Treadmill running regulates adult neurogenesis, spatial and non-spatial learning by ErbB4 signaling

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Research

Abstract

Recent studies have shown exercise is effective for adult hippocampus neurogenesis and memory. However, the molecular mechanism of exercise is unclear. In this study, AG1478, an ErbB4 inhibitor, was used to explore the involvement of ErbB4 receptors. Four weeks post-running, cognitive impairment was analyzed using T-maze, Morris water maze (MWM) and contextual fear discrimination learning tests, followed by histological assessment of the proliferation and survival of hippocampal neurons using Ki67, NeuN and BrdU immunostaining respectively. Expression of total and phosphate ErbB4 protein level was evaluated by Western blotting. The results showed that AG1478 significantly impaired the performances in T-maze and MWM (spatial learning and memory), contextual fear conditioning and discrimination learning paradigm (non-spatial working and reference memory), enhanced neurogenesis loss, downregulated the expression of p-ErbB4 and total ErbB4 protein, which could be reversed by running. Taken together, our study suggested that running ameliorates cognitive impairment and neurogenesis via ErbB4 signaling.

Introduction

Adult neurogenesis in the subgranular zone of the dentate gyrus (DG) plays important roles in cognitive functions[1], and its impairment is involved in neuropsychiatric disorders, such as bipolar disorder and schizophrenia[2, 3]. Neural stem cells maintenance and differentiation are regulated by GABA release from interneurons, altered GABA signaling are found in in various pathological conditions [4]. Physical exercise and enriched environments can improve cognitive functions through stimulation of hippocampal neurogenesis [5, 6], but the mechanism is still largely unknown. In our previous study[7], we found parvalbumin positive GABAergic interneurons in DG were critical for exercise to increase adult neurogenesis and to improve schizophrenia related phenotypes in mouse model. How exercise affects parvalbumin interneuron function needs to be further investigated.

Neurotrophic factor neuregulin 1 and its receptor ErbB4 are susceptibility genes of schizophrenia and bipolar disorders [8, 9]. ErbB4 is exclusively expressed in GABAergic interneurons in hippocampus, and mainly in parvalbumin positive type, regulating the spine formation and synaptic plasticity in glutamatergic neurons[10, 11], specifically knockout ErbB4 in parvalbumin interneurons is enough to induce cognitive impairments and inhibits adult hippocampus neurogenesis [12]. Exercise can increase neuregulin 1/ErbB signaling in skeletal and cardiac muscle and promote muscle repairment[13, 14], we also observed exercise could increase ErbB4 expression in hippocampus, but whether neuregulin1/ErbB signaling mediates exercise effects on adult neurogenesis is still unknown.

To address ErbB4 functions in neurogenesis and their role for behavioral control on running, we have begun to dissect its functions by pharmacological means. Here, we report the mice behaviorally and addressed the putative physiological and morphological correlates of the observed disturbances of AG1478-treated mice. Based on the above-mentioned disturbances of hippocampal functions of ErbB4 receptor, we focused our analysis mainly on hippocampus-dependent behavior and memory formation. In

fact, our data thus reveal an altered performance of AG1478-treated mice associated with increased anxiety-like behavior, spatial reference memory impairments is associated with decreased adult neurogenesis in these animals, thus exploring the cellular targets of running on neurogenesis.

Materials And Methods

Animal

C57BL/6 male mice (7 weeks old) were purchased from the Experimental Animals Center of Tongji Medical College, Huazhong University of Science and Technology. All mice experiments in this research were carried out in accordance with the recommendations of, and were approved by Animal Welfare Committee of Huazhong University of Science and Technology.

Mice aged between 7 and 13 weeks were used for all experiments. Animals were housed less than 5 mice per cage at 22-24°C with 40-70% humidity, on a schedule of 12h/12h light/dark cycle, with water and food available ad libitum. Mice were all backcrossed with C57BL/6 mice for more than 10 generations.

Experimental Design

A schematic experimental design is shown in Figure1. AG1478 (HY-13524, MCE) or saline, at a dose of 50 mg/kg body weight, were chronically administered intraperitoneally (i.p.) every other day for four weeks. The timing and dosage of AG1478 administration was based on a previous study [15].

Mice in running groups underwent adaptive run-training sessions in individual lanes of a treadmill (FT-200,Taimeng, China) (5 m/min for 45 min) for 5 days, and running sessions (5 m/min for 10 min, 8 m/min for 30 min, 5 m/min for 10 min) for the next 4 weeks to prevent stress-induced inhibition of hippocampal neurogenesis [16]. Mice in the static groups were left for the same duration on the treadmill without running.

BrdU Injections

5'-bromo-2'-deoxyuridine (BrdU; Sigma) in saline were administered i.p. every four hours (100 mg/kg), twice one day, for six days. Animals were sacrificed four weeks later after last BrdU injection.

Behavioral Analysis

No body weight, whisker number, and motor coordination difference was found in any groups during behavioral analysis. All behavioral tests were performed during the light period, all mice were handled for at least 5 minutes twice a day for three days prior to the behavioral test [17]. Behavioral analysis was carried out with 12-week-old mice by investigators unaware of their genotype and groups.

Animals were tested in a sequential order of least disruptive (absence of noxious stimulation: open field and T-maze) to most disruptive (eg., MWM, fear conditioning test, and contextual fear discrimination

learning, which involved physical stimuli, such as loud noise, foot shock or cool water).

Open field test (OFT)

To provide measures of locomotor activity and general anxiety-like behavior, locomotor activity was recorded in the open-field, made of a rectangular chamber (45×45×45 cm). The mouse was gently placed on the center square and allowed to freely explore the arena for 5min. The total distance travelled during a session, the time spent moving and total duration spent in the center were measured by an automated video tracking system (TMV-100S, TaiMeng, China) above the open field. After each trial, the apparatus was swept out with 75% alcohol to avoid the presence of olfactory cues. Total distance traveled was measured.

Forced-choice Spontaneous Alternation in T-Maze Test

A forced-choice paradigm was employed to encourage a higher level of alternation [18].The apparatus was an enclosed maze with 3 arms, a start arm (38 × 7 cm), a central choice area (7 × 7 cm), and two symmetrically choice arms (30 × 7 cm). In brief, each trial consisted of a 5 min acquisition phase, an intertrial-interval (ITI; 2 min) and a final 5 min test phase. ITIs of 2 min were included to assess the persistence of short-term spatial memory [19]. During the acquisition phase, the mouse was placed in the start arm facing the wall and allowed to explore the apparatus. As soon as the animal entered (with all four paws) one of the two choice arms, the door of that compartment was closed for 30 seconds. Then the mouse was removed gently from the maze to homecage for the ITI. At test, the block was removed and the mouse was placed back in the start arm to perform a second choice trial. The novel arm in the second trial was the right choice After each trial, the arena was cleaned thoroughly using 70% ethanol to remove any scent cues, which might identify the novel arm. The test was performed in the room where the animals were housed and comprised 10 trials. Correct percentage (%) = total novel arm in the second trials/ total trials × 100%.

