data suggest a previously unidentified cross-talk between BDNF and the Ca^{2+}-stimulated cAMP signaling. On one hand, lack of intact cAMP signaling in AC1 KO mice alters the exon-specific up-regulation of BDNF mRNA after running. On the other hand, enhancement of BDNF overrides the defective cAMP signaling and rescues the impaired LTP and object memory in AC1 KO mice.

AC1 expression is only detected in the central nervous system (Xia et al. 1993) and concentrated at the post-synaptic density (Conti et al. 2007). Previous studies found that AC1 KO mice are defective in spatial memory retention, but show normal CA1 LTP in hippocampal slices (Wu et al. 1995; Wong et al. 1999). Here, we found that, in anesthetized AC1 KO mice, a single HFS at 100 Hz failed to induce significant LTP in vivo. In contrast, two HFS trains induced LTP that is comparable to the WT level. The stimulation-dependent synaptic phenotype is correlated with the training-dependent memory defects. While AC1 KO mice showed normal memory following passive avoidance training, the object recognition memory, which does not involve reinforcement with noxious stimulus, was impaired in AC1 KO mice. These data suggest that strong synaptic stimulation or association training procedures may recruit other molecular regulators to compensate the defects in cAMP signaling.

It has been established that BDNF up-regulation in brain neurons is a prominent molecular outcome following physical exercise (Neeper et al. 1995). Our data show that the pattern of exercise-induced changes of BDNF is regulated by AC1 in vivo.

The BDNF gene contains nine 5′ noncoding exons (exons I–IXA) and one 3′ coding exon (exon IX). After splicing, the mature BDNF mRNA contains only one of the noncoding exons fused with the common coding exon. Thus, all BDNF mRNA isoforms are translated to the same proBDNF protein, which is further processed to form mature BDNF (Aid et al. 2007). Among all the BDNF mRNA isoforms, the most abundant forms are exon I-, II-, IV-, and VI-containing BDNF mRNA (BDNF-I, II, IV, and VI). We found that, partially consistent with the results from studies with other long-term exercise paradigms (Adlard et al. 2004; Zajac et al. 2010), 1-mo running increased BDNF-I, II and IV in mouse hippocampus. It is hypothesized that the different isoforms driven by different promoters may allow temporal and spatial regulation of BDNF transcription by multiple signaling pathways (Greenberg et al. 2009). For instance, the Ca^{2+}-dependent up-regulation of BDNF-I in hippocampal neurons is mediated by the upstream regulatory factor binding element (USFBE) and the cAMP responsive element (CRE) (Tabuchi et al. 2002). Transcriptional factors including calcium responsive factor (CaRF), upstream regulatory factor (USF), CRE-binding protein (CREB), and methyl CpG binding protein 2 (Mecp2) may regulate BDNF-IV transcription (Tao et al. 2002; Chen et al. 2003; Martinowich et al. 2003; Zheng et al. 2012). Here, we found that the running-induced BDNF-I and IV up-regulation requires Ca^{2+}-stimulated cAMP signaling. This result is consistent with the hypothesis that Ca^{2+}-stimulated ACs impinge on cAMP-CREB signaling (Sindreu et al. 2007) and regulate gene expression (Wieczorek et al. 2012). There is also evidence suggesting that BDNF-I is an in vivo downstream target of Ca^{2+}-stimulated ACs, as the learning-induced BDNF-I transcription is absent in AC1 and AC8 double knockout mice (Zheng et al. 2012). Consistently, BDNF-I up-regulation induced by short-term running was absent in AC1 KO mice. However, the up-regulation of BDNF-II was intact in the hippocampus of AC1 KO mice following long-term running. Our data not only suggest that genetic alteration does affect the molecular outcome of physical exercise, but also implicate that the existence of multiple BDNF isoforms driven by different promoters provides redundancy to minimize the impact of deficit in certain signaling pathways.

Numerous studies have shown that physical exercise benefits multiple aspects of mental health including improvement of memory, neurogenesis, and attenuation of neurodegeneration. This is likely due to the fact that physical exercise/movement does not occur without neuronal activation in the brain. It involves multiple brain regions for planning and posture adjustment and balance, sensation and feedback on whether the movement is appropriate, motivation, emotion, and reward reflecting physical exercise as a demanding as well as enjoyable experience. Conversely, increased blood flow and circulating hormones to the brain during exercise would affect many aspects of neuronal function. As reported by previous studies, exercise also increases BDNF in the peripheral tissues including the skeletal muscle [(Pence et al. 2016) but also see (Jimenez-Maldonado et al. 2016)]. Although previous works and our data show local increase of BDNF mRNA in brain tissues, it remains unclear on whether exercise-induced peripheral BDNF protein can circulate, cross the blood brain barrier, and affect brain neuron function.