Contextual and Cued Fear Conditioning Test

The contextual and cued fear conditioning test is the behavioral paradigm used to assess associative fear learning, hippocampus-dependent and hippocampus-independent memory function memory in rodents [20]. Briefly, the mouse was placed in the conditioning chamber using the Freeze Monitor system (San Diego Instruments, San Diego, CA, USA) for 3 min as an accommodation period and then one tone-foot-shock pairing (tone, 30 s, 65 dB, 1 kHz; foot-shock, 2 s, 0.75 mA) was delivered. The mouse was allowed to explore the chamber for another 30 s after the shock to study postshock freezing.

The contextual fear conditioning test was assessed 24 h after the training by placing the mice back in the same test chamber for 3 min to assess short-term memory.

The cued-fear conditioning test was assessed 2 h after the contextual fear conditioning test in a novel chamber changed in the shape, color, and smell and the training tone was delivered for 3 min.

Freezing behavior, defined as the absence of all visible movement of the body except the movement necessitated by respiration, was scored by an observe software. At the end of testing, the chamber was cleaned with 75% alcohol to avoid the presence of olfactory cues.

Morris Water Maze

To assess hippocampal-dependent spatial learning and memory, mice were tested in the Morris water maze (MWM,XR-XM101; Shanghai Softmaze Information Technology Co., Ltd., Shanghai, China) (130 cm diameter, 45 cm high) containing opaque water (24–26 °C) and a circular platform (10 cm diameter, approximately 1 cm below the water surface) located in the center of the target quadrant. The MWM was virtually divided in four equal imaginary quadrants by the AnyMaze software. The test was executed as described previously [21]. Briefly, the test consisted of a four-day hidden platform training test and a one-day single probe test. In the training test, the mice were allowed to face to the pool wall in a randomized starting place in the pool to find the hidden platform. The mouse was allowed 120 s to find the platform upon which they sat for 20 s. If the mouse did not find the platform within 120 s, it was gently guided there and allowed to stay for 30 s. Twenty-four hours after the last training session, the platform was removed from the pool and the mouse was placed in the opposite quadrant, and a 60-s probe trial was performed. The escape latency, the percentage of time spent in the target and the number of platform crossings were recorded.

Contextual Fear Discrimination Learning

This paradigm tests the animal's ability to distinguish between two similar contexts [22]. Pattern separation is a fundamental computational function of the DG [23, 24], which depends on normal adult neurogenesis.

The shock-associated training context A (shock) and the similar (no-shock) context B shared many features, including an exposed stainless steel grid floor and roof. The similar context differed from the training context in that four plastic inserts were used to cover the walls. A non-alcoholic antiseptic solution was used to clean the grids between trials. In pilot experiments, mice were exposed to the training context where they received a single 2-s 0.75 mA foot shock , 185 s following placement in the sound proof chamber (29 × 29 × 24 cm; Coulbourn instruments, Allentown, PA, USA, model H10-11M-7C-SF). For discrimination learning, mice were again exposed to training context A. One hour later, mice were placed in the similar context and left for 180 s, and were never shocked. Freezing levels were measured by video camera each day and computed as a Discrimination ratio: (Freezing training context - Freezing similar context) / (Freezing training context + Freezing similar context). A score of 0 indicated complete lack of discrimination, i.e., freezing levels were the same in the similar and training contexts (Freezing similar context = Freezing training context).

Immunofluorescence

Mice were anesthetized with chloral hydrate and perfused transcardially with 4% paraformaldehyde (PFA) in phosphatebuffered saline (PBS, pH7.4), and tissues were fixed overnight in 4% PFA at 4°C. After cryoprotected in 30% sucrose, brain tissues were frozen in OCT and cut into 40 μm by a cryostat (Thermo Scientific, HM550).

The free-floating sections were rinsed three times in PBS, and blocked in PBS with 0.3% Triton and 10% goat serum and 0.1% Triton-X 100 (PBST) for 60 min at room temperature. Sections were then incubated with primary antibodies in PBST at 4 °C overnight. After washing with PBS for 3 times, samples were incubated with donkey anti-rabbit IgG conjugated with Alexa Fluor 594 (711-585-152, 1: 200, Jackson ImmunoResearch), goat anti-rabbit IgG conjugated with Alexa Fluor 488 (SA00006-2, 1: 250, Proteintech) in PBST for 1 hr at room temperature. Samples were mounted with mounting medium, antifading (with DAPI) (S2110, Beijing Solarbio Science & Technology), and images were taken by Zeiss Axioplan light microscope. Quantification of labeling was determined by counting all fluorescent cells in every sixth coronal section.

Immunocytochemistry used following primary antibodies: rabbit anti-KI67 (ab15580, abcam, 1:500), rat anti-BrdU (FITC conjugated; ab74545, Abcam; 1:300).

Western Blot

One day after the behavioral tests, mice were anesthetized with 5% chloral hydrate (8 ml/kg) and the tissues were rapidly collected. The tissue homogenates were prepared on ice in RIPA buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5% sodium deoxycholate, 1% NP40, 1 mM PMSF, and 1 μg/ml protease inhibitor cocktail. For immunoblotting p-ErbB4, homogenates subjected to immunoprecipitation with ErbB4 antibody and protein-A (Roche) at 4°C overnight. Homogenates or bound proteins were resolved on SDS/PAGE and transferred to PVDF membranes, which were incubated in Tris-Phosphate buffer (TBS) containing 0.1% Tween-20 and 5% milk (TBST) for 1 hr at room temperature before the addition of primary antibodies for incubation overnight at 4 °C. After wash, the membranes were incubated with HRP-conjugated secondary antibodies (BL003A, 1: 30000, Biosharp) in TBS for 1 hr at room temperature. Primary antibodies used and blotting conditions were: rabbit anti-phosphate-ErbB4 (Ab76132, 1: 1000, Abcam), rabbit anti-PVALB (A2791, 1: 1000, Abclonal), rabbit polyclonal anti-a-Tubulin (AC003, 1: 500, abclonal). Immunoreactive bands were visualized using enhanced chemiluminescence (1705060, Biorad, United Kingdom), scanned with MicroChemi 4.2 (DNR Bio-imaging Systems, ISRAEL) and semiquantified with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

All Statistical analysis was performed by GraphPad Prism 6.0 Software(GraphPad Software, San Diego, CA). Data were analyzed by unpaired two-tailed Student's t-test or analysis of variance (ANOVA). Significant main effects or interactions were followed up with Tukey's post hoc test. Data were presented as mean ± SEM. Statistical differences were considered to be significant when P < 0.05.

Results

Treadmill running reversed AG1478 effect on ErbB4 phosphorylation, but not expression

To test the molecular mechanisms responsible for promoting adult hippocampal neurogenesis effects of running, AG1478 (HY-13524, MCE), an ErbB signaling inhibitor, at a dose of 50 mg/kg body weight, were chronically administered i.p. 2 h prior to treadmill running every other day for four weeks. Animals were randomly selected and divided into four groups according to the treatment: 1) vehicle+static: mice subjected to saline injection and static treadmill, 2) AG1478+static: mice subjected to AG1478 injection and static treadmill, 3) vehicle+run: mice subjected to saline injection and running treadmill, 4) AG1478+run: mice subjected to AG1478 injection and running treadmill (Figure 1A). In agreement with the previous study[25], The two running groups gained significantly weight loss, however, AG1478 did not influence the body weight (Figure 1B).

One day after the behavioral tests, mice were anesthetized and the tissues were rapidly collected. ErbB4 phosphorylation (Y1258) and total ErbB4 protein expression in hippocampus were decreased in running groups after AG1478 treatment, suggesting the effectiveness of AG1478 (Figure 1C-1E). Treadmill running significantly upregulated ErbB4 phosphorylation and the total ErbB4 expression, however, AG1478 treatment could abolish the effect of treadmill running on ErbB4 expression but not ErbB4 phosphorylation. Interestingly, the ErbB4 phosphorylation level was significantly decreased in AG1478+run group when compared to vehicle+run group, but similar with the vehicle+static group(Figure 1E), suggesting treadmill running reversed AG1478 effect on ErbB4 phosphorylation level to normal.

AG1478 Abolished NSCs survival but not proliferation

To investigate whether running enhances neurogenesis through ErbB4 signaling, Ki67, a marker of proliferating cells, was observed at the end of training (Figure 2A). In line with numerous reports [26], we observed treadmill running enhanced the proliferation of NSCs in the DG (vehicle+run). AG1478 decreased Ki67-positive cells in the DG, this effect could be reversed by treadmill running (Figure 2B).

To further investigate cell survival in parallel, BrdU, an exogenous marker, was injected during the first week of treadmill running to label proliferating progenitor cells (Figure 1A). BrdU$^+$ cells exhibited with an elliptical shape distributed from basal to apical portions of the GCL (Figure 2C). Although treadmill running increased survival of NSCs, in line with previous reports, AG1478 blocked the treadmill effects on NSCs survival (Figure 2D).

Treadmill running effect on anxiety and working memory abolished by AG1478

We initially investigated the locomotor activity and anxiety-like behaviors using the open field test. Briefly, the mice were allowed to freely explore an open field for 5 min. General activity and anxiety were assessed by calculating the total distance traveled, total movement time and time spent in the center of the field, respectively. Compared to animals receiving saline or AG1478 in static, running animals

exhibited lower locomotor activity, lower movement time, but AG1478 could not affect the locomotion (Figure 3A - 3C). Interestingly, although treadmill running increased the time in the center of arena, AG1478 completely blocked this treadmill running effects, without affecting the time in the center of arena in static mice (Figure 3C), suggesting treadmill running effect on anxiety requires neuregulin 1/ErbB4 signaling.

To analyze the effect of ErbB4 on working memory, we examined spontaneous alternations by T-maze test (Figure 3E). As indicated in Figure 3F, animals treated with AG1478 had a significantly lower percentage of correct entries compared to vehicle counterparts, indicating that AG1478 impaired aspects of working memory. Running treatment (vehicle+run) attenuated the working memory deficits by AG1478 administration (AG1478+run), and restored T-maze performance to control levels, while blocking ErbB activation interrupted this benefit action (Figure 3F). These observations demonstrate that enhanced working memory of running might have efficacy on cellular target of ErbB4.

Treadmill running effect on spatial memory partially compromised by AG1478

The Morris water maze is the most frequently used technique to detect spatial memory [27-29]. In the acquisition trial (Figure 4A), mice were subjected to learn the location of a submerged platform placed in target quadrant. Significant differences in escape latency for the hidden platform were found between the static and running groups. In the probe trial (Figure 4B and 4C), when the platform was removed, the number of times the mice of the two running groups crossed the platform increased significantly compared with those in the two static groups. There was no significant difference between AG1478+static group and vehicle+static group. However, this parameter in AG1478+static group was significantly reduced by running (AG1478+run). This evidence indicated that the spatial memory of the running mice was enhanced by ErbB4 signaling.

To further investigate whether it is hippocampus-dependent, we then examined non-spatial memory using a contextual fear conditioning and cued fear conditioning paradigm (Figure 4D). In this task, animals learn to fear a training chamber (context A) by associating it with an aversive stimulus (foot shock). Contextual fear memory was measured 24 h after training. Memory is expressed as percent of freezing responses. The AG1478-treated mice displayed significantly altered fear response in contextual fear conditioning compared with the control (vehicle+static), and running showed significantly higher freezing levels (Figure 4E), indicating that AG1478 impaired contextual fear memory. Two hours later, mice were tested for their memory towards the auditory cue presented in a new shape context (context B), which is hippocampus independent (Figure 4F). However, in this cued-conditioning test, freezing responses of the AG1478-treated mice in static to a tone were similar to the control mice; in addition, no abnormal nociceptive responses were found between the static and running group, this parameter in the AG1478+vehicle group was the same as in AG1478+run ones. These data clearly showed that hippocampus-dependent, but not hippocampus independent, fear memory of the running mice was enhanced by ErbB4 signaling.

Treadmill running effect on pattern separation compromised by AG1478

Previously, Contextual fear-discrimination learning has been demonstrated to be dependent upon the function of adult-generated DG cells [30, 31] and essential for the accuracy of memory encoding[32]. In the current study, we observed altered hippocampal neurogenesis in mice of two running groups when compared to two relative controls. To further assess the functional role of hippocampal neurogenesis on running mice by ErbB4 signaling, we used a contextual fear-discrimination paradigm to analyze their behavior. In this task (Figure 5A), mice were placed in the training chamber (context A) and received a foot shock for training. Then the mice were returned to the same context (context A) with a foot shock or to a modified context (context C) with no foot shock and allowed to explore each context for 3 min on the following five days. Subsequently, their freezing behaviors were evaluated. There was no difference in freezing behavior of four groups for the first three days (Data not shown). On the fourth day, analysis of freezing behavior in both contexts over days revealed that the mice of three groups were able to distinguish between contexts A and C. In contrast, the mice of AG1478+static group showed an only trend towards lower freezing in context C relative to context A (Figure 5B). On the fifth day, all mice showed significantly comparable levels between the two contexts. Among these, the mice in the two running groups showed a significantly decreased freezing response in context C relative to context A (Figure 5C). As a result, mice treated with AG1478 in the two groups displayed significantly decreased discrimination ratio compared to the running mice on the fourth day (Figure 5C). On the fifth day, mice in all groups exhibited a significant increase in the discrimination ratio between the two similar contexts. Running significantly enhanced discrimination ratio when compared to vehicle-treated ones. AG1478-treated mice displayed marked impairment in their ability to discriminate between these similar contexts when compared to the relative controls, and this AG1478-induced deficits could be reversed by running (Figure 5D). Following these data, specific behavioral aspects of these AG1478-induced changes are highly correlated with neurogenesis.