Previous studies support the enhancement effects of exercise on LTP only at the dentate gyrus (DG) but not the CA1 synapses in wild-type animals with normal health conditions (van Praag et al. 1999; Vasuta et al. 2007). However, physical training can revert the reduced CA1 LTP in rodents following disturbance such as epilepsy or sleep deprivation (Arida et al. 2004; Zagaar et al. 2013). This is possibly due to that the CA1 synapses may become more responsive to the beneficial effects of exercise following neuronal insult. Consistent with this idea, the defective CA1 LTP in AC1 KO mice was more sensitive to and enhanced by voluntary running. It is interesting to note that BDNF-II has been reported to increase only in the CA1 region after running (Oliff et al. 1998). Intriguingly, infusion of exogenous BDNF fully rescued and voluntary running partially rescued the LTP deficit in AC1 KO mice. This may be because that the amount of infused BDNF exceeded the level of exercise-induced BDNF. It is also possible that the enhanced BDNF-II following long-term exercise does not directly target to dendrites (Chiaruttini et al. 2008), and hence the restricted local BDNF-II translation in soma may exert less effect on synaptic functions (Waterhouse and Xu 2009).

The function of hippocampus in object recognition memory is under debate (Clark et al. 2000; Winter et al. 2007; Langston and Wood 2010; Barker and Warburton 2011). While some studies have shown normal object recognition memory in rodents with permanent hippocampal lesion [(Duva et al. 1997; Forwood et al. 2005) but also see (Clark et al. 2000)], a more recent study suggests that animals with hippocampal damages may recruit other extrahippocampal structures for the nonspatial recognition memory (Cohen et al. 2013). In undamaged brains, neuronal activity in hippocampus is required for acquisition, consolidation, and retrieval of object recognition memory, which is blocked by intrahippocampal infusion of muscimol or the protein synthesis inhibitor anisomycin (Cohen et al. 2013). Here, we demonstrate that intrahippocampal infusion of BDNF rescued object recognition memory deficits in AC1 KO mice. Our data not only suggest the function of Ca^{2+}-stimulated cAMP signaling but also provide strong support for the role of hippocampus in nonspatial object recognition memory.

It is interesting to note that infusion of exogenous BDNF did not alter LTP and object recognition memory in WT mice. These results are not necessarily contradictory to the conclusions from previous studies (Figurov et al. 1996; van Praag et al. 1999). In contrast to using acute brain slices in most of the previous reports, we determined LTP in vivo in anesthetized mice. The degree of in vivo synaptic potentiation is significantly lower than that detected in brain slices. Comparing to the single HFS-induced LTP, stronger stimulation such as 2 HFS trains did not significantly enhance LTP in WT mice (Fig. 1; Supplemental Fig. S2). However, 2 HFS trains enhanced LTP in AC1 KO mice. One possible explanation is that our experimental setup is not sensitive enough to detect BDNF effects on LTP in WT mice, and impaired LTP in AC1 KO mice is more sensitive to strong stimulation and the increase of BDNF. Consistently, object memory formation in AC1 KO but not WT mice showed better response to BDNF infusion.

Numerous signaling molecules impinge on LTP and memory not necessarily through BDNF-dependent mechanisms. We show that, without affecting the basal BDNF level, AC1 is required. However, the increased BDNF level, after running and supplement of exogenous BDNF, can override the cAMP requirement. This is an interesting result, indicating that exercise and BDNF can facilitate plasticity and memory in the absence of intact cAMP signaling, which plays essential roles under basal conditions. However, our data do not exclude the possibility that synaptic targeting of BDNF and stimulation-induced BDNF release, which are important for certain aspects of synaptic plasticity such as LTP (An et al. 2008; Zheng et al. 2012), are altered and contribute to the defective LTP and object recognition memory in AC1 KO mice.

We acknowledge that reliable wheel running activity requires isolation housing, which may affect animal behavior. Mouse in either the sedentary (equipped with a locked running wheel) or the exercising group was singly housed, and thus the effects of isolation were taken into account and controlled. Our data also show that WT and AC1 KO mice responded similarly to stress and acute stimulation in the tail suspension test, light–dark test, and open filed test. Further, measurements of memory in many previous studies have been done with singly housed animals

following wheel running. The performance in object recognition was not significantly different between the group housed (Fig. 2) and singly housed sedentary AC1 KO mice mouse (Fig. 3), and the singly housed AC1 KO mice showed robust object recognition memory after exercise (Fig. 3).