Discussion

Growing evidences have demonstrated that the NRG1-ErbB4 pathway has effect on neurogenesis in both the embryonic and adult rostral migratory stream (RMS) of the mouse [33], the ErbB4 (JM-a)-derived 4ICD fragment promotes neuronal progenitor migration [34]. Specially, a number of mature newborn neurons (BrdU$^+$NeuN$^+$), as well as the number of DCX-positive and GFP-positive immature neurons was decreased in the hippocampus of mice after deleting ErbB4 in PV neurons[12], suggests a possible role for ErbB4 and NRGs, as modulators of the proliferation and migration of neural stem and progenitor cells. These findings might be beneficial to understand the etiology of SCZ. The positive relationship between ErbB4 and neurogenesis aligns with previous findings that this receptor is involved in mitogenic signaling mediated by NRG2 in the SVZ and RMS [35]. Several hundred phosphorylation sites that were significantly regulated upon stimulation of cells expressing both receptors [36]. Phosphorylation of the ErbB4 receptor leads to the recruitment of adaptor/effecter molecules [37] and activates numerous downstream signaling pathways crucial to neuronal development, neuronal migration, axonal navigation, and synaptic function [38]. In the present study, we determined that the ErbB4 protein is activated through phosphorylation in the hippocampus of the running mice, In contrast, AG1478 suppressed this

phosphorylation levels in the hippocampus (Figure 1D), indicating running improve the brain function by activation of ErbB4. These included phosphorylation events mediated through STAT3 activation - a typical pathogenic pathway [39, 40], ERK MAP kinase and Akt pathway activation [36], which account for a variety of phosphorylation-dependent signaling mechanisms known to contribute to cell survival and proliferation.

NRG-1 engages the ErbB receptor tyrosine kinases through its EGF-like domain, activation of ErbB receptors impacts cell proliferation, migration, differentiation, and apoptosis in a variety of cell types[41]. Then we examined neurogenesis at study. BrdU labeling experimental data showed ablation of ErbB4 in PV neurons promotes the hippocampal neural progenitor cell (NPC) proliferation [12]. Ki67 is expressed in the cell cycle during all except the G0 and early G1 phases and used to provide evidences of proliferation [42]. To pharmacologically dissect ErbB4 functions on running and to investigate its role in behavior and neurogenesis, we treated mice with AG1478, an inhibitor of ErbB signaling, to inhibit ErbB4.Our report of putatively dividing and synthetic cells indicates that proliferation and survival cells in DG of the running mice as previously suggested. However, AG1478 block the running-induced activations of ErbB4, and AG1478-treated mice exhibit decreased proliferation of hippocampal neural progenitor cells with deficits in neuronal survival. These further suggest the underlying mechanism of running may be related to the activation of ErbB signaling. Neurogenesis is a multistep process whereby precursor cells undergo lineage-directed cell division to produce immature neurons, of which only a fraction are selected to survive and contribute to hippocampal function. Future studies, employing thymidine analogs, may be needed to make firmer conclusions about the exact time course of running effects on various neurons.

To understand contributions of various pathologies to observed behavioral phenotype of cognitive function, we sought to determine which aspects of cognition and pathology could or could not be rescued. The behavioral analysis of AG1478-treated mice showed anxiety-related behavior, with no change in locomotor activity in an OFT task. What's more, when subjecting AG1478-treated mice to T-maze and the Morris water maze task, we found evidence for a significantly change in sensorimotor gating, working memory and spatial learning by running. Since both vehicle-treated and AG1478-treated mice performed poor in these spatial learning tasks compared to the two running once, we further observed non-spatial learning. We observed decreased contextual fear memory without alterations in cued fear conditioning, whereas running-treated mice on the other hand showed a significantly change in all these paradigms, suggesting a change of hippocampus-dependent.

Since decreases in adult hippocampal newborn neurons impair pattern separation, whereas increases facilitate pattern separation and executive function [43, 44], we decided to render the contextual fear-discrimination learning paradigms to test pattern separation. In fact, these AG1478-treated mice show decreased discrimination ratio for the two similar contexts, whereas this parameter was enhanced by running. These behavioral changes are associated with decreased neurogenesis by ki67-positive and BrdU$^+$NeuN$^+$ label. In support of our neurogenesis results, running rescued deficits of AG1478-treated mice in pattern separation. These physiological changes are likely to increase granule cell responsiveness to stimulation during the acquisition and/or retrieval of contextual and spatial tasks [45, 46]. Disturbed

maturation of the DG has been identified as a critical process in schizophrenia and depression [47] and future studies will have to address the potential involvement of ErbB4-mediated cellular processes in these psychopathologies.

In summary, the cognitive impairment in AG1478-treated mice mainly reflected in the short-term and working memory, spatial learning ability and contextual fear conditioning. This was consistent with that the acquisition trial and probe trial in the water maze and pattern separation in contextual fear discrimination learning may be more sensitive and preferred to assess the function of memory. The present study strongly suggests that, ErbB4 inhibition is capable of inducing neurogenesis deficits in the adult hippocampus and at the same time impair hippocampus-dependent memory. These findings further underscore the interactions between ErbB and neurogenesis may underlie the pathogenesis of SCZ, shedding an additional interesting light on the potential target in the prevention and treatment of this mental disorder.

Declarations

Ethics approval and consent to participate

The animal study was reviewed and approved by the Animal Welfare Committee of Huazhong University of Science and Technology.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Funding

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

YL conceived the study and participated in the experiment design. YY performed the experiments, carried out the functional analysis, and drafted the manuscript. YS and BL contributed to the experiment design

and manuscript preparation. All authors read and approved the final manuscript.

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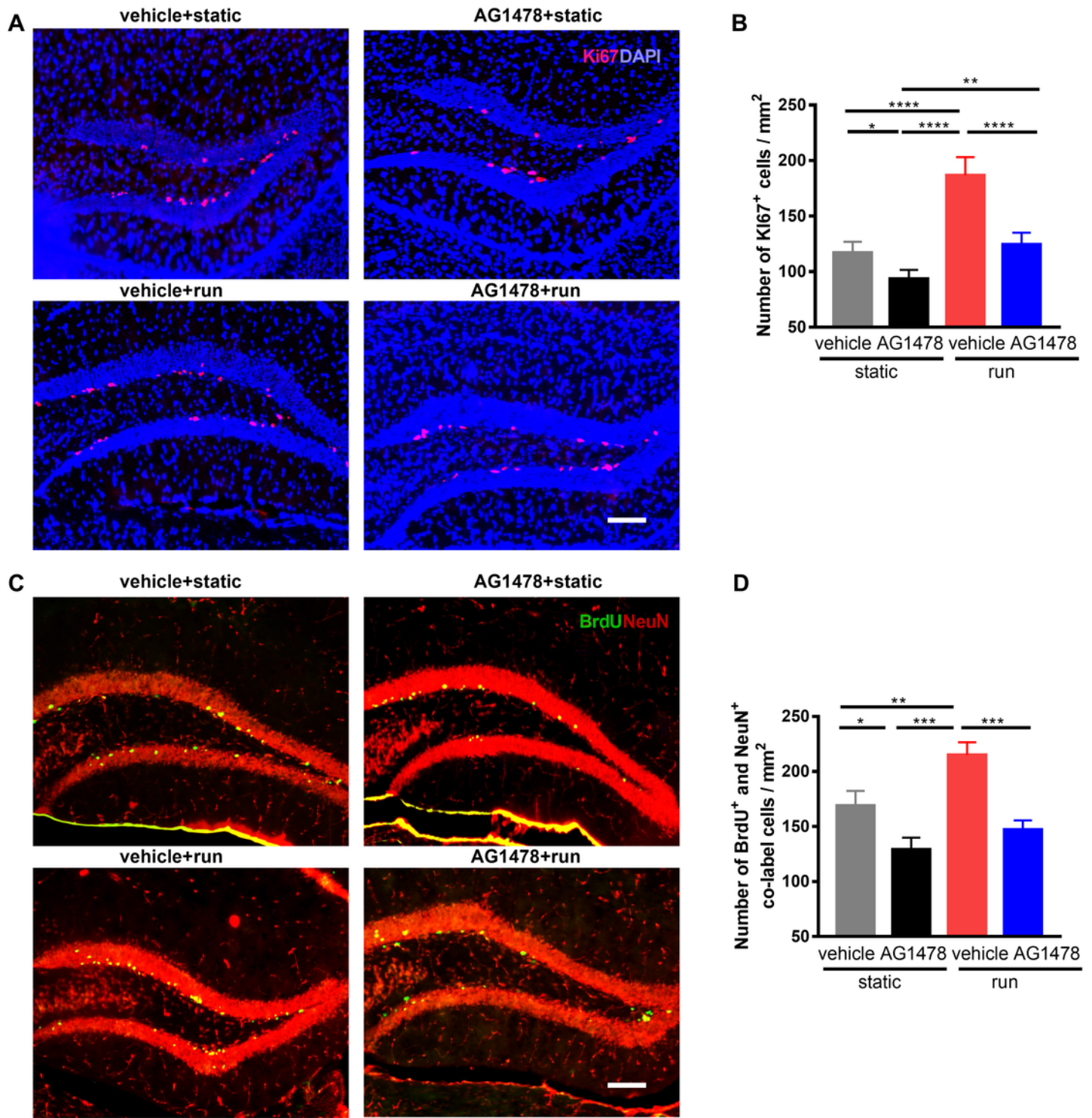
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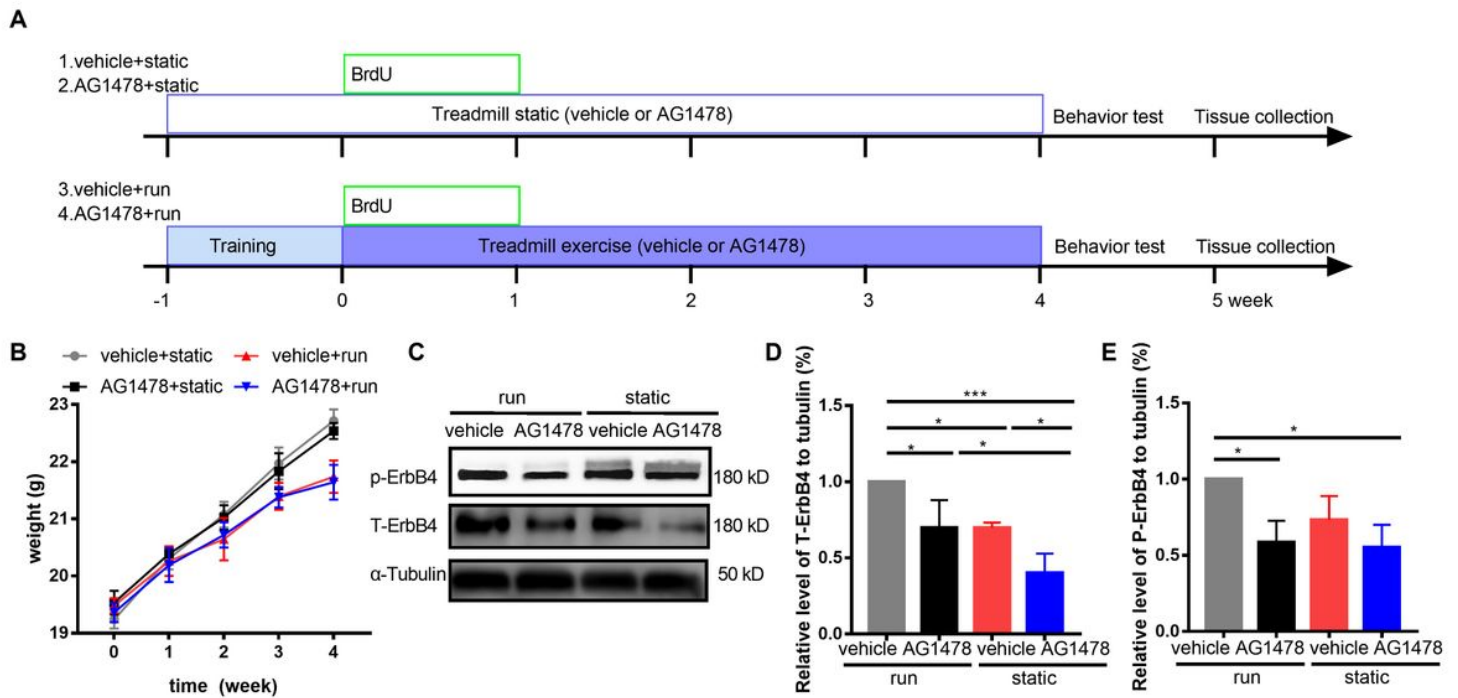
Figure 1

Treadmill running reversed AG1478 effect on ErbB4 phosphorylation, but not expression. A. Schematic experimental design. The schematic shows the time course of the mice subjected to vehicle or AG1478 and running or static treadmill (4 weeks), behavioral test and morphological analyses. A. The body weight of both the vehicle+run group and AG1478+run group weighs less than the relevant control group (vehicle+static group and AG1478+static group), while AG1478 does not affect it. Data are expressed as mean ± SEM, and there were ten mice in each group. Two-way ANOVA, $F_{3,180}$ = 44.33, $P < 0.0001$; post hoc test: vehicle+static vs. vehicle+run: $p < 0.0001$, vehicle+static vs. AG1478+run: $p < 0.0001$, AG1478+static vs. vehicle+run: $p < 0.0001$, AG1478+static vs. AG1478+run: $p < 0.0001$. ****, $P < 0.0001$. B. Representative western blots of total ErbB4 and phosphate-ErbB4 in the hippocampus. a-Tubulin served as loading control. C. Quantitative analysis of the expression of total ErbB4 in the hippocampus. Data are expressed as mean ± SEM, and there were three mice in each group. One-way ANOVA, $F_{3,8}$ = 46.05, $P < 0.0001$; post hoc test: vehicle+run vs. AG1478+run: $p = 0.0433$, vehicle+run vs. vehicle+static: $p = 0.0435$, vehicle+run vs. AG1478+static: $p = 0.0008$, AG1478+static vs. AG1478+run: $p = 0.0480$, vehicle+run vs. AG1478+static: $p = 0.0478$. *, $P < 0.05$, ****, $P < 0.0001$. D. Quantitative analysis of the expression of phosphate-ErbB4 in the hippocampus. Data are expressed as mean ± SEM, and there were three mice in each group. One-way ANOVA, $F_{3,8}$ = 7.515 $P = 0.0103$; post hoc test: vehicle+run vs. AG1478+run: $p = 0.0181$, vehicle+run vs.AG1478+static: $p = 0.0181$. *, $P < 0.05$.