In conclusion, our study identifies new functions of AC1 in regulating hippocampal LTP, hippocampus-dependent memory formation, and the activity-dependent up-regulation of BDNF-I and IV expression. More importantly, our study demonstrates that physical exercise is sufficient to overcome the defective cAMP signaling and up-regulate BDNF-II in AC1 KO animals. It is interesting to examine whether the benefit of voluntary running as well as BDNF can be extended to ameliorate cognitive impairments in neurological disorders such as Rubinstein–Taybi syndrome, in which the cAMP-mediated signaling is altered.

Materials and Methods

Animals

The AC1 KO mice were generated as described previously (Wu et al. 1995) and maintained on the C57BL/6 background. Animals were housed in the specific pathogen free facility with a 12 h-light/12 h-dark cycle, and had free access to food and water. The Institutional Animal Care and Use Committee at Michigan State University and Kunming Medical University approved all behavioral paradigms and procedures. Young adult male mice (2.5–3.5 mo of age) were used for all experiments. To avoid carry-over effects, separate cohorts were used for each behavioral test; no animal was repeatedly used for more than one paradigm. All mice were handled for at least a week before behavioral examinations.

Electrophysiology

The in vivo electrophysiological recording was performed as previously described (Zhang et al. 2011). Briefly, the mouse was anesthetized with 100 mg/kg Nembutal sodium solution (Ovation) and mounted to a stereotaxic frame (David Kopf Instruments). A pair of electrodes (100 μm outer diameter Teflon-coated wires) was inserted into the brain through drilled holes. The stimulating electrode was placed at the Schaffer collaterals of the dorsal hippocampus (AP, −1.7–1.9 mm; ML, 1.7–1.9 mm; DV, 1.6–2.0 mm from skull surface). The recording electrode was placed at the ipsilateral striatum radiatum of the CA1 area (AP, −1.7–1.9 mm; ML, 1.2–1.3 mm; DV, 1.5–1.9 mm). Electrophysiological signals were delivered and recorded by a PowerLab System (ADInstruments). Stimulating pulses of 100-μsec duration were delivered at an intensity that evoked one-half of the maximum amplitude. The pulses were first maintained at 0.033 Hz to obtain a stable baseline for at least 30 min. LTP was induced by either a single high-frequency stimulation (HFS, 100 Hz for 1 sec) or two HFSs (100 Hz for 1 sec with a 1-min interval). After induction, the sampling rate was returned to 0.033 Hz. For BDNF infusion, a 24-gauge stainless cannula was attached to the recording electrode. Of note, 0.5 μL of BDNF (1 μg/μL) or vehicle was injected at a rate of 0.05 μL/min before the recording.

Object recognition

During training, two objects with different shapes were presented to the mouse for 10 min. During testing (1 h after training), one of the old objects was replaced by a novel object. The number of sniffing/approaches was scored and expressed as "object preference" and "object discrimination index" as previously described (Wang et al. 2004; Cohen et al. 2013).

Voluntary wheel running

Mouse was singly housed in its home cage, which was equipped with either a locked (for the sedentary control group) or unlocked (for the exercised group) running wheel. Mouse had free access to the running wheel during the whole period of the 1 mo (long-term) exercise or 1 day (short-term) exercise. Cyclometers (Cat Eye, Mity 8) were mounted on the unlocked running wheel, and used for daily recording of running time, running distance, and velocity. WT and AC1 KO mice showed similar running activity (as shown in Supplemental Fig. S8).

Passive avoidance

During the training for passive avoidance task (Ding et al. 2014), a mouse was placed in the brightly lit half and allowed to explore freely for 1 min before the trap door was opened. Immediately after the mouse entered the darkened half, it received a mild foot shock (0.7 mA, 2 sec). When the mouse was tested, it was placed in the lit half and its latency to cross over to the darkened half was recorded.

Light–dark test

Light–dark test was performed as described in our previous studies (Wang et al. 2009; Ding et al. 2014). Mouse was introduced to the dark chamber and habituated for 2 min before the door that connects the dark and lit chamber was opened. The mouse was allowed to move freely between the dark and lit chamber for 5 min. The total number of transition to the lit chamber, the latency of animals' initial crossover to the lit chamber, and total time spent in the lit chamber were recorded.