Figure 2

AG1478 Abolished NSCs survival but not proliferation. B. Representative images of Ki67+ cells and DAPI in DG. Scale bar, 100 μm. C. Quantification of Ki67+ cells reveals a significant decrease in their total number in AG1478+static mice when compared to vehicle+static mice, which are increased by running. Data are expressed as mean ± SEM, and there were 5 mice in each group. One-way ANOVA, $F_{3,8} = 58.58$, $p < 0.0001$; post hoc test: vehicle+static vs. AG1478+vehicle: $p = 0.0255$, vehicle+static vs. vehicle+run: p

< 0.0001, AG1478+static vs. vehicle+run: p < 0.0001, AG1478+static vs. AG1478+run: p = 0.0034, vehicle+run vs. AG1478+run: p < 0.0001. *, P < 0.05, ****, P < 0.0001. D. Representative images of BrdU+ cells and their colocalization with NeuN in DG. Scale bar, 100 μm. E. Quantification of BrdU+NeuN+ cells reveals a significant decrease in their total number in AG1478+static mice when compared to vehicle+static mice, which are increased by running. Data are expressed as mean ± SEM, and there were 5 mice in each group. One-way ANOVA, F3,16 = 55.14, P < 0.0001; post hoc test: vehicle+static vs. AG1478+vehicle: p = 0.0002, vehicle+static vs. vehicle+run: p < 0.0001, vehicle+static vs.AG1478+run: p =0.0325, AG1478+static vs. vehicle+run: p < 0.0001, vehicle+run vs. AG1478+run: p < 0.0001,*, P < 0.05, ****, P < 0.0001.
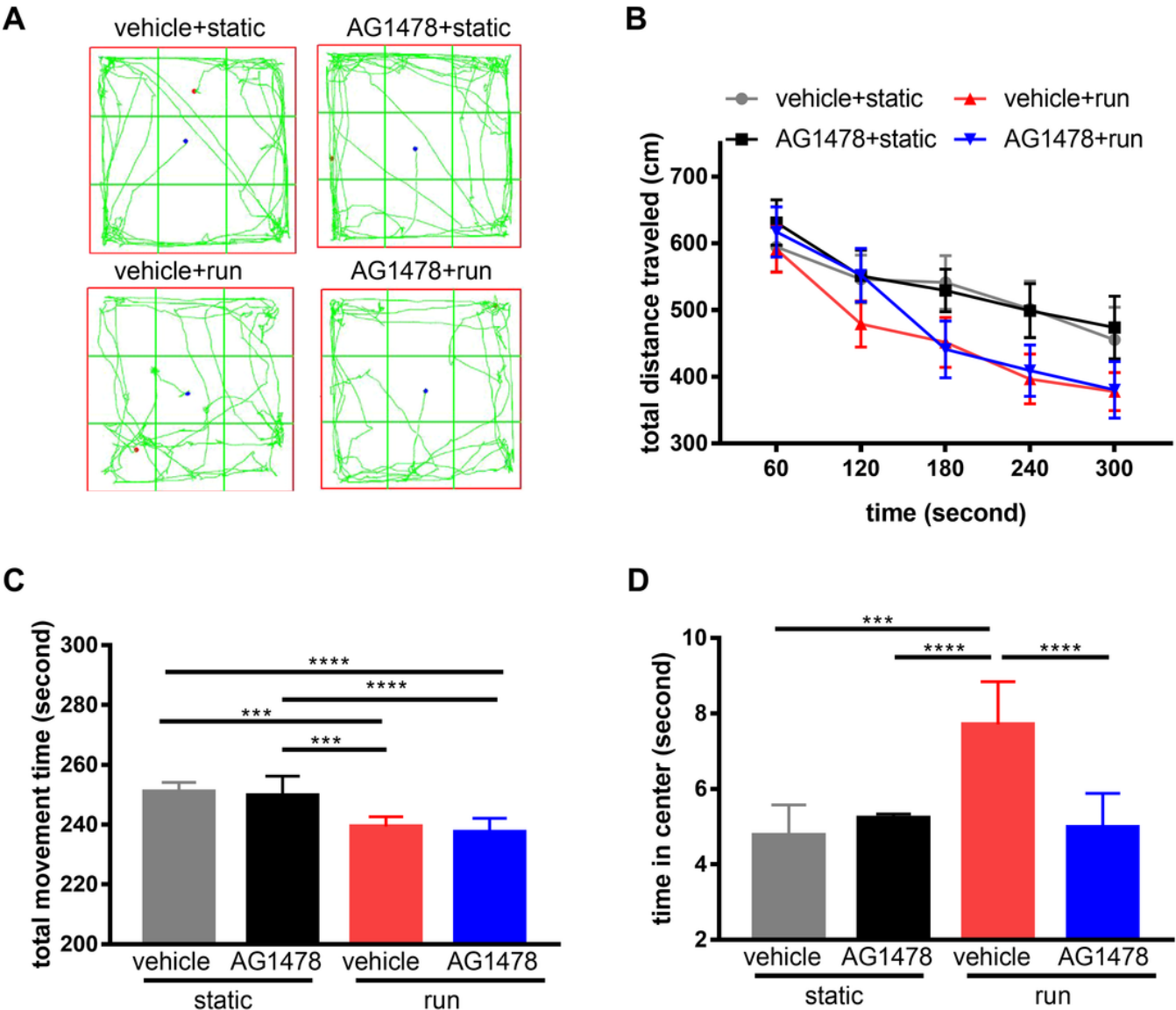

Figure 3

Treadmill running effect on anxiety and working memory abolished by AG1478. A. Representative traces of mice movement during the open field test. Test duration: 5 min. B. In the open field, the total distance

travelled of both the vehicle+run group and AG1478+run group are lower than the relevant control group (vehicle+static group and AG1478+static group), while AG1478 donot affect it. Data are expressed as mean ± SEM, and there were 8, 9, 9, 9 mice in vehicle+static, AG1478+static, vehicle+run and AG1478+run group, respectively. Two-way ANOVA, $F_{3,155}$ = 40.81, p < 0.0001; post hoc test: vehicle+static vs. vehicle+run: p < 0.0001, vehicle+static vs.AG1478+run: p < 0.0001, AG1478+static vs. vehicle+run: p < 0.0001, AG1478+static vs. AG1478+run: p < 0.0001. ****: p < 0.0001. C. In the open field, the total movement time travelled of AG1478+vehicle group is more than the vehicle+static group, which is significantly decreased by running. Data are expressed as mean ± SEM, and there were 8 mice in each group, respectively. Two-way ANOVA, $F_{3,28}$ = 18.52, P < 0.0001; post hoc test: vehicle+static vs. vehicle+run: p = 0.0001, vehicle+static vs.AG1478+run: p < 0.0001, AG1478+static vs. vehicle+run: p = 0.0005, AG1478+static vs. AG1478+run: p < 0.0001. ***: p < 0.001,****: p < 0.0001. D. The mice of vehicle+run group spent significantly more time in the center of the field when compared with all the other three group' s mice, although there was no significant difference among the three groups. Data are expressed as mean ± SEM, and there were 8, 9, 9, 8 mice in vehicle+static, AG1478+static, vehicle+run and AG1478+run group, respectively. Two-way ANOVA, $F_{3,30}$ = 23.94, P < 0.0001; post hoc test: vehicle+static vs. vehicle+run: p < 0.0001, AG1478+static vs. vehicle+run: p < 0.0001, vehicle+run vs. AG1478+run: p < 0.0001. ****: p < 0.0001.
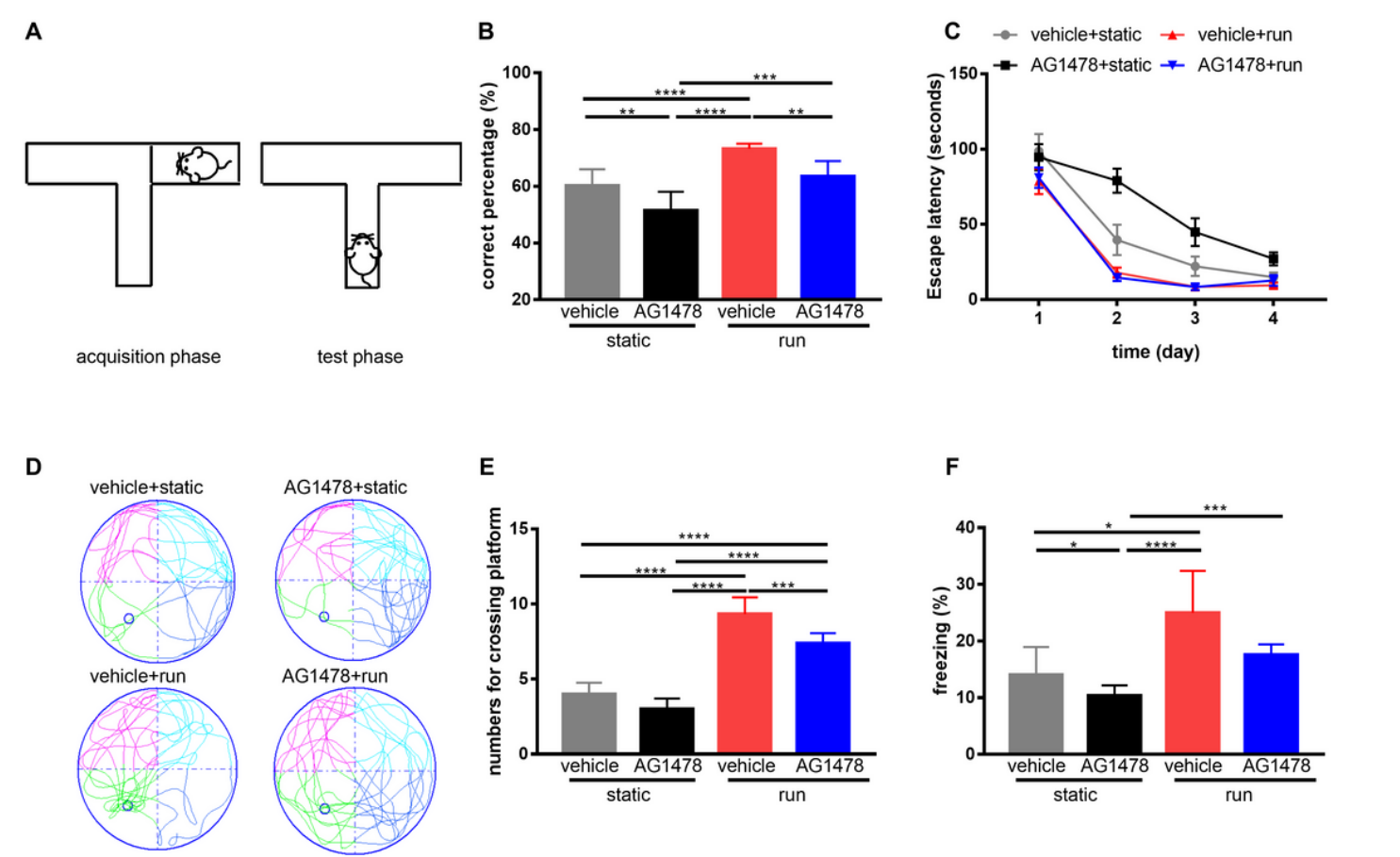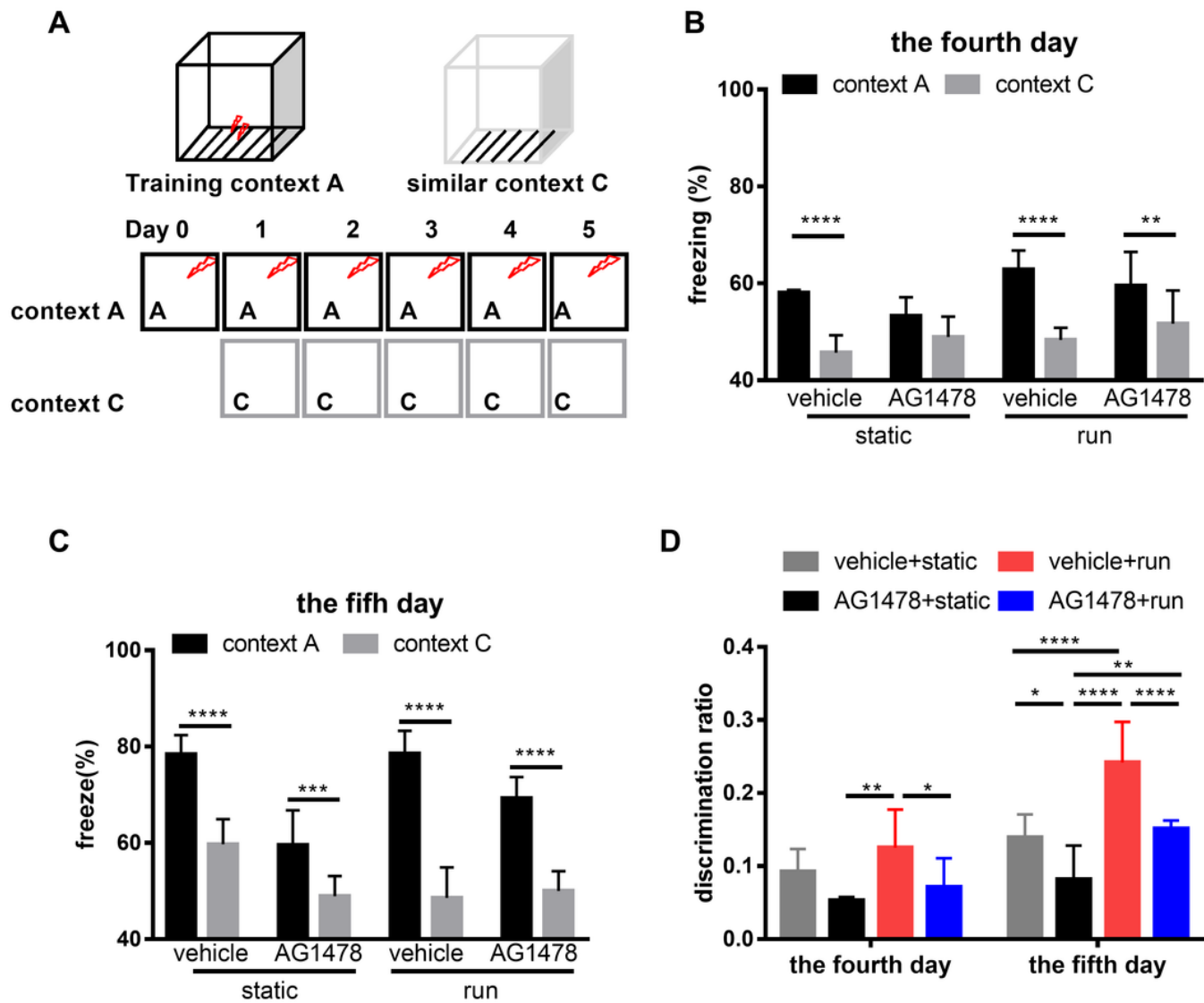