Locomotor activity in open field

To determine locomotor activity in a novel environment, we subjected mice to open field test. During the 15 min test, total ambulatory distance, distanced traveled in the center area of the open filed arena, and number of entries to the center area were recorded by an automated activity-monitoring system (Tru Scan Activity System, Coulbourn Instruments).

Tail suspension and Social interaction test

Mouse was subjected to tail suspension test, as described in Chen et al. (2015), for 6 min. Immobility during the last 4 min or the entire testing session was recorded. The three-chamber social interaction test, as described in Chen et al. (2015), was used to determine social activity. During the 10-min test, mouse was first introduced to the middle chamber, and allowed to travel freely in the three-chamber apparatus. The entry to and time spent in the chambers holding a stranger mouse (in a wire enclosure) or a novel subject (in a wire enclosure) were recorded. The direct interaction between the test mouse and the stranger mouse, as measured by the sniffing time, was also recorded.

General locomotor activity recording in home cage

Animals were singly housed in Plexiglas cages (34 × 28 × 17 cm) with a motion sensor mounted on top of each cage. Home cage locomotor activities were recorded in 5-min bins using VitalView (Minimitter, Inc.) for 12 d. The following parameters were examined as collective data in the last 7 d: total daily activity, daytime activity, and nighttime activity. Daily activity was calculated by averaging the total amount of activity per day. The actograms depicting the daily activity pattern were created using the ClockLab (Actimetrics, Inc.).

Reverse-transcription PCR (RT-PCR)

Hippocampus was removed from the sedentary and exercised mice and homogenized in TRIzol solution (Invitrogen). Total RNA was extracted following the manufacturer's instruction and reverse transcribed into cDNA using the SuperScript III Reverse transcription kit (Invitrogen). The cDNA was subjected to quantitative PCR, which was performed on a Bio-Rad iQ5 system using the SYBR-Green supermix (Bio-Rad Laboratories). The annealing temperature for the reaction is 55°C, and the $2^{-\Delta\Delta Ct}$ value was used to determine the mRNA expression levels. Different sets of

primers were used to amplify exon-specific BDNF mRNAs and GAPDH. The primers used to amplify GAPDH, exon 1-, and exon IV-containing BDNF mRNA were described in our previous studies (Zheng et al. 2011, 2012). Primers used for exon II-containing BDNF mRNA were 5′-CGGTGTAGGCTGGAATAGAC-3′ (forward) and 5′-GCCTTCATGCAACCGAAGTA-3′ (reverse). Primers used for exon VI-containing BDNF mRNA were 5′-GTGACAA CAATGTGACTCCAC-3′ (forward) and 5′-GCCTTCATGCAACCG AAGTA-3′ (reverse). As all the BDNF mRNA isoforms contain the same coding region, total BDNF mRNA level was determined by primers amplifying the coding region: 5′-GCGGCAGATAAAAAG ACTGC-3′ (forward) and 5′-TCAGTTGGCCTTTGGATACC-3′ (reverse). We chose GAPDH as the control gene, because its mRNA is stable in the rodent hippocampus during wheel running (Intlekofer et al. 2013). The *Ct* value of hippocampal GAPDH also did not show detectable variation in this study (data not shown). The expression level of distinct BDNF mRNAs was normalized to GAPDH.

Surgery and microinjection

Mice were anesthetized by ketamine (7.0 mg/mL) and xylazine (0.44 mg/mL) through intraperitoneal injection (22 μL/g body weight), and mounted on a stereotaxic frame (David Kopf Instruments). Two 24-gauge stainless steel cannulae with 30-gauge dummies were bilaterally implanted into the CA1 regions (1.50 mm posterior, 1.65 mm lateral, and 1.78 mm ventral to the bregma). The cannulae were fixed by dental cement and the mice were allowed to recover for at least 7 d before microinjection. 30 min before the object recognition memory training, 0.5 μL of recombinant BDNF (purchased from R&D Systems, 1 μg/μL in PBS) or vehicle (PBS) was infused into the dorsal hippocampus through the cannulae at a rate of 0.05 μL/min using a motorized syringe pump (Hamilton). The positions of cannula tips were verified by histological analysis after the behavioral experiments.

Data analysis

The individuals performing the behavioral and molecular experiments were blinded to the genotypes and treatments. The identity of the genotypes and treatments were decoded before performing data analysis. Data are presented as mean ± SEM. Student's paired *t*-test was used to assess difference between two groups. Two-way repeated measures ANOVA was used for analyzing running behavior and electrophysiology results. One-way or two-way ANOVA and post hoc analysis were used for comparison among multiple groups.

Competing interest statement

The authors declare no competing financial interest.

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