Figure 4

Treadmill running effect on spatial memory partially compromised by AG1478. A. Schematic illustration of the T-maze test. B. In the T-maze test, AG1478 significantly decrease the correct percentage of the right arm, which are enhanced by running. Data are expressed as mean ± SEM, and there were 9 mice in each group. One-way ANOVA, $F_{3,32} = 26.53$, $P < 0.0001$; post hoc test: vehicle+static vs. AG1478+vehicle: $p = 0.0056$, vehicle+static vs. vehicle+run: $p < 0.0001$, AG1478+static vs. vehicle+run: $p < 0.0001$, AG1478+static vs. AG1478+run: $p = 0.0002$, vehicle+run vs. AG1478+run: $p = 0.0022$. **: $p < 0.01$, ****: $p < 0.0001$. C. In the acquisition trial of the Morris water maze, AG1478 significantly increase the escape latency to find platform in the AG1478+static group compared with those in the vehicle+static group, which are shortened by running. The mice of all group Data are expressed as mean ± SEM, and there were 8, 9, 9, 8 mice in vehicle+static, AG1478+static, vehicle+run and AG1478+run group, respectively. Two-way ANOVA, $F_{3,120} = 190.5$, $P < 0.0001$; post hoc test: vehicle+static vs. AG1478+satic: $p < 0.0001$, vehicle+static vs. vehicle+run: $p < 0.0001$, vehicle+static vs. AG1478+run: $p < 0.0001$, AG1478+static vs. vehicle+run: $p < 0.0001$, vehicle+static vs. AG1478+run: $p < 0.0001$. ****: $p < 0.0001$. D. Swimming trajectory images of the mice in the probe trial. E. In the probe trial of the Morris water maze, AG1478 significantly decrease the number of times the mice in the AG1478+static group crossed the platform compared with those in the vehicle+static group, which are enhanced by running. The mice of all group Data are expressed as mean ± SEM, and there were 8, 9, 9, 8 mice in vehicle+static, AG1478+static, vehicle+run and AG1478+run group, respectively. Two-way ANOVA, $F_{3,30} = 108.5$, $P < 0.0001$; post hoc test: vehicle+static vs. vehicle+run: $p < 0.0001$, vehicle+static vs. AG1478+run: $p < 0.0001$, AG1478+static vs. vehicle+run: $p < 0.0001$, vehicle+static vs. AG1478+run: $p < 0.0001$, vehicle+run vs. AG1478+run: $p = 0.0002$.***: $p < 0.001$, ****: $p < 0.0001$. F. The AG1478-treated mice displayed significantly altered fear response in contextual fear conditioning compared with the control (vehicle+static), and running showed significantly higher freezing levels. Data are expressed as mean ± SEM, and there were 8, 10, 9, 8 mice in vehicle+static, AG1478+static, vehicle+run and AG1478+run group, respectively. Two-way ANOVA, $F_{3,31} = 16.99$, $P < 0.0001$; post hoc test: vehicle+static vs. vehicle+run: $p = 0.0002$, AG1478+static vs. vehicle+run: $p < 0.0001$, AG1478+static vs. AG1478+run: $p = 0.0122$, vehicle+run vs. AG1478+run: $p = 0.0122$. *: $p < 0.05$, ***: $p < 0.001$, ****: $p < 0.0001$.

Figure 5

Treadmill running effect on pattern separation compromised by AG1478. A. Mice were tested in a contextual fear discrimination learning paradigm. Briefly, in two similar contexts, foot shook was only present in context A. After several trials training in context A and C, mice could discriminate context A but not C as the cue of foot shook tested by freezing. B. The fourth day of contextual fear discrimination learning. All mice can discriminate between the context A and context C, expect the mice in the AG1478+static group. Data are expressed as mean ± SEM, and there were 8 mice in each group. Two-way ANOVA, $F_{1,56}= 74.18$, $P < 0.0001$; post hoc test: the discrimination between context A and context C: vehicle+static: $p < 0.0001$, vehicle+run: $p < 0.0001$, AG1478+run: $p = 0.0043$, *: $p < 0.05$, ****: $p < 0.0001$. C. The fifth day of contextual fear discrimination learning. All mice can discriminate between the context A and context C. Data are expressed as mean ± SEM, and there were 8 mice in each group. Two-way ANOVA, $F_{1,56}= 232$, $P < 0.0001$; post hoc test: the discrimination between context A and context C:

vehicle+static: p < 0.0001, AG1478+static: p = 0.0005, vehicle+run: p < 0.0001, AG1478+run: p < 0.0001, ***: p < 0.001, ****: p < 0.0001. D. The discrimination ratio indicates that the learning ability of the mice changed along the learning period, AG1478 significantly decrease the discrimination ratio in the AG1478+static group compared with those in the vehicle+static group, which are enhanced by running in the fifth day. Data are expressed as mean ± SEM, and there were 8 mice in each group. Two-way ANOVA, $F_{3,56}$= 25.15, P < 0.0001; post hoc test: in the fourth day: AG1478+static vs. vehicle+run: p = 0.0022, vehicle+run vs. AG1478+run: p = 0.0325 . in the fifth day: vehicle+static vs. AG1478+static: p = 0.0206, vehicle+static vs. vehicle+run: p < 0.0001, AG1478+static vs. vehicle+run: p < 0.0001, vehicle+static vs. AG1478+run: p = 0.0032, vehicle+run vs. AG1478+run: p < 0.0001 . *: p < 0.05,***: p < 0.001, ****: p < 0.0001.

